# IRRIGATION AND NITROGEN MANAGEMENT OF SOYBEAN IN HIGHLY PRODUCTIVE SOILS

BY

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## DISSERTATION

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#### ABSTRACT

"Intensively managed" is a phrase becoming more common and describes an approach in which soybean [Glycine max (L.) Merrill] producers try to increase soybean yields through various applications of in-season inputs or other alterations of typical production practices. Two areas of interest include water limitations of soybean yield under rainfed conditions in highly productive Illinois soils and use of fertilizer nitrogen (N) to supplement soybean when mineralized soil N and biologically fixed N may be insufficient. Three studies on productive soil in Urbana, IL over six years (2008, 2009-2010, 2012-2014) tested irrigation's impact seeding rate or seed treatment and different in-season products (fungicide, insecticide, nitrogen fertilizer, and foliar macro and micro-nutrients) with and without irrigation. Additionally, nine site-years from a combination of years (2014-2017) and locations (Brownstown, Chillicothe, Monmouth, and Urbana) examined the impact of N applied at different timings (planting, R1, R3, R5, and planting+R1+R3+R5) over a range of Illinois soils. Soybean yield was increased by irrigation at Urbana, IL by an average of 685 kg ha<sup>-1</sup> (15.8%) in three years with moderate precipitation deficits (2008, 2012, and 2013), but not in three years (2009, 2010, and 2014) with more consistent rainfall. Across all six years, irrigation increased soybean yield by an average of 295 kg ha<sup>-1</sup> (6.2%). Three applications of foliar fungicide (2009-2010) or two applications of fungicide plus insecticide (2012-2014) consistently increased yield, by 311 kg ha<sup>-1</sup> (6.2%) and 269 kg ha<sup>-1</sup> (5.5%), respectively. Seeding rate and seed treatment had no effect on yield. Irrigation interacted with other management factors only in 2008, when separate treatments of fungicide and nitrogen fertilizer increased yield only under irrigation. Fertilizer N applied four times (at planting, R1, R3, and R5) increased yield at four of nine sites, but yield increases were insufficient to pay for repeated applications of N fertilizer. Applying N at planting on coarsertextured soils at Chillicothe increased yield by 1,830 kg ha<sup>-1</sup> (16%) in 2015 and by 1,351 kg ha<sup>-1</sup> (26%) in 2016 but had no effect in 2017. Managing soybean with irrigation, fungicide and/or insecticide, and nitrogen fertilizer increased yields, but responses were typically modest and not consistent. Moderate yield benefits combined with considerable costs make these practices unprofitable at current soybean prices for most Illinois producers.

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## **CHAPTER 1: GENERAL INTRODUCTION**

## **1.1 INTRODUCTION**

Increasing production of all crops to meet food, fuel, and fiber needs of the rising global population is a concern. Many crops meet this need, including soybean [Glycine max (L.) Merrill]. In 2017, 4.01 million ha of soybean were grown in Illinois, exceeded by only corn [Zea mays L.] (USDA-NASS, 2018). Historical yield gain has been attributed to a combination of genetic improvements, improved technology and management, and increased atmospheric CO2 concentration (Specht et al., 1999). Specht et al. (2014) attributed two-thirds of yield improvement to continual releases of new soybean cultivars and one-third to advances in agronomic practices. Consideration of agronomic practices allows producers to actively improve yields.

In the last twenty years soybean price received peaked at \$.53 kg<sup>-1</sup> in 2012 with prices averaging \$.39 kg<sup>-1</sup> between 2008 and 2017, but only \$.22 kg<sup>-1</sup> between 1998 and 2017 (USDA-NASS, 2018). Recent high yields along with yield contests with prizes for breaking the 6,724 kg ha<sup>-1</sup> (100-bushel) barrier has encouraged producers to "intensively manage" soybean.

Studies were implemented to quantify areas of interest for potential improvement in soybean management. Corresponding with the widespread drought in 2012, the effectiveness of irrigation on productive Illinois soils and its impact on management factors including but not limited to seeding rate, fungicide and nitrogen applications were tested. Additional resources were used to investigate the impact of N timings on soybean yield on several Illinois soils.

## **1.2 LITERATURE REVIEW**

#### Irrigation

Timing irrigation by both growth stage and soil moisture deficits, through multiple irrigation techniques, have effectively increased yield. Irrigating at various reproductive growth stages often increased yield (Kendig et al., 2000, Eck et al., 1987; Kadhem et al., 1985a; Korte et al., 1983a), and Korte et al. (1983a) found that irrigating during pod elongation increased yield most consistently. Regular irrigation timings included through reproductive growth regardless of moisture also increased yield (Kadhem et al., 1985a ,Korte et al., 1983a), though sometimes

varying by year (Kadhem et al., 1985a). Use of specific growth stages to apply irrigation has been effectives, but this research has usually been implemented in areas with water limitations. Applying irrigation based on soil water deficits, usually initiated around flowering, similarly benefited soybean yields (Ball et al., 2000; Heatherly and Pringle, 1991; Heatherly, 1988; Heatherly and Elmore, 1986).

Physiological changes contributing to higher yields were more common for seed number than seed mass. Less water stress during flowering and pod formation generally resulted in more seeds (Eck et al., 1987; Kadhem et al., 1985b; Korte et al., 1983b). Irrigation after pod formation resulted in greater seed mass (Kadhem et al., 1985b; Korte et al., 1983b). Full season irrigation timed from water deficits, consistently had higher seed numbers benefiting yield (Frederick et al., 1991; Heatherly and Pringle, 1991; Heatherly, 1988; Heatherly and Elmore, 1986), whereas seed weight response was less consistent and only occasionally increased yield (Heatherly and Pringle, 1991).

## **Plant density**

Achieving optimum soybean plant density is important as producers seek to maximize economic efficiency. For Illinois producers, 247,000 plants ha<sup>-1</sup> at harvest has been suggested as a minimum density in which soybean yield reaches a maximum (Nafziger, 2009). Several studies across the Midwest looking at seeding rate in Illinois, Kansas, and Nebraska have found maximum yield near 375,000 (Cooper, 1977), 284,000 (Devlin et al., 1995), and 112,000 seeds ha<sup>-1</sup> (Elmore, 1998). De Bruin and Pedersen (2008a) found maximum yield with a harvest density of 462,000 plants ha<sup>-1</sup>, but more than 95% of maximum yield was reach with just 259,000 plants ha<sup>-1</sup>. In other instances, soybean yield responded to high densities. Herbert and Litchfield (1984) found maximum yields with a harvest density as high as 680,000 plants ha<sup>-1</sup> (800,000 seeds ha<sup>-1</sup>). Seeding rate research has shown a range of results, but the differences demonstrate that seeding rates are generally flat and the ability of soybeans to compensate.

Changes in seed number and size have both been associated with greater yield from increasing seeding rates. Egli (1988) found that seed number increased with increasing yield and density, while De Bruin and Pedersen (2008b) found consistent seed number with greater seed size. De Bruin and Pedersen (2008a) also measured greater seed mass as yields increased; however, Wright et al. (1984) and Elmore (1998) both found that seed mass decreased, at first rapidly and then more slowly as plant density continued to increase. Egli (1988) also found

positive and negative seed size responses depending on the year. Soybean plants have the ability to use seed number or size to increase yield depending on the conditions.

## Seed treatment

Seed treatments are often used to combat a broad spectrum of early and mid-season pathogens and insect species (Gaspar et al., 2015), especially as planting shifts earlier to cooler and wetter soils (Esker and Conley, 2012). Seed treatments with a combination of fungicide and insecticide have been effective at improving plant stands and/or yields (Gaspar et al., 2015; Esker and Conley, 2012; Cox and Cherney, 2011), though sometimes results are mixed (Gaspar et al., 2014) and there are even cases where seed treatments are ineffective (Cox et al., 2008). When seed treatment does increase yield, the benefit is often small and profitability varies. Gaspar et al. (2015) and Esker and Conley (2012) found that seed treatment improved net returns, while Cox and Cherney (2011) concluded that the practice was not profitable. Bacteria (*Bradyrhizobium japonicum*) inoculum has also been applied as seed treatment to promote fixation of N<sub>2</sub>. In fields with a history of soybean production, results have been mixed. Schulz and Thelen (2008) found that inoculation increased yields were in six of 14 site-years, but De Bruin et al. (2010) found that inoculation did not increase yield in 63 of 73 sites across Indiana, Minnesota, Nebraska and Wisconsin. Seed treatments have benefited yield, but small yield increases and sometime inconsistent responses make the economics less certain.

#### **In-season fertilization**

Supplying adequate nutrition is also critical to maximizing yield. Soybean plants utilize large amounts of nitrogen (N), grain contains between 55-58 mg N per kg of yield (Gaspar et al., 2017; Bender et al., 2015; IPNI, 2018). The plants satisfy these requirements through N available in the soil and from biological fixation of atmospheric N<sub>2</sub> through a symbiotic relationship with bradyrhizobium. Fixation typically accounts for 50-60% of N used by a plant (Salvagiotti et al., 2008). The ability of a soybean plant to use both soil N and fixed N is advantageous and well established; however, when soil levels of N are high, less N is fixed (Russelle, 2008). Inhospitable environmental conditions such as water stress can also limit fixation of N (Purcell et al., 2004; Purcell and King, 1996).

As previously stated, multiple factors have led to yield improvement since soybean was first introduced to the U.S. Recently there has been concern about soybean plants' ability to fix enough  $N_2$  as soybean yield reach higher levels. Interestingly, Wilson et al. (2013) found yearly

yield increases of maturity group III cultivars, between 1924 and 2008, to be 20% higher when supplied with a non-limiting source of nitrogen.

Yield responses to fertilizer N on soybean have been inconsistent. N applied near planting by deep banding (Salvagiotti et al., 2009), as starter (Osborn and Riedell, 2006), as broadcast over multiple planting dates (Taylor et al., 2005), as starter on double crop soybeans (Starling et al., 1998), and as pre-plant with incorporation (Ham et al., 1975) have frequently increased yields. Likewise, trials (Salvagiotti et al., 2009; Wesley et al., 1998; Wood et al., 1993) including applications during reproductive growth have also increased soybean yield. However, other trials have shown that similar application timings near planting (Slater et al., 1991; Bharati et al., 1986) and during reproductive growth (Barker and Sawyer, 2005; Freeborn et al., 2001) didn't increase yields. Welch et al. (1973) conducted a number of studies investigating direct and residual N sources, N rates, and application timings, and reported only occasional yield responses at high (450 kg N ha<sup>-1</sup> or more) N rates.

Some researchers have reported that split applications increased yields. La Menza et al. (2017) increased soybean yield by supplied nitrogen through five applications to meet plant N demand. Wilson et al (2013) investigated a range of previously released varieties and that found a non-limiting source of N applied through two applications increased soybean yield. Beard and Hoover (1971) on the other hand reported that nitrogen rates split between pre-plant and flowering did not increase yield.

Compensation by the plants was not frequently described throughout much of the literature. Salvagiotti et al. (2009) found greater seed mass associated with greater yield. Ham et al. (1975) found greater seed mass from N and occasionally greater seed number. Starling 1998 saw no differences in seed size.

An in-depth review by Salvagiotti et al. (2008) emphasized that N applications likely can help overcome environmental constraints including the low soil N, poor nodule establishment, plant water stress, and soil pH. It was further suggested that soybean were more likely to response to N fertilization at high yield levels. Either deep banding slow-release N below the nodulation zone or N applications during late reproductive growth to avoid inhibiting N<sub>2</sub> fixation were suggested as promising application strategies.

The role of foliar applications of macro and micro nutrients in supplying the soybean crop nutrition has also been explored, especially during seed fill. Macro nutrient research

focused on nitrogen (N), phosphorus (P), potassium (K), and sulfur (S). Nelson et al. (2005) used applications of K to increase soybean yield with the greatest success on sites with low to medium soil K levels. After examining different nutrient ratios, frequencies, and timings, Garcia and Hanway (1976) found that yield and seed number showed the greatest increases when applied multiple times in the ratio of 10:1:3:0.5 (N:P:K:S) during seed fill. Mallarino et al. (2001) also found small yield increases from foliar N, P, and K applications, but only for three treatments across eighteen sites.

Some studies applying foliar N, P, K, and S applications during seed fill had even less success. Poole et al. (1983) found only one increase of sixteen site years; Boot et al. (1978) found higher nutrient concentrations in the leaves, but no yield differences; and Parker and Boswell (1980) decreased yields, attributing changes to damaged foliage. Results varied greatly, but some papers, unsurprisingly, noted that positive responses were more common with deficient soils. Conclusions often suggested that returns seldom offsets costs, though probability would increase when reducing costs by tank-mixing foliar fertilizer with post-emergence herbicides.

Micronutrients commonly included in foliar applications include boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn). Schon and Blevins (1990) increased yields in multiple experiments with B, whereas Reinbott and Blevins (1995) could only increase yield if Mg was included with B. On the other hand, Freeborn et al. (2001) found no response to B. Of those that increased yield, pod number followed suit. Use of foliar Fe has often been tested on calcareous soil with a history of Fe deficiency. Niebur and Fehr (1981) used multiple applications of Fe to prevent yield loss while evaluating soybean genotypes. Goos and Johnson (2000) increased soybean yield in certain site-cultivar combinations.

Gettier et al. (1985) and Randall et al. (1975) established trials on sites with visible Mn deficiency and low soil Mn, respectively. In both studies, foliar Mn increased yield and seed mass and, in most cases, seed number. Boswell and Anderson (1969) and Parker and Harris (1962) found that addition of Mo increased yields frequently on acidic soils. Campo et al. (2009) concentrated chiefly on enriching seed with Mo for improving yields in subsequent season and concluded that in most cases, Mo-rich seeds did not require any further supply of Mo as fertilizer. Enderson et al. (2015) reported no increases in soybean yield with foliar Zn over many sites (twenty-three soil series.) Rose et al. (1981) reported yield increases at three sites and attributed this to having established trials on alkaline soils likely to be deficient in Zn.

Several researchers also looked at adding micronutrient combinations of B, Cu, Mn, Mo, and Zn (Enderson et al. 2015); B, Fe, and Zn (Haq and Mallarino, 2005; Mallarino et al., 2001); and B, Co, Cu, Fe, Mn, Mo, and Zn (Poole et al., 1983) to the soybean crop with no success. Enderson et al (2015) found that adding micronutrients often increased nutrient concentrations in seeds and occasionally in the leaves but did not increase yields. Poole et al. (1983) found that micronutrients decreased yield in one cultivar-in one year.

It appears that the likelihood that micronutrients will increase soybean yields is highly dependent on soil factors, including pH unfavorable to availability of certain micronutrients or to low micronutrient levels in the soil. Even when yield responded to micronutrient applications, the yield increase is often inadequate to cover the cost of material plus application.

#### **In-season pest management**

Plant protection is also an important aspect of maintaining plant health and there is interest in prophylactic applications of pesticides to prevent pest damage in soybean. Current recommendations are described as an integrated pest management (IPM) approach in which regular monitoring is combined with several control methods and pesticides are only utilized when economically warranted. Effective management of pathogens include selecting resistance varieties, using high-quality seed, utilizing tillage when necessary, fungicides, scouting, and proper insect and weed control (Bradley, 2009).

Research on pathogens and fungicides were conducted in low and high-pressure environments. Under substantial disease pressure, from above normal precipitation, Nelson and Meinhardt (2011) found that pyraclostrobin increased soybean yields 20 to 27% without subsurface drainage and up to 36% with. The severity of septoria brownspot and frogeye leaf spot were reduced 2-8%. Cooper (1989), also working with septoria brownspot, increased soybean yield between 7.7 and 15.5% depending on row spacing and variety with benomyl.

In other cases, fungicide effectiveness was investigated under low disease pressure. Mahoney et al. (2015) found a 4.1% yield response across all site-years from fungicide. Fungicide yield responses, though consistent across cultivars (Marburger et al, 2016) and frequently positive, have varied by site-year (Bradley and Sweets, 2008), location (Nelson et al., 2010), region (Orlowski et al., 2016), and fungicide timing (Henry et al., 2011). In another case, Swoboda and Pedersen (2009) didn't find a yield response, nor did they find any nonfungicidal physiological effects. Villamil et al. (2012), using data from the Illinois Soybean Association's

Yield Challenge program, reported that foliar applications of fungicide, fungicide and insecticide, or insecticide were commonly associated with increased soybean yields (219 kg ha<sup>-1</sup>). Many soybean studies found at least occasional yield increases with fungicide in low disease environments, but some researchers advised that applications were not economically justifiable (Orlowski et al., 2016; Mahoney et al., 2015; Bradley and Sweets, 2008).

Key insect pests for Illinois soybean include the bean leaf beetle, *Cerotoma trifurcate* (Forster); Japanese beetle, *Popillia japonica* Newman; soybean aphid, *Aphis glycines Matsumura*; and twospotted spider mite, *Tetranychus urticae Koch* (Steffey and Gray, 2009). Again, the IPM approach is recommended, with regular scouting to tract insect density for timely treatments when the economic thresholds are reached. Pest management thresholds are based on a combination of insect densities and soybean plant defoliation, which change as plants develop.

Bean leaf beetle can cause economic injury to soybean through the entire growing season resulting different economic thresholds based on beetle numbers, defoliation, and pod injury. Smelser and Pedigo (1992), for example, found that at R6 economic injury levels ranged from 14.9 to 42.5 beetles m<sup>-2</sup> depending on costs. Japanese beetle control follows general defoliation thresholds because correlation between Japanese beetle damage and yield is not clear (Steffey and Gray, 2009). While the economic injury level is much higher, Ragsdale (2007) found an economic threshold of 273±38 to make applications because soybean aphid densities can increase very rapidly. Twospotted spider mites are often only noticed in localized or droughty condition, though prompt treatment is warranted regardless of when infestation occurs. Rodriguez et al. (1983) found that the earlier the infestation occurs between V2 and R5, the larger the yield penalty.

In most cases economic thresholds for insects have been established, but prophylactic and preventative applications also been tested. Orlowski et al. (2016) evaluated insecticide use over many site-years in three regions. Insecticide significantly increased soybean yield with high break-even probabilities in the northern region (MI, MN, and WI). Within that region threshold levels of insects (aphid) only occurred at 5 of 18 site –years, so researchers cautioned against applications at sub-optimal thresholds to unnecessary resistance development. Johnson et al. (2008) was not able to increase yields by targeting specific generations of soybean aphid and bean leaf beetle, whether the pest density eventually reached the economic threshold or not. Examining a wide range of paired plots in the Illinois Soybean Association's Yield Challenge

program, Villamil et al. (2012) found that applications of insecticide were associated with yield increases. Johnson et al. (2009) found that both a prophylactic treatment of insecticide, fungicide, and herbicide tank-mixed as well as the IPM approach increased yield (and lowered aphid density), but concluded that the IPM approach still had the higher probability of success. Henry et al. (2011) found a 150 kg ha<sup>-1</sup> yield increase from a R4 timing of lamda-cyhalothrin when pressure was low, but concluded that insecticide use would not be economical even if the grower was risk-adverse.

#### Interactions

Evaluation of potential interactions between many factors has been limited. Several studies found at least an occasional yield increase from irrigation; however, irrigation did not impact the optimum plant density (Ball et al., 2000; Boquet, 1990; Doss and Thurlow, 1974).

The most common interaction investigated with the various in-season inputs has been with irrigation. Three studies (Purcell et al., 2004; Purcell and King, 1996; Al-Ithawi et al., 1980) found greater responses to N applications in the non-irrigated treatment compared to irrigated treatment in at least one year, likely because N<sub>2</sub> fixation is sensitive to moisture stress. The yield increase Purcell and King (1996) found was associated with greater seed number.

While evaluating fungicide use with furrow and sprinkler irrigation, Heatherly and Sciumbato (1986) indicated that foliar fungicide should only be used on soybean with adequate water through reproductive development. Nelson and Meinhardt (2011) found that fungicide increased soybean yields equally for subirrigation and non-irrigated treatments, possibly from above normal precipitation. Slater et al. (1991) found that fungicide consistently increased yield for one cultivar when irrigated at a high frequency, but found more variation with another cultivar, lower irrigation frequency, and below normal precipitation.

Klubertanz et al. (1990) examined plant water stress and the impact energy of rainfall on spider mite population dynamics. Rainfall did not change mite density, but mite intensity was greater in stressed plots due somewhat to lower leaf area. Simulating both insect and disease damage by defoliation, Caviness and Thomas (1980) found that on a percentage basis defoliation decreased yield similarly across irrigation treatments.

There has been limited research on relationships between applications of fungicide and insecticide or between applications of fertilizer with fungicide or insecticide. Henry et al. (2011) found that fungicide and insecticide both increased soybean yield under low pest pressure but did

not interact. Nelson et al. (2010) found no interaction between foliar fungicide and foliar K applications but noted a positive yield response to both individual products in one of two years. Yet, another study did find a significant interaction. Riedell et al. (2011) found that using high-N fertilizer under hot and dry conditions may increase bean leaf beetle damage. There seems to be little research testing potential interactions and even fewer examples of them.

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## CHAPTER 2: IRRIGATION AND OTHER INPUTS ON SOYBEAN 2.1 ABSTRACT

While soybean [Glycine max (L.) Merrill] grown in Illinois are not as widely irrigated as in states with soils more prone to drought, there is interest in finding out how much irrigation might further increase yields on highly productive soils. We conducted, on highly productive soils at Urbana, IL, a series of soybean studies to examine the effects of irrigation and several other management factors on soybean yield. Rainfall patterns and totals were inconsistent, with periods of droughty conditions in 2008, 2012, and 2013, but not in 2009, 2010, or 2014. Across all six years, irrigation increased soybean yields by 295 kg ha<sup>-1</sup> (6.2%), but the difference averaged 685 kg ha<sup>-1</sup> over the three dry years and -96 kg ha<sup>-1</sup> over the three wetter years. Increasing seeding rate in five of the study years (2009-2010 and 2012-2014) and use of seed treatment (in 2008) had no effect on soybean yields, with or without irrigation. Applying three applications (at R3, R5, and R6) of fungicide (2009-2010) and two applications (at R1 and R3) of fungicide plus insecticide (2012 to 2014) increased yield by 311 kg ha<sup>-1</sup> (6.2%) and 269 kg ha<sup>-1</sup>  $^{1}$  (5.5%) over the control, respectively. Over the same years, two applications of fertilizer N, with or without foliar nutrients, did not impact soybean yield. In 2009-2010 and 2012-2014, responses to in-season applications were not affected by irrigation. In 2008, both fungicide and N applications increased yield only under irrigation. Across years with significant yield increases from irrigation, 92% yield improvement was attributed to greater seed number, but seeds were also larger, especially when droughty conditions persisted to maturity. Yield components for other yields responses to other management factors were not quite as consistent. While irrigation increased soybean yield on productive Illinois soils in three years with significant precipitation deficits, it had no effect on yield in the three wetter years, indicating that use of irrigation for soybean is not likely to be profitable over years in soils with high water-holding capacity. The amount of water available from rainfall and irrigation to the crop at different stages did not consistently affect soybean response to other management factors, which indicates that minimizing water as a potential yield limitation did not move other factors into position of most limiting.

## **2.2 INTRODUCTION**

Irrigated soybean is not very common in Illinois; in 2012, only 45,000 ha, or 1.3 percent of the 3,620,000 ha of soybean were irrigated (USDA-NASS, 2018). Corn [*Zea mays* L.] was irrigated at a higher rate (2.8%) and on more than three times the area compared to soybean in 2012. Irrigation percentages are much higher for soybean in states like Nebraska (41.6% irrigated) and Arkansas (72.9%) where drought is more common and where supplies of water for irrigation are ample.

Research examining irrigation's impact on soybean when grown on productive Illinois soils with high water holding capacities is limited. The recent drought in 2012 brought more attention to this shortcoming, and with center pivot irrigated crop area increasing 29,000 ha (13%) from 2012 to 2014 (Bridges et al., 2015) soybean irrigation requires closer examination. Additionally, questions have also developed about irrigation's impact on management of other factors such as seeding rate and in-season applications of foliar fungicide and insecticide.

In regions with periods in which rainfall is often inadequate, irrigation has regularly increased soybean yield, when application is timed to individual growth stages (Kadhem et al., 1985a; Korte et al., 1983a), using multiple timings (Kadhem et al., 1985a, Korte et al., 1983a), and is based on soil water deficits (Ball et al., 2000; Heatherly and Pringle, 1991; Heatherly, 1988). Less water stress during flowering and pod formation generally resulted in more seeds (Kadhem et al., 1985b), but irrigation after pod formation increased seed mass (Kadhem et al., 1985b; Korte et al., 1983b). Season long irrigation frequently increased seed number (Heatherly and Pringle, 1991; Heatherly, 1988) but only occasionally increased seed mass (Heatherly and Pringle, 1991).

As producers consider more active management of soybean, seeding rate is regularly discussed and easily modified. Plant densities often have a wide range of optimum due to a soybean plants ability to compensate resulting in relatively flat responses. Research has shown that in certain years, seeding rates as low as 112,000 seeds ha<sup>-1</sup> can maximize yield (Elmore, 1998). De Bruin and Pedersen (2008) found maximum yield with a harvest density of 462,000 plants ha<sup>-1</sup>, with 259,000 plants ha<sup>-1</sup> sufficient to achieve more than 95% of maximum yield. As seeding rate increases yield, plants have increased both seed number (Egli, 1988) and seed size

(De Bruin and Pedersen, 2008). In the southern U.S., irrigation has not influenced optimum plant density (Ball et al., 2000)

Seed treatments protect soybean plants as they germinate and emerge by combating a broad spectrum of early and mid-season pathogens and insect species (Gaspar et al., 2015), especially when planting shifts in cooler and wetter soils (Esker and Conley, 2012). Seed treatments with a combination of fungicide and insecticide have been effective at improving plant stands and/or yields (Gaspar et al., 2015; Esker and Conley, 2012), though sometimes seed treatments are ineffective (Cox et al., 2008).

Current recommendations are described as an integrated pest management (IPM) approach in which regular monitoring is combined with multiple control methods and pesticides are only utilized when economically warranted. Though, as producers more actively manage soybean during the growing season, foliar fungicide and insecticide use has increased. In 2015, 7% of Illinois soybean producers used in-season insecticide and 12% used fungicide, whereas in 2004 fungicide use was not surveyed and insecticide use was at 1% (USDA-NASS, 2018). Fungicide effectiveness has been investigated under low disease pressure and yield responses, though consistent across cultivars (Marburger et al, 2016) and frequently positive (Mahoney et al., 2015) have varied by region (Orlowski et al., 2016) and timing (Henry et al., 2011). Additionally, researchers advised that applications were not economically justifiable (Orlowski et al., 2016; Mahoney et al., 2015). More frequent soybean yield responses to fungicide have also been observed when used in conjunction with irrigation (Heatherly and Sciumbato, 1986). In most cases economic thresholds for insects have been established, but prophylactic and preventative applications also been tested. With mostly below threshold insect densities (soybean aphid, Aphis glycines Matsumura), Orlowski et al. (2016) reported increased soybean yields with high break-even probabilities in only one of three regions. Others have reported that prophylactic applications have a lower break-even probability (Johnson et al., 2009) or would not be economical even if the grower were risk-adverse (Henry et al., 2011).

In-season applications have not been limited to pesticides; in 2015, 21% of farmers reported having applied N to soybean fields (USDA-NASS, 2018). Though some producers may have applied N with the intention of planting corn or as component of another fertilizer (such as ammoniated phosphates used as P sources), there is interest in in-season application of fertilizer. Soybean plants utilize large amounts of nitrogen (N), and grain contains about 55 g N per kg

(Gaspar et al., 2017). Typically, biological fixation of atmospheric N<sub>2</sub> through a symbiotic relationship with *Bradyrhizobium* accounts for 50-60% of the N used by the soybean plant (Salvagiotti et al., 2008) with the rest coming from the soil. Due to the plants supply of photosynthate to bacteria, there is concern about soybean plants' ability to simultaneously reach high yields and fix enough N<sub>2</sub> for those same yield levels. The ability of a soybean plant to use both soil N and fixed N is advantageous and well established; however, when soil levels of N are high, less N is fixed (Russelle, 2008). Inhospitable environmental conditions such as water stress can also limit fixation of N compared to those with adequate soil moisture (Purcell et al., 2004). Yield responses to fertilizer N on soybean have been inconsistent. N applied during reproductive growth (Salvagiotti et al., 2009; Wesley et al., 1998) have frequently increased yields. However, other have reported no yield increase with similar application timings during reproductive growth (Barker and Sawyer, 2005; Freeborn et al., 2001).

Use of foliar fertilization to supply the soybean crop nutrients is another area of interest, especially during reproductive growth. Garcia and Hanway (1976) found that yield and seed number showed the greatest increases when nutrients were applied multiple times in the ratio of 10:1:3:0.5 (N:P:K:S) during seed fill. Other studies with foliar applications of N, P, K, and S during seed fill had less success (Mallarino et al., 2001; Poole et al., 1983). Foliar micronutrient combinations of B, Cu, Mn, Mo, and Zn (Enderson et al. 2015) and B, Co, Cu, Fe, Mn, Mo, and Zn (Poole et al., 1983) have not increased soybean yields. With so few yield responses to foliar fertilization, chances that this input would offset product and application costs are very low.

As irrigation availability increases, evaluation of irrigation responses on productive Illinois soils become more important. This series of studies was undertaken (i) to evaluate irrigation's impact on soybean yield and plant characteristics; and (ii) to assess the effect of irrigation on the response of soybean to seeding rate and in-season applications of nutrients and fungicide/insecticide.

## 2.3 MATERIALS AND METHODS

#### **Environments and production practices**

Six field experiments were conducted over the period of 2008 through 2014 at the University of Illinois Crop Sciences Research and Education Center near Urbana (40.088407, -

88.228822), the first trial in 2008, a second in 2009 and 2010, and a third for three years (2012-2014). Soybean varieties, soil characteristics, latitude and longitude, and productivity index were compiled for all years (Table 2.1). Monthly growing-season precipitation was recorded, as well as the 30-year precipitation averages. The previous crop in all cases was corn [Zea mays L.]. Fall and spring tillage was performed on the corn residue. Soybean varieties were glyphosate-resistant, and locally adapted mid-maturity variety. In 2008 seed treatment as a factor within the study, whereas studies in 2009, 2010, 2012, 2013, and 2014 had seed treated with a fungicide and insecticide seed treatment. Trial maintenance included pre-emergence and post-emergence herbicides to keep plots weed free. Hand weeding was supplemented where needed. In early August of 2012, a rescue treatment of Cheminova Dimethoate 4E to control spider mites was also sprayed across the entire trial.

#### **Experimental design and data collection**

#### Experiment 1, 2008

In 2008, the trial was designed as a split-split plot with irrigation as the main plot in a randomized complete block design with four replications. The irrigation treatments were either rainfed or sprinkler irrigated. About 25 mm of water was applied weekly beginning at the start of the third week of July (stage R2) and continuing through the end of the first week in September (R6). Seed treatment was assigned to sub-plots, with or without seed treatment. Seed treatment included Cruiser Maxx (Syngenta Crop Protection, Greensboro, NC) fungicide-insecticide seed treatment and Optimize (EMD Crop BioScience, Milwaukee, WI) inoculant. Soybeans were seeded at 630,000 seeds ha<sup>-1</sup> in plots 7, 15-cm rows (2.7 m) wide by 12 m long. In-season applications of N fertilizer and foliar fungicide were compared to a control within the seed treatment sub-sub-plots. Nitrogen application consisted of 112 kg N ha<sup>-1</sup> as granular urea (46-0-0) broadcast at R2 and again at R5, for a total of 224 kg N ha<sup>-1</sup>. The fungicide application included 110 g ai ha<sup>-1</sup> of pyraclostrobin (Headline<sup>®</sup>, BASF Ag Products, Research Triangle Park, NC) applied twice, at R3 and R6.

Four of seven rows were harvested for yield with a plot combine. Yields were adjusted to 13% moisture and seed samples were collected to determine seed mass. Seed number per unit of area was calculated form harvest weight and seed mass.

#### Experiment 2, 2009-2010

In 2009 and 2010 at Urbana, the trial was designed as a split-split plot. Irrigation treatments, rainfed or sprinkler irrigation, were randomized in a complete block arrangement with six replications. On even days of the month, 0.64 cm of irrigation was applied over a one-hour period each day, beginning at stage R2, on 16 July in 2009 and 2 July in 2010, and ending after the midpoint of R6, on 12 September in 2009 and 2 September in 2010. Seeding rates of 309,000 and 618,000 seeds ha<sup>-1</sup> were assigned to the subplots. In-season applications of N fertilizer and fungicide were compared to a control in the sub-subplots. The N application included 112 kg N ha<sup>-1</sup> as granular urea (46-0-0) broadcast plus 18.3 kg N ha<sup>-1</sup> as a foliar N (diluted with water for a total volume of 140 L ha<sup>-1</sup>) application of polymethylene urea (25-0-0) (CoRoN<sup>®</sup>, Helena Chemical Company, Collierville, TN). Two N applications were made, one at R2 and one at R5, for a total of 260.6 kg N ha<sup>-1</sup>. The fungicide application included 110 g ai ha<sup>-1</sup> of a pyraclostrobin fungicide, Headline<sup>®</sup> (BASF Ag Products, Research Triangle Park, NC) applied at R3, R5, and R6. Plots were 7, 15-cm rows (2.7) wide by 12 m long.

At maturity, plant heights were measured and lodging notes were taken on a scale of 1 (no lodging) to 5 (lodged flat). Harvest densities were also counted in 1.31 and 2.62 m of row in 2009 and 2010, respectively. The center four rows of plots were harvested for yield using a plot combine and adjusted to 13% moisture. Seed samples were collected to determine seed mass and using harvest samples with seed mass, seed number per m<sup>2</sup> were also calculated. Protein and oil concentrations of the seed samples were also measured by a FOSS 1229 whole grain analyzer (FOSS North America, Eden Prairie, MN) using near-infrared (NIR) spectroscopy.

#### *Experiment 3, 2012-2014*

This trial was also designed as a split-split-plot, with four blocks. Irrigation treatments were assigned to main plots in a randomized complete block design. Irrigation treatments were rainfed (not irrigated) or irrigated as needed to prevent water stress. Soil moisture was monitored on a volumetric basis with probes provided by John Deere Water (formerly part of Deere and Co). Water was applied through drip tape spaced 76 cm apart (every two rows) on the soil surface, anytime volumetric soil moisture dropped below 28% in the top m of soil. Seeding rates of 173,000, 296,000, and 420,000 seeds ha<sup>-1</sup> were assigned to subplots within irrigation treatments. Due to a planting error with the lowest seeding rate in one year, data form only the two higher rates were analyzed.

Sub-subplots 7, 15-cm rows (2.7) wide by 8 m long consisted of in-season applications of factorial combinations with and without fertilizer and with or without pesticide. The fertilizer application included a combination soil applied N and foliar nutrients applied at two separate growth stages as described by Fehr and Caviness (1977). N fertilizer as urea (46-0-0) coated with Agrotain<sup>®</sup> Ultra (3.13 ml kg<sup>-1</sup>) (Koch Agronomic Services, LLC) was broadcast at a rate of 51.5 kg N ha<sup>-1</sup> twice, once each at V5 and R3. 4.7 L ha<sup>-1</sup> per application of additional foliar applied nutrients in the form of Task Force<sup>®</sup> 2 (Loveland Product, INC. <sup>®</sup> Greeley, CO) were applied at R1 and R3. Foliar rates at each timing were 625, 455, 284 g ha<sup>-1</sup> of total N, available phosphate, and soluble potassium as well as 5.68, 2.84, 2.84, 2.82, 1.14, 0.0284, and 0.0284 g ha<sup>-1</sup> of iron, manganese, zinc, copper, boron, molybdenum, and cobalt. The pesticide application included 110 g ai ha<sup>-1</sup> of a pyraclostrobin fungicide, Headline<sup>®</sup> (BASF Ag Products, Research Triangle Park, NC), and 28 g ai ha<sup>-1</sup> of a pyrethroid insecticide, Warrior II<sup>®</sup> (Syngenta Corporation, Wilmington, DE), applied both at R1 and R3.

Plant density was counted in 3 m of row at maturity. Five plants were also harvested individually at maturity to measure height and harvest index. The center four rows of plots were harvested for yield by a plot combine and adjusted to 13% moisture. Seed samples were collected to determine the mass of 300 seeds. Seed mass and harvest weight were used to calculate number of seeds per m<sup>2</sup>. From the seed samples protein and oil concentrations were measured using NIR spectroscopy with a FOSS 1229 whole grain analyzer (FOSS North America, Eden Prairie, MN).

#### **Statistical Analysis**

Data analysis was conducted with Statistical Analysis Software 9.4 (SAS) (SAS Institute Inc., 2013) using the MIXED procedure and the Type 3 method. Due to the nature of an irrigation study and varying precipitation patterns, year was analyzed as a fixed class variable in studies with multiple years, and treatment interactions with year were also tested. Blocks were considered random, and in studies with multiple years, block was nested in year. The independent factors of each study were class variables arranged in a split-split plot design. Mean separations (alpha = 0.05) for the class variables were conducted by comparing treatments through LSMEANS statements with the PDIFF option in PROC MIXED.

## **2.4 RESULTS**

## **Experiment 1**

Irrigation, in-season applications, and the interaction between irrigation and in-season applications affected yield (Table 2.3). Irrigation increased yield 589 kg ha<sup>-1</sup> (15.0%) across other treatments. Across irrigation, fungicide and N applications increased yields by 127 kg ha<sup>-1</sup> (3.0%) and 256 kg ha<sup>-1</sup> (6.2%), respectively. The interaction demonstrated that without irrigation, in-season applications of N and fungicide did not impact soybean yields (Table 2.3). Under irrigation, N and fungicide applications significantly increased soybean yields, by 410 kg ha<sup>-1</sup> (9.5%) and 178 kg ha<sup>-1</sup> (4.1%), respectively. Seed treatment did not affect soybean yield (Table 2.3), likely because late planting (30 May) resulted in good conditions for plant establishment.

Irrigated plots had 307 seeds m<sup>-2</sup> (14.7%), but similar seed mass to non-irrigated plots. Applications of fungicide increased seed mass 12 mg seed<sup>-1</sup> (6.6%) but resulted in 76 (3.4%) fewer seeds m<sup>-2</sup>. Applications of N increase seed mass 6 mg sd<sup>-1</sup> (3.2%). The interaction between irrigation and in-season applications for yield did not translate into an interaction for seed mass or seed number (Table 2.3).

## **Experiment 2**

Yields were greater in 2009 than in 2010, but irrigation did not increase soybean yield in either year, and the only treatment to affect yield across the two years was fungicide applications (Table 2.4). Three applications of fungicide (at R3, R5, and R6) increased soybean yield by 311 kg ha<sup>-1</sup> (6.2%) over the control. Across the two years, yields following N applications were not statistically different from either the control or the fungicide-treated plots, but yield with N was similar to that with fungicide in 2009, and similar to that of the control in 2010 (Table 2.4). Consistent rainfall (Table 2.2) for most of the growing season likely reduced the impact of irrigation on soybean yield. In 2009, the only period of below normal rainfall was at the very end of the growing season, and in 2010 rainfall was relatively consistent throughout the growing season. Regular precipitation may have led to excellent conditions for N mineralization and N fixation as well and potentially limited any benefits of N applications (Table 2.4). Excellent season-long rainfall also may have created a hospitable environment for pathogen development,

thereby contributing to the yield response to fungicide applications. Seeding rate did not affect yield, but there were large differences in stand establishment between the two years.

In 2009, high (618,000) and low (309,000 seeds ha<sup>-1</sup>) seeding rates resulted in stands at harvest of 190,000 and 126,000 plants ha<sup>-1</sup>, respectively. Stands were much higher in 2010, when the high and low seeding rate resulted in stands of 555,000 and 282,000 plants ha<sup>-1</sup>, respectively. The low stand at the lower seeding rate in 2009 apparently did not limit yields, however, under the cool, wet conditions of that growing season.

Soybean seed numbers were 648 m<sup>-2</sup> (23.4%) lower in 2009 than 2010, but yields were higher in 2009 due to the fact that seeds weighed 47 mg seed<sup>-1</sup> (32.2%) more (Table 2.4). Across the two years, the higher seeding rate decreased seed number by 212 seeds m<sup>-2</sup> (6.8%) and increased seed mass by 11 mg seed<sup>-1</sup> (6.7%); these two components exactly offset one another, leaving yields the same. The effects of irrigation and in-season applications on yield components did not consistently mirror yield responses, however. Of the in-season applications, only fungicide (three applications) increased yield. Seed size was 8 mg (4.9%) and 9 mg (5.5%) greater for nitrogen and fungicide applications compared to the control, respectively. Response of seed number to in-season applications varied by year. Nitrogen applications lowered seed number by 204 seed m<sup>-2</sup> (5.9%) compared to the control in 2010, but not in 2009.

Both protein and oil concentration showed a year by in-season application interaction, with no response in 2009, but lower protein and higher oil compared to the control following fungicide and nitrogen applications in 2010 (data not shown). Across years, the higher seeding rate also had higher protein. Oil concentration was lower with the higher seeding rate in 2009, but higher at the higher seeding rate in 2010.

Soybean plant height was 98 cm in 2009 and 124 cm in 2010. Plants at the higher seeding rate were 4 cm (4.0%) taller than at the lower seeding rate, with greater competition resulting in taller plants.

Lodging score significantly increased, from 2.1 at the lower seeding rate (309,000) to 2.9 (618,000 seeds ha<sup>-1</sup>) in 2010, but in 2009, seeding rate had no effect on lodging, with a score of 2.3 at both seeding rates (Table 2.4). Thus lodging was correlated to plant height and plant stand, but plants at the lower seeding rate in 2010 stood as well as the plants at the lower seeding rate in 2009, despite having more than twice the density. Lodging did not respond significantly

to irrigation within either year, but irrigated treatments in 2009 lodged less than irrigated treatments in 2010.

## **Experiment 3**

Soybean yields in this 3-year study responded positively to irrigation, though the response was not consistent over years (Table 2.5). Soybean yield also responded to in-season applications of fungicide/insecticide (Table 2.5). Averaged across irrigation, yields were significantly higher in 2012 and 2014 than 2013 (Table 2.5). Yield levels in 2013 were likely the lowest because of late planting (10 June) and precipitation deficits during seed fill period (Figure 2.2). With a little earlier planting (26 May) but with particularly well-distributed rainfall (Figure 2.3), yields were very high in 2014. There was significant moisture stress in the non-irrigated treatment in June and July of 2012 (Figure 2.1), but timely planting (10 May) and adequate precipitation during seed fill helped to maintain good yields even without irrigation.

Irrigation increased yield across the three years of this experiment by an average by 530 kg ha<sup>-1</sup> (11.2%), from 4,724 to 5,254 kg ha<sup>-1</sup>. Irrigation in both 2012 and 2013 increased yield substantially, by 842 kg ha<sup>-1</sup> (18%) in 2012 and by 624 kg ha<sup>-1</sup> (14.3%) in 2013. There were lengthy periods in both years during which rainfall was limited (Table 2.2). In 2012, a total of only 60 mm of rain (26% of normal) fell during June and July. In 2013, a total of only 25 mm of rain fell in August and September, compared to the normal amount of 180 mm. In 2014, rainfall was above normal in June and July, and, while less than normal (36 mm) in August, the crop was never under visible stress in 2014, and soybean yield did not respond to irrigation. Irrigation water amounts reflected the shortages of rainfall, and totaled 242, 176, and 48 mm in 2012, 2013, and 2014, respectively.

There was no yield response to seeding rate (Table 2.5), and none of the interactions with seeding rate were significant. Across other treatments, the final harvest densities for the two seeding rates of 296,000 and 420,000 seeds ha<sup>-1</sup> were 284,000 and 393,000 plants ha<sup>-1</sup>, respectively.

Soybean yield responded modestly to in-season applications, with responses relatively consistent across years. There were no yield differences between the control and the fertilizer (foliar plus dry) treatment, but the treatment combining fungicide and insecticide (applied twice) increased yield in both irrigated and non-irrigated treatments (Table 2.5). Compared to the control, the combination of fungicide and insecticide increased yield by 269 kg ha<sup>-1</sup> (5.5%).

Although irrigation responses differed by year, applying water on productive Illinois soil did not change the responsiveness of yield to seeding rates or application of in-season fungicide and insecticide or fertilization.

Irrigation interacted with year to affect both seed number and seed mass (Table 2.5). The early water deficits in 2012 (Figure 2.1) occurred during flowering, pod formation, and the beginning of seed development, and resulted in fewer seeds in the non-irrigated treatments, with large increases of 540 seeds m<sup>-2</sup> (20.9%) with irrigation compared to without irrigation. Late season rain replenished soil water, and resulted in seed mass being similar with and without irrigation. In 2013, soil water deficits (Figure 2.2) occurred later in the season, with irrigation applications distributed equally before and after R5. Irrigation applications during pod and seed development not only increased seed number, by 174 seeds m<sup>-2</sup> (8.1%), but also increased seed mass, by 12 mg seed<sup>-1</sup> (5.8%) compared to the non-irrigated plots. With rainfall relatively high and consistent throughout the 2014 season, little irrigation was applied (Figure 2.3), and yield, seed number and seed mass were unaffected by irrigation (Table 2.5).

There was no yield response to seeding rate, but seed mass was slightly (1.4%) higher at the higher seeding rate. Across years, in-season applications of fungicide and insecticide increased seed number by 85 seeds m<sup>-2</sup> (3.5%) over the control, and seed mass by 5 mg seed<sup>-1</sup> (2.5%). The increase in mass varied by year, however and was only significantly larger in 2013, at 10 mg seed<sup>-1</sup> (5.0%) (data not shown).

Patterns in yield components were most noticeable for the irrigation treatments because the yield changes were relatively large and differences in irrigation timing and quantity were distinct. This resulted in increases in seed number when large amounts of irrigation water were applied early (2012) and increases in seed mass when large amounts of irrigation were applied late in the growing season (2013). Of factors with more limited yield responses, yield components were less consistent. Regardless, soybean plants demonstrated their ability to adjust throughout development.

In 2012-2014, increasing seeding rate increased protein concentration, but did not affect oil concentrations (data not shown). Protein and oil concentrations both differed by year, irrigation treatment, and in-season application of (fertilizer). In 2012, the fertilizer treatment increased protein concentration without irrigation. In 2013, the fertilizer treatment lowered

protein and increased oil without irrigation. In 2014, fertilizer lowered protein regardless of irrigation treatment, and only increased oil with irrigation.

In-season applications of fertilizer by itself, or in combination with fungicide and insecticide, lowered the harvest index, by 0.2 (3.8%) compared to the control. It is likely that the fertilizer applications increased vegetative growth, while not impacting seed yield. Effects of irrigation on HI differed by year, from no effect in 2014 (when yield was unaffected by irrigation) to higher HI with irrigation in 2013 and lower HI with irrigation in 2012. Early water deficits and irrigation helped promote vegetative growth in 2012, whereas late deficits and irrigation in 2013 allowed soybean to better fill seeds after without increasing vegetative biomass (Table 2.5).

Both irrigation treatment and year, and their interaction affected plant height (Table 2.5) Irrigation increased soybean plant height by 44 cm (37%) in 2012 and by 6 cm (6%) in 2013, with these increases directly related to the amount of irrigation water (244 mm in 2012 and 177 mm in 2013) provided. The larger increase in 2012 could also be attributable to timing – 11 of the 13 irrigation events occurred prior to R5 that year. In 2013, with the dry weather coming later in the season, four of eight irrigation events were after R5; most of the water was applied as vegetative growth was slowing. In 2014, there were no differences between height, likely because irrigation was very limited (48 mm) and occurred late (after R5) when vegetative growth was complete.

Lodging increased under irrigation, but the amount of increase was not consistent over years (Table 2.5). In 2012, the soybean crop was planted early and experienced early drought stress requiring significant irrigation (242 mm) mostly before vegetative growth terminated. This irrigation pattern increased plant height and also helped increase lodging from 1.8 to 2.5 (on a scale from one to five). A considerable amount of water was also applied in 2013 (177 mm), but lodging (1.1) was consistent across the trial. This could be partially attributed to shorter heights from late planting and little height difference between irrigation treatments when half of the irrigation events were applied after vegetative growth. Soybean were planted timely in 2014 and received above normal precipitation through much of the vegetative growth. The little irrigation (48 mm) applied occurred late during reproductive growth (after R5). This resulted in some lodging (1.8), but no differences between irrigation treatments.
#### **2.5 DISCUSSION**

Irrigation significantly increased yields in three (2008, 2012, 2013) of six years with two different irrigation methods. This pattern demonstrated that on productive Illinois soils with large water holding capacities, yield responses are only possible with periods of substantial precipitation deficits. There was only 17 mm of precipitation over five weeks in 2008, and two consecutive months of limited rainfall in 2012 and 2013 (Table 2.2) led to the yield increases associated with irrigation. Like 2012 and 2013, literature has shown that soybean yields increase by timing water applications to soil water deficits (Ball et al., 2000; Heatherly and Pringle, 1991; Heatherly, 1988). As in 2008, regular timings for irrigation (initiated after R1) also benefited yield (Korte et al., 1983a). Yield increases from irrigation in the three years with significant responses averaged 685 kg ha<sup>-1</sup> (15.8%), but in the three years without a response to irrigation, the yield difference averaged -96 kg ha<sup>-1</sup>, and across all sites, the yield response was only 295 kg ha<sup>-1</sup> (6.2%).

Responses in yield components to irrigation treatments demonstrated a soybean plant's ability to adjust seed number and weight in response to conditions. Precipitation deficits occurred during June and July, August, and August and September for 2012, 2008, and 2013, respectively. Our results are in line with other reports that soybean plants produce more seeds per unit area when water stress is relieved early, during flowering and pod formation, and plants produce greater seed mass when water stress is relieved late, after pod formation (Kadhem et al., 1985b; Korte et al., 1983b). Several trials timing season-long irrigation to soil water deficits, initiated by flowering at the latest, also found that yield increases from irrigation were associated greater seed numbers (Heatherly and Pringle, 1991; Heatherly, 1988) and occasional increases in seed mass (Heatherly and Pringle, 1991).

Though not measured in this study, insect and disease pressure was not noticeable, but three applications of fungicide (experiment 2, 2009-2010) or two applications of insecticide and fungicide (experiment 3, 2012-2014) still increased yield. Villamil et al. (2012) reported a similar response to fungicide across a large number of farm sites in Illinois. The yield increases found in this study are similar to those from a prophylactic application of fungicide and insecticide between R1 and R2 (Johnson et al., 2009). Henry et al. (2011) also found yield increases, though smaller, from separate treatments of fungicide and insecticide applied at R4.

Fungicide applications alone have likewise increased yield (Mahoney et al., 2015), but there have also been differences by region (Orlowski et al., 2016) or timing (Henry et al, 2011).

In 2008, rainfall was low during August during most of the pod development. Irrigation may have raised canopy moisture and led to greater disease pressure, resulting yield responses to fungicide applications. In 2009 and 2010, the consistent yield response across both irrigation treatments was likely due to the fact there was consistent precipitation through most of the growing season in both years creating similar levels of pressure in each irrigation treatment. With irrigation provided by drip tape in 2012-14, difference in canopy wetness would have been small, and pathogen pressure unaffected by irrigation. Previous research observed that furrow and sprinkler (Heatherly and Sciumbato, 1986) irrigation increased the frequency at which soybean yield responded to fungicide.

Since no nutrient deficiencies were observed and the fields for these experiments did not have a history of nutrient or pH problems, finding that soybean yield did not respond to fertilizer applications alone in five of the six years was not surprising. Visible symptoms of N deficiency symptoms would be rare in such soils, since the supply of mineralized N is high, and soybean plants also nodulate and fix N readily. The lack of yield increases from N applications during reproductive growth was similar to results reported by Barker and Sawyer (2005) and Freeborn et al. (2001). In contrast, some researchers have reported higher soybean yield with N applications (Salvagiotti et al., 2009; Wesley et al., 1998). The lack of yield response to a combination of foliar nutrients and dry N in experiment 3 was similar to that reported following applications of N:P:K:S (Mallarino et al., 2001; Poole et al., 1983) or different combinations of micronutrients (Enderson et al. 2015; Poole et al., 1983).

In experiment 1, N increased yield only under irrigation. Others have reported that N fertilizer increased yield in non-irrigated plots (Purcell et al., 2004); this has been attributed to drought sensitivity of N fixation. As mentioned in the previous paragraph, literature has shown variability in yield response to N fertilizer and so the inconsistency in this research is not unexpected. The only yield increase from N occurred in 2008 with irrigation, which seems to indicate that N fertilizer had the potential to increase yields in 2008, but N fertilizer was only readily available to the plant with applications of sprinkler irrigations.

Yield components of in-season applications partially mirrored yield increases. Three applications of fungicide in experiment 2 increased yield and seed mass while maintaining

consistent seed number. This increase in seed mass with fungicide applications has also been reported by Mahoney et al. (2015) and Henry et al. (2011) and could be from a more effective or longer seed fill period. In experiment 3, the combination of fungicide and insecticide also increased seed mass in 2013; however, seed number increased across all years and could possibly be attributed in part to insecticide applications as Henry et al. (2011) found. In regions (northern) with significant yield response, Orlowski et al. (2016) found that fungicide increased seed mass and insecticide increased seed mass and seed number. Within experiment 1, the yield response to fungicide or N separately only increased yield when irrigated, but yield components were significant across irrigation treatments. Seed mass was associated with improved yield, similar to previously reports (Mahoney et al., 2015; Henry et al., 2011). Seed mass was also higher than the control for N applications, which was also reported by Salvagiotti et al. (2009), though not tested with irrigated plots unlike our results; contrasting our increase in seed mass, the yield improvement was due to a greater number of seeds likely due to earlier application of large amounts of N (V6+R2).

Optimal seeding rates have been reached with as few as 112,000 seeds ha<sup>-1</sup> in specific instances (Elmore, 1998), so discovering consistent yields with seeding rates at 296,000 or greater was not surprising. Seed treatment did not impact yield in experiment 1, a result similar to that reported by Cox et al. (2008), but unlike other reports of higher yields with seed treatments (Gaspar et al., 2015; Esker and Conely, 2012). The soybean were planted (30 May) late enough to experience favorable conditions for stand establishment, whereas seed treatment provides protection with early planting into cooler and wetter soils (Esker and Conley, 2012). Irrigation also did not impact the yield response to seeding rate (or seed treatment), which was consistent with previous irrigation research on seeding rates in the southern U.S. (Ball et al., 2000).

As higher seeding rates have improved soybean yield, plants have had greater seed number (Egli, 1988) and greater seed mass (De Bruin and Pedersen, 2008a; Egli, 1988). Without yield improvement in experiment 2, plants compensated with fewer but heavier seeds in the higher seeding rates. In experiment 3, the higher seeding rate increased seed mass slightly in experiment 3 as well but did not have a corresponding decrease in seed number as expected.

The most consistent yield increases came from irrigation and from in-season treatments that included foliar fungicide. The yield increase from irrigation across years was 295 kg ha<sup>-1</sup> (6.2%) and of the three years significantly improving soybean yield the greatest increase was 842 kg ha<sup>-1</sup> (17.9%) in 2012. Using a cash market price of \$.35 kg<sup>-1</sup>, gross revenue was \$103.17 ha<sup>-1</sup> greater with irrigation and ranged up to \$294.68 ha<sup>-1</sup> in the dry year of 2012. Improvement in gross revenue from three applications of fungicide (experiment 2) and two applications of fungicide and insecticide (experiment 3) were \$108.88 (311 kg ha<sup>-1</sup>) and 93.98 \$ ha<sup>-1</sup> (269 kg ha<sup>-1</sup>), respectively.

Costs of the inputs tested in these experiments are substantial. Scherer (2015) estimated the cost of running irrigation on 60.7 ha over 25 years to be \$336.1 ha<sup>-1</sup> yr<sup>-1</sup> including capital, annual ownership, and operating costs. University of Nebraska Extension also surveyed retailers for pesticide costs, the average prices for Headline was \$89.83 L<sup>-1</sup> (2016a) and for Warrior II was \$100.4 L<sup>-1</sup> (2016b). These prices result in cost of \$39.34 and \$14.05 per hectare per application, respectively. From an Iowa State Custom Rate Survey (Plastina, 2017), the median cost of application with a self-propelled sprayer was \$17.3 ha<sup>-1</sup>. Without any applications costs, three applications of fungicide and two applications of a combination of fungicide and insecticide would cost 118.02 and \$106.78 ha<sup>-1</sup>, respectively. The response in 2008 requiring both irrigation and in-season applications to increase yield, would require even more expense without an increase in net revenue.

On average, the costs of irrigation would substantially exceed the gross returns from irrigating soybean on productive soils. Even with the largest response we found, the net return to irrigation was negative. Returns on fungicide and insecticide applications are closer to breakeven, but even without applications costs they did not increase income either.

#### **2.6 CONCLUSIONS**

Across years, soybeans in the studies reported here experienced a range of soil moisture conditions, with significant precipitation deficits both early and late in the season, as well as growing seasons with excellent water availability throughout. Irrigation only increased yield when there were significant precipitation deficits and the use of fungicide or the combination of fungicide and insecticide also provided a modest increase in soybean yield throughout most of the trials. While there may be opportunities to increase yield, results did not demonstrate that

irrigation or repeated applications of fungicide with or without insecticide would be economical on productive Illinois soils, nor did it consistently increase yield response to other management factors.

# 2.7 TABLES

Year	Variety	Planting Date	Latitude, Longitude	Soil Series	Taxonomic classification	$\mathrm{PI}^\dagger$
2008	93M70	29-May	40.088407, -88.228822	Drummer sicl	Fine-silty, mixed, superactive, mesic Typic Endoaquolls	144
2009	93M70	12-May	40.085980, -88.228684	Drummer sicl	Fine-silty, mixed, superactive, mesic Typic Endoaquolls	144
2010	93M70	6-May	40.088407, -88.228822	Drummer sicl	Fine-silty, mixed, superactive, mesic Typic Endoaquolls	144
2012	P93Y70	10-May	40.089274, -88.228654	Flanagan sil	Fine, smectitic, mesic Aquic Argiudolls	144
2013	P93Y82	10-Jun	40.088649, -88.229419	Drummer sicl	Fine-silty, mixed, superactive, mesic Typic Endoaquolls	144
2014	P93Y82	26-May	40.087522, -88.230769	Drummer sicl	Fine-silty, mixed, superactive, mesic Typic Endoaquolls	144

Table 2.1. Variety, planting date, field location, soil description, and soil productivity index (PI) by year at Urbana, Illinois.

<sup>†</sup>Optimum Crop Productivity (Index) Ratings for Illinois (Olson, 2000).

			Pre	cipitatio	n				Irrigation amount					
Month	2008†	2009	2010	2012	2013	2014	Ave <sup>†</sup>	2008	2009	2010	2012	2013	2014	
							mm							
May	149	130	78	90	118	105	124							
June	133	108	199	46	135	229	110				31			
July	202	156	91	14	88	203	119	45	51	96	152	20		
August	17	137	40	142	12	36	100	96	96	96	59	85	31	
September	202	16	77	142	13	89	80	26	38	6		72	17	
Total	703	547	485	434	366	662	533	167	185	198	242	177	48	

Table 2.2. Monthly rainfall by year for Urbana, Illinois along with 30-year averages.

†Monthly data (Illinois State Water Survey, 2018)

1 30 Year Averages from 1981 to 2010 (Illinois State Water Survey, 2017)

		Yiel	d	Seed N	Jumber	Seed	Mass
		kg ha	a <sup>-1</sup>	seed	s m <sup>-2</sup>	mg s	eed <sup>-1</sup>
Irrigation							
Non-Irrigat	ted (NIRR)	3932	b	2081	b	189	NS
Irrigated (I	RR)	4521	а	2388	a	190	
Seed Treat	ment						
Untreated		4253	NS	2239	NS	191	NS
Treated		4200		2230		188	
In-season a	oplications						
Control (C)		4099	с	2238	a	183	с
Fungicide (	F)	4226	b	2162	b	196	а
Nitrogen (N	[)	4355	а	2303	a	189	b
<u>Year</u>	In-season applications						
NIRR	C	3873	с				
	F	3948	с				
	Ν	3975	с				
IRR	С	4325	с				
	F	4503	b				
	Ν	4735	а				

Table 2.3. Yield, seed number, and seed mass values by irrigation, seed treatment, in-season applications, and interaction between irrigation and in-season applications from experiment 1 (2008).

\* Different letters indicate significantly different values (alpha=0.05) within each factor

 $\dagger NS = not significant at alpha = 0.05$ 

		Yield		Seed Nu	mber	Seed N	lass	Height		Lodg	ging:
		ka k	na <sup>-1</sup>	seeds	m <sup>-2</sup>	ma se	ed <sup>-1</sup>	C	m		
Vear		Kg I	ia	secus	111	ing set	cu	C	111		
2009		5309	а	2764	h	192	я	98	h	23	NS
2002		4954	u h	3412	a	145	u h	124	a	2.5	110
2010		1951	U	5112	u	115	U	121	u	2.5	
Water	r Regiment										
Non-l	rrigated (NIRR)	5234	NS	3129	NS	171	NS	111	NS	2.4	NS
Irriga	ted (IRR)	5029		3046		167		111		2.4	
Seedi	ng Rate (seeds ha <sup>-1</sup> )										
309,00	00	5131	NS	3194	a	163	b	109	b	2.2	b
618,00	00	5132		2982	b	174	a	113	а	2.6	а
T	<b>1</b>										
In-sea	son applications	4000	1	2100	NG	1.62	1	110	NG	2.2	NG
Contro		4988	b	3109	IN2	163	D	110	N2	2.3	N2
Fungi	cide	5299	a	3140		172	a	112		2.5	
Nitrog	en	5108	ab	3014		1/1	а	111		2.4	
Year	Irrigation										
2009	NIRR			2730	с					2.4	ab
	IRR			2798	с					2.1	b
2010	NIRR			3529	а					2.3	ab
	IRR			3294	b					2.6	а
Year	In-season applications										
2009	Control			2766	c						
	Fungicide			2747	c						
	Nitrogen			2780	c						
2010	Control			3453	а						
	Fungicide			3533	a						
	Nitrogen			3249	b						
<b>X</b> 7											
Year	Seeding Rate (seeds ha <sup>-1</sup> )										
2009	309,000									2.3	b
0010	618,000									2.3	b
2010	309,000 618,000									2.1	b
	010,000									2.9	a

Table 2.4. Yield, seed number, seed mass, plant height, and lodging values by year, irrigation, seeding rate, in-season applications, and interactions between year and irrigation, year and in-season applications, and year and seeding rate from experiment 2 (2009-2010).

\* Different letters indicate significantly different values (alpha=0.05) within each factor

 $\dagger NS = not significant at alpha = 0.05$ 

\$Lodging score (1=upright, 5=down)

		Yie	ld	Seed Number		Seed N	Seed Mass		vest ex	Height		Lodging‡	
		kg h	a <sup>-1</sup>	seeds	m <sup>-2</sup>	mg see	ed <sup>-1</sup>			cr	n		
Year		0				8							
2012		5111	a*	2850	a	180	b	0.48	c	131	а	1.8	a
2013		4677	b	2216	c	211	a	0.55	a	101	c	1.1	b
2014		5179	а	2424	b	214	a	0.53	b	121	b	1.8	a
<u>Irrigation</u>													
Non-Irrig	gated (NIRR)	4724	b	2376	b	199	b	0.52	NS	109	b	1.3	b
Irrigated	(IRR)	5254	a	2617	а	204	a	0.52		126	а	1.8	a
Seeding	Rate (seeds ha <sup>-1</sup> )												
297,000		4997	NS	2518	NS	200	b	0.52	NS	117	NS	1.5	NS
420,000		4982		2475		203	a	0.52		119		1.6	
In-season	applications												
Control		4849	b	2448	c	199	b	0.53	а	117	NS	1.5	NS
Fertilizer	(Fert)	4937	b	2469	bc	202	ab	0.51	b	117		1.6	
Fungicid	e/Insecticide (FI)	5117	а	2533	ab	204	а	0.54	а	117		1.6	
Fert + FI		5054	а	2595	а	201	ab	0.51	b	120		1.6	
Year													
2012	NIRR	4690	cd	2814	b	182	c	0.49	c	109	c	1.0	c
	IRR	5532	a	3549	а	178	c	0.47	d	153	а	2.5	а
2013	NIRR	4365	d	2032	c	205	b	0.55	b	98	d	1.0	с
	IRR	4989	bc	2252	с	217	a	0.56	а	104	c	1.1	с
2014	NIRR	5118	b	2323	с	212	a	0.53	b	121	b	1.8	b
	IRR	5241	ab	2371	с	216	a	0.54	b	120	b	1.9	b

Table 2.5. Yield, seed number, seed mass, harvest index, height, and lodging values by year, irrigation, seeding rate, in-season applications, and the interactions between year and irrigation for experiment 3 (2012-2014).

\* Different letters indicate significantly different values (alpha=0.05) within each factor

 $\dagger NS = not significant at alpha = 0.05$ 

\$Lodging score (1=upright, 5=down)

## **2.8 FIGURES**



Figure 2.1. Volumetric soil moisture (%) throughout the soybean growing season in 2012 with irrigation events ( $\triangle$ ) and specific reproductive growth stages ( $\blacktriangle$ ) indicated.



Figure 2.2. Volumetric soil moisture (%) throughout the soybean growing season in 2013 with irrigation events ( $\triangle$ ) and specific reproductive growth stages ( $\blacktriangle$ ) indicated.



irrigation events ( $\triangle$ ) and specific reproductive growth stages ( $\blacktriangle$ ) indicated.

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# CHAPTER 3: NITROGEN FERTILIZER ON SOYBEAN 3.1 ABSTRACT

Soybean [*Glycine max* (L.) Merrill] plants utilize large amounts nitrogen (N) typically supplied by the soil and biological N<sub>2</sub> fixation. Though N fertilization of soybean has been extensively investigated, there is renewed interest as soybean yields increase, along with concerns about having adequate nutrients to support such yields. We conducted a series of experiments examining soybean yield response to N application timings (at planting, R1, R3, R5, and at all four stages) at nine site-years, covering a range of Illinois soil types, between 2014 and 2017. Yield responses to individual N applications were rare, but at one location with irrigated loam soil, N as urea at planting increased yields by 1,830 kg ha<sup>-1</sup> (16%) and 1,351 kg ha<sup>-1</sup> (26%) in 2015 and 2016, respectively, but not in 2017. Applying N fertilizer four times (planting+R1+R3+R5) also increased soybean yields at three sites with productive silt loam soils, by an average across sites of 337 kg ha<sup>-1</sup> (6%). Even though there were yield some yield increases, especially on lighter-textured soil, most responses were small, and insufficient to cover the cost of the application. Until we can better predict the soil and circumstances under which yield increases can be expected, the application of fertilizer N to soybean in productive regions of the Corn Belt is not a profitable practice.

#### **3.2 INTRODUCTION**

Soybean [*Glycine max* (L.) Merrill] is widely grown in both the US and Illinois. The Illinois soybean area has averaged about 4 million hectares over the past five years (USDA-NASS, 2018), exceeded only by corn [*Zea mays* L.], which has averaged 4.65 million hectares over the same time period. Soybean utilizes large amounts of N per unit of yield, with the grain alone containing 53-58 g N per kg (Gaspar et al., 2017; Bender et al., 2015; IPNI, 2018), and total plant uptake of about 63 g kg<sup>-1</sup> of grain yield in high-yielding soybeans (Gaspar et al., 2017).

Nitrogen supplied from soil organic matter via mineralization and by biological  $N_2$  fixation has generally been considered adequate to support high soybean yields. Soil N is supplied through microbe=mediated mineralization of soil organic N to an inorganic form

available for plant uptake. Biologically fixed N comes from the symbiotic relationship between the bacterium *Bradyrhizobium japonicum* and the soybean plant. *Bradyrhizobium* bacteria infect the root, and then develop inside nodules formed on soybean roots; they, in exchange for photosynthate, break the very strong triple covalent bond in N<sub>2</sub> and fix the N in organic compounds that circulate to provide N to the plant. This process is of great importance and is generally considered adequate to provide 50 to 60% of the soybean plant's N (Salvagiotti et al., 2008). The ability of a soybean plant to use both soil inorganic N and fixed N from bacteria has economic and environmental benefits, but bacteria fix less N when soil contains large amounts of inorganic N (Russelle, 2008). Environmental stresses such as drought stress can also limit fixation of N (Purcell et al., 2004; Purcell and King, 1996), and might also decrease soil mineralization and root access to soil N, thereby limiting N availability.

Illinois soybean yields have been high in recent years, averaging 3,572 kg ha<sup>-1</sup> over the past five years, and reaching 3,967 kg ha<sup>-1</sup> in 2016 (USDA-NASS, 2018). Individual yields in excess of 6,700 kg ha<sup>-1</sup> have been reported. As soybean yield increases, there has been renewed concern about the plants' ability to simultaneously produce high yields and support the high N fixation required for such yield. More frequent N fertilization of soybean demonstrates this concern. N fertilization of Illinois soybean hectares topped 20% for the first time in a 2015 survey with a relatively small rate of 26 kg N ha<sup>-1</sup> of fertilized hectares (USDA-NASS, 2018). Some of this is from applications of fertilizer materials such ammoniated phosphates primarily used to supply other nutrients, and some likely results from changing to soybean made after applying N to the intended crop. Wilson et al. (2013) found that genetic improvement measured by planting together maturity group (MG) III cultivars with year of release ranging from 1923 to 2008, was 20% (4.6 kg ha<sup>-1</sup> yr<sup>-1</sup>) higher with an unlimited supply of N (560 kg N ha<sup>-1</sup>) than without fertilizer N.

A large amount of research has been invested to examine fertilizer N applications on soybean. Frequently investigated are early season vegetative applications and applications during reproductive growth. There has been success improving soybean yield with both earlyseason (Salvagiotti et al., 2009; Osborne and Riedell, 2006; Taylor et al., 2005; Starling et al., 1998) and reproductive (Salvagiotti et al., 2009; Wesley et al., 1998; Wood et al., 1993) timings. Responses have also been flat with both early-season (Slater et al., 1991; Bharati et al., 1986) and reproductive (Barker and Sawyer, 2005; Freeborn et al., 2001) applications as well.

Research in Illinois specifically has been limited, but one paper (Welch et al., 1973) had very little success improving soybean yields in Illinois with N fertilization while working with topics including residual N, direct applications, and planting date and N rates. It is also important to note that even within studies there were cases of year to year variation and responses were sometimes shown as cases pooled across years or sites.

Looking at both yield studies and those investigating fixation's relationship with fertilizer, have helped develop insights into more effective N fertilization techniques. Starling et al. (1998) noticed that soybean responded to starter N fertilizer on soils low in N. Alilthawai et al. (1980) also found that N fertilization can more effectively increase soybean yields when residual N in the soil is not too high. Again, Wood et al. (1993) observed that yield responses to N fertilizer were more frequent in soils with lower soil nitrate-N at planting, even though applications were made later during reproductive growth.

One large N fertilization review (Salvagiotti et al., 2008) suggested that soybean yield would more likely increase from N by avoiding inhibition of nodule formation or fixation, especially in cases where yield was highest. Two noted methods included deep banding of slow release fertilizers early in the season and broadcasting N over the crop late during reproductive growth (seed fill) as nodulation activity decreased. This review was followed with testing. Salvagiotti et al. (2009) found deep banding to be effective and in addition to improving yield only decreased the supply of fixed N from 50 to 46% when compared to the control. Surface applied N at planting and V6 (Fehr and Caviness, 1977) decreased the amount of fixed N down to only 32%.

With relatively high crop prices prior to 2015 and potential yield improvement, interest in N fertilization of soybean remains relatively high. The purpose of this study was to evaluate the magnitude, frequency, and predictability of N responses over a range of Illinois soil environments.

#### **3.3 MATERIALS AND METHODS**

#### **Environments and production practices**

Field experiments were established between 2014 and 2017. Locations included the Brownstown Agronomy Research Center (2015), Northwestern Illinois Agricultural Research and Demonstration Center near Monmouth (2015), the University of Illinois Crop Sciences

Research and Education Center near Urbana (2014-17), and on a farmer's field near Chillicothe (2015-17). At Chillicothe the trial was grown on an irrigated loam (sandy loam in 2017) soil with about 2% organic matter (OM); at Brownstown the trial was on silt loam soil with less than 2% OM; and at Monmouth and Urbana, trials were on very productive silt loam soils with more than 3.5% OM. Field locations, soil descriptions, and soil productivity indexes (PI) were compiled for all site-years (Table 3.1). Management practices are listed in Table 3.2. Monthly precipitation for May through September, along with 30-year precipitation averages are in Table 3.3. The previous crop in all cases was corn [Zea mays L.], followed by fall and spring tillage, except at Chillicothe in 2015, where soil was not tilled before planting. Soybean cultivars were glyphosate-resistant, locally-adapted, mid-maturity cultivars, treated with seed treatment containing fungicide and insecticide. The seed was not inoculated with *Bradyrhizobium*. At Chillicothe in 2017, the seed was also treated with ILeVo<sup>®</sup> (fluopyram) (Bayer CropScience AG) for control of soybean sudden death syndrome (Fusarium virguliforme). Plots consisted of four rows spaced 76 cm apart, with length between 6.9 and 11.7 m. Trial maintenance included preemergence and post-emergence herbicides, supplemented with hand-weeding as needed, to keep plots weed free.

The center two rows of plots were harvested for yield using a plot combine, with yield adjusted to 13% moisture. Whole-plant samples, soil samples, and Normalized Difference Vegetation Index (NDVI) measurements were taken at R6, except at Urbana in 2014, when only soil samples and NDVI measurements were taken. At Chillicothe in 2016, Sudden Death Syndrome (SDS) caused by *Fusarium virguliforme* was severe, and consequently severity (1-9) and incident (percent) ratings were taken to determine a SDS index. The index was calculated as SDS Index = (rating, 1-9) x (incidence, 0-100)/9. Plant and soil samples were taken in two blocks only.

#### **Experimental design**

The trial was designed as a randomized complete block design (RCBD) with four blocks and six treatments. Supplemental N was applied in the form of urea fertilizer granules coated with Agrotain<sup>®</sup> Ultra (Koch Agronomic Services, LLC), a urease inhibitor. There were four application timings: at planting, R1, R3, and R5. Four treatments included a single application of N at each of these stages, and a fifth treatment included N applied at each of the four timings resulting in a 4X rate. There was also an untreated control. N rates per application were 112 kg

N ha<sup>-1</sup> in 2015 and 52 kg N ha<sup>-1</sup> in 2014, 2016, and 2017. After finding responses to applied N at Chillicothe in 2015, two additional N rates (26 and 103 kg N ha<sup>-1</sup>) were applied in 2016 and 2017, with an additional rate of 155 kg N ha<sup>-1</sup> at this site in 2017.

#### **Statistical Analysis**

Data analysis was conducted with Statistical Analysis Software 9.4 (SAS) (SAS Institute Inc., 2013) using the MIXED procedure and the Type 3 method. Due to the nature of differing soil types and different N rates by year, each site-year was analyzed separately. All of the blocks were considered random. Mean separations (alpha = 0.10) for the fixed variable (treatment) were conducted by comparing treatments through LSMEANS statements with the PDIFF option.

Soil N was analyzed by site-year, but to increase statistical power, soil N content was also considered by N rate across site-years and analyzed in PROC MIXED by treatment. The relationships with plant N and NDVI at R6 as well as others were further investigated utilizing PROC CORR and PROC REG in SAS to examine simple linear relationships.

### **3.4 RESULTS AND DISCUSSION**

#### Brownstown

Soybean yields ranged from 3,442 to 6,121 kg ha<sup>-1</sup> across the study sites. The trial at Brownstown was conducted only in 2015 and yields were relatively high for the soil at this site (Table 3.1), averaging 4,106 kg ha<sup>-1</sup>. Compared to the control, the only significant treatment effect at this site was a yield increase of 408 kg ha<sup>-1</sup> (10%) from the R3 N application. The R3 treatment may have coincided with a time of stress or the N from the R3 applications became available in a timely manner. Soil N values at R6 in the top 61 cm were lower than those check for the N applied at planting or at R1 at this site (Table 3.5). This supports the possibility that early (planting, R1) N applications restricted nodule development or fixation so that fixation capacity was reduced later in the growing season and plants scavenged more N from the soil. It is also possible that early N promoted excess vegetative growth resulting in a lower harvest index reducing yield. Like many N applications results are often mixed. There have been past results where R3 applications have both improved yield as was seen here (Wesley et al., 1998) and had little effect on yield (Barker and Sawyer, 2005; Freeborn et al, 2001). The R5 N application resulted in a yield level between those of the check and at the R3 timing, statistically equivalent to both and higher only than the yield following planting-time N or four applications of N. Relatively high yields from the R5 timing, second to only R3 timing, in conjunction with no yield increase and high soil N content (Table 3.5) suggest that R5 N may have been applied late enough to avoid hurting plant development (similar to R3), but too late for the crop to utilize efficiency.

Repeated applications of N resulting in a 4X rate of 448 kg N ha<sup>-1</sup>, did not increase soybean yields in 2015 at Brownstown (Table 3.4). Not only was the treatment statistically equal to the check, but it also was the lowest yielding treatment at this location. Yields may have also been inhibited by excess vegetative growth and lower harvest index similar to the early individual applications.

The soil at this site has a silt loam surface texture but a natural claypan at a depth of 20 to 30 cm that might influence N dynamics and the amount of N in the soil as nodules are forming. It is well-established that high nitrate-N in the soil reduces potential yield increases to fertilizer N (Al-Ithawi et al., 1980) and a detrimental effect on fixation. Though yield didn't necessarily mirror ours, Taylor et al. (2005) and Starling et al. (1998) have noted the decrease in nodulation associated with planting-time N applications. Additionally, Salvagiotti et al. (2009) found that two surface applications (at planting and at V6) reduced the proportion of fixed N used in the plant from 50 to 32% when compared to the check. So while most treatments did not increase yield at Brownstown, especially those applied early in the growing season, there is potential when timings coincide with mid to late reproductive growth to avoid inhibition of fixation or excess vegetative growth, but yet supply N with enough time for uptake and yield benefit.

#### Chillicothe

The Chillicothe site produced the largest yield increases from fertilizer N. N applied at planting time increased yields 1,506 kg ha<sup>-1</sup> (35%) in 2015 and of 1,324 kg ha<sup>-1</sup> (31%) in 2016. Repeating N applications four times also increased yield, by 1,830 and 1,351 kg ha<sup>-1</sup> in 2015 and 2016, respectively; repeated applications did not produce significantly greater yield increases than application at the planting timing alone. This indicates that it was the planting-time application that drove the yield increase, not the additional N applied plater in the 4x treatment. The yield increase from planting time N applications is surprising, given the expectation that surface-applied N at planting would decrease nodulation, N fixation, and probably yields due to lack of adequate N later in the season.

2015 and 2016 yield responses to individual applications other than at planting varied by year, but within year responses were similar within year (Table 3.4). In 2015, the yields from applications at R1, R3, and R5 ranged from 496 to 533 kg ha<sup>-1</sup> (12%) greater than the check, but were statistically equivalent to the check (Table 3.4) even when compared as a group (P = .1001). In 2016 all three timings significantly improved yield over the check and ranged from 607 to 822 kg ha<sup>-1</sup> (14 to 19%). There was more variation in 2015 with a standard error of 4.1 and CV of 10% than in 2016 with a standard error of 2.8 and CV of 7.1%, which may have made it more difficult to separate differences.

The 2016 soybean responded (P = <.0001) to planting-time N rates in a manner similar to that of a non-leguminous crop such as corn (Figure 3.1). With soybean and N prices of \$0.35 kg<sup>-1</sup> and \$1.10 (kg N)<sup>-1</sup>, respectively, the optimum N rate would have been about 98 kg N ha<sup>-1</sup>, and predicted yield at that rate 5,218 kg ha<sup>-1</sup>.

After the first two years of finding similar responses to applied N on the loam soil at Chillicothe, we developed the hypothesis that fertilizer N applied at planting was available to grow a larger, more productive plant, but did not raise soil nitrate level enough to inhibit N nodule development and subsequent N fixation. Low soil nitrate levels in the nodulation zone may also come from movement of fertilizer N to below this zone. With lower organic matter and soil conditions often dry or cool near the surface, these soils have lower N mineralization capacities. The results in this study were similar to other reported responses to N fertilizer oncoarse textured soils (Starling et al., 1998; Wesley et al., 1998; Wood et al., 1993), which in many cases have low soil N. Salvagiotti et al. (2008) suggested that deep banding N fertilizer would move N below the nodulation zone, and found in a follow-up study (Salvagiotti et al., 2009) that the decrease in fixation from deep banding N was much less than that from surfaceapplied N, though both treatments improved soybean yield.

The Chillicothe trial location in 2017 was about 4.3 km from the previous two years and on a sandy loam soil (Table 3.1) instead of loam soil. Yields were excellent in 2017, with the check averaging 5,288 kg ha<sup>-1</sup>, about 990 and 1,846 kg ha<sup>-1</sup> higher than 2015 and 2016, respectively. The yield response to N applications, however, was not nearly as large as in previous years. The only treatment to improve soybean yields in 2017 was the four-time application of N (Table 3.4). Though yield increase of 580 kg ha<sup>-1</sup> (11%) from this treatment was larger than at most other sites, improvement was more modest than in previous years at

Chillicothe. The response to N rates applied at planting in 2017 was linear (P = .0158) and very flat (Figure 3.1). Using the prices mentioned above, N application would have had to increase yield by 3.1 kg per kg of N; the response was only 2.7 kg of yield for each kg of N applied, so the addition of N did not pay for itself with added yield. After two years of large yield responses to applied N, the lack of yield response to N applications in 2017, especially coming as it did at very high yield levels, raises questions regarding how much consistency we can expect from N applications on lighter-textured soils.

The most noticeable difference between soybean trials at Chillicothe was the pattern and severity of SDS. There was no notable SDS pressure in 2017. In 2015 SDS appeared late in the growing season and did not appear severe enough to warrant ratings, though the cooperator had another cultivar in the same field with more severe symptoms. In 2016, SDS was severe throughout the trial with visible differences among treatments enough to justify taking SDS ratings. Yields were positively correlated to NDVI measured at R6 for all three years, and were negatively correlated to SDS rating in 2016 (Figure 3.2). The relative values of the coefficient of determination (R<sup>2</sup>) showed that years with more severe SDS symptoms had stronger correlations between NDVI and yield (Figure 3.2). In 2016, this translated into significantly lower SDS ratings and higher yields for plots with N applications. The SDS index of the check in 2016 was 56, while the planting time N and repeated applications of N were significantly lower, with ratings of 31 and 22, respectively (data not shown).

In 2016, the cooperator at Chillicothe also had an ILeVo<sup>®</sup> seed treatment by seeding rate experiment in the same field. The trial had a different cultivar than our trial with less resistance to SDS, and four seeding rates, but as part of larger study, it was not replicated in this field. Though there was SDS throughout the field hurting yield, visual strips across the field clearly showed greater symptoms without ILeVo<sup>®</sup>. Across seeding rates ranging from 198,000 to 371,000 seeds ha<sup>-1</sup>, yield was 1,033 kg ha<sup>-1</sup> (40%) higher with the ILeVo seed treatment.

After large yield increases from N fertilizer at Chillicothe in the first two years, limit responses in 2017 complicated the results. Soil type, variety, seed treatment, and apparent SDS pressure changed by year and may have been responsible for varying responses. There was true no comparison for these factors, but observations in 2016 and 2017 demonstrate a need for further investigation. The inverse relationship between SDS rating and N treatment (and yield) in 2016 and the use of ILeVo throughout the trial in 2017 with subsequent little SDS evidence

and limited yield response to N empirically suggests that both N applications and the ILeVo seed treatment could help promote a larger healthier plant to fight SDS and improve yield. There has not been any known research investigating this potential relationship. Further consideration of this relationship would require a factorial of treatments with and without N applications, ILeVo seed treatment, and possibly varieties, but the number of N timings could be reduced to ease logistics to continue this research.

#### **Monmouth and Urbana**

Yield responses at the two highly productive sites of Monmouth and Urbana were relatively consistent. Check yields ranged from 4,688 to 6,121 kg ha<sup>-1</sup> (Table 3.4) over five site-years, with the highest yield level at Urbana in 2015. Soybean grown on these productive soils responded significantly only once to treatments involving a single N application, and this was a decrease in yield of 351 kg ha<sup>-1</sup> (6%) from application at planting. This decrease in yield coincided with the very high check yield, and occurred in the year when a higher N rate (112 kg N ha<sup>-1</sup>) was used.

The review by Salvagiotti et al. (2008) did not list any papers reporting significant yield declines regardless of N timing. Several experiments (Osborne et al., 2006; Taylor et al., 2005; Starling et al., 1998) specifically tested planting-time N applications, and found that they frequently increased yield, though these studies were located outside Corn Belt. Applications were typically made as starter or broadcast over the crop at planting, and even when N increased yield, it also caused fewer nodules to form (Taylor et al., 2005; Starling et al., 1998) and less N fixation. It is possible that large amounts of N applied at planting in our study decreased soybean nodulation and fixation penalizing yield or excess N applied at planting may have increased biomass early in the season reducing N availability prematurely. With check yields at such a high level, the plant may have also been more sensitive to any changes in availability or periods with low N.

There were no other responses to individual applications of N at Monmouth and Urbana, but the treatment with repeated N applications significantly increased yield in three of these five site-years (Table 3.4). Yield was 436 kg ha<sup>-1</sup> (9%) and 338 kg ha<sup>-1</sup> (6%) higher than the check with four N applications at Urbana in 2014 and 2015, respectively. At Monmouth in 2015, yield from this treatment was 237 kg ha<sup>-1</sup> (4%) higher than in the check. Yield improvement from repeated applications may have been facilitated by lowering the demand of photosynthate for N

fixation and thus increasing the amount available to fill seeds. The yield improvement was relatively small compared to the quantity of N applied through four applications, though, so while these responses are interesting, the treatment would have lowered net returns at every site. The greatest yield improvement on these highly productive soils was 436 kg ha<sup>-1</sup> at Urbana in 2014, which at a soybean price of \$.35 kg<sup>-1</sup> would have increased revenue by \$153 ha<sup>-1</sup>. The total amount of N applied in this case was 208 kg ha<sup>-1</sup> and with a cost of \$1.10 kg N<sup>-1</sup>, the total cost would have been \$229 ha<sup>-1</sup>. Even using the greatest yield improvement without application costs, the net return was not positive.

Repeated applications of N have not been the most commonly tested application strategy, thought it has sometimes been included in designs intending to supply a non-limiting N source as part of a larger experiment. Reports from yield contest-winning fields suggest that repeated applications of N are a very common strategy to force yields higher, even though most such exercises provide no way to assess the response to any individual treatment. There have been reports in the literature of yield responses to repeated applications of N similar to our results, but results still are still mixed overall. Beard and Hoover (1971) tested N rates with split applications between pre-plant and flowering and found no yield response. The most recent research has shown several instances of yield improvement from repeated applications of N studies (La Menza et al., 2017; Wilson et al., 2013; Salvagiotti et al., 2009). The studies include examples of using five application timings to mirror plant N demand (La Menza et al., 2017) and two applications to supply a non-limiting source of N while investigating N requirement changes for a historical range of varieties (Wilson et al., 2013). These results demonstrated that soybean yield can be limited by N supply, whether or not yield responses are economical.

#### **Supplemental Data**

The R6 plant N and NDVI measurements have frequently shown positive, but often weak, correlation with yield. Of individual associations with yield, the strongest link occurred with NDVI at Chillicothe (Figure 3.2). This was likely due to SDS development and its negative impact on the canopy quality. The data shows that years with more severe SDS (2016>2015>2017) had stronger correlations between NDVI and yield. The treatments in 2016 with no N had the most severe SDS symptoms followed by later season N applications, with more minor symptoms in those receiving N at planting.

Through linear regression, the positive relationship between yield and plant N (Figure 3.3a) and the negative relationship between yield response to N and check yields (Figure 3.3b) were quantified. The regression line between yield and plant N had a coefficient of determination (R<sup>2</sup>) of .78. The majority of the variation was attributed to differences between sites and the linear coefficient describing the relationships between yield and plant N only had a partial-R<sup>2</sup> of .05. Though the positive relationship between plant N and yield was weak, it was also not surprising. Gaspar et al. (2017) found that for each kg increase in yield, total N uptake increased 1.45 kg and higher yielding plants with more N delayed remobilization and took up a greater proportion of N after R5.5. So while applying N fertilizer may not be an effective way to supply soybean N, Gaspar emphasized using production practices supporting greatest N<sub>2</sub> fixation and N mineralization through the growing season.

Across sites larger yield responses in our study were weakly ( $R^2$ =.25) associated lower yields (Figure 3.3b). As mentioned earlier there is concern about a plant's ability to supply N at higher yields, but at the same time past research has shown that N can help lower yielding soybean overcome stresses such as drought (Purcell et al., 2004; Purcell and King, 1996) or low soil N (Starling et al., 1998; Wood et al., 1993; Alilthawai et al., 1980). The negative relationship between yield improvement and yield level was weak, but demonstrated that within our study the lower yielding sites had the greatest N limitations and with certain treatments stress could be reduced.

Soil sampling also offered additional insight into the data. Data was grouped across sites and separated into 112 kg N ha<sup>-1</sup> (2015) and 52 kg N ha<sup>-1</sup> (2014, 2016, and 2017) N rates, but similar trends were seen (Figure 3.4). Soil N content was similar for plants receiving no N or N before R5. Soil N was significantly higher when applications were made at R5 and then again even higher for the treatment with repeated applications of N. Plants seem to use N applied before R5 completely with or without yield improvement and the treatments with repeated applications or an individual application at R5 don't necessarily use all of the fertilizer N. This pattern in soil N often results in little benefit for soybean yield and simply adds another opportunity to potentially lose N to the environment.

#### **3.5 CONCLUSIONS**

With a few notable exceptions, we found little increase in soybean yield from individual N applications made at different times. Repeated applications increased yield more often, but the increases were not adequate to cover the cost of the fertilizer. The biggest exception was found on irrigated loam soil at Chillicothe, where soybean yields showed large responses to planting-time applications of urea in two out of three years. On a lighter-textured soil in 2017, with even higher yields, planting-time N produced no yield increase. So while there may be opportunities to improve yield with N fertilizer, the unpredictability of the response indicates that this strategy is ineffective as a way to increase net returns to inputs for Illinois producers.

# **3.6 TABLES**

Table 3.1. Field location, soil description, and soil productivity index (PI) by year and location in Illinois.

Year	Location	Latitude, Longitude	Soil Series	Taxonomic classification	PI†
2014	Urbana	40.089282, -88.228671	Flanagan sil	Fine, smectitic, mesic Aquic Argiudolls	144
2015	Brownstown	38.953296, -88.960411	Cisne sil	Fine, smectitic, mesic Mollic Albaqualfs	109
2015	Chillicothe	40.915561, -89.513812	Warsaw 1	Fine-loamy over sandy or sandy-skeletal, mixed,	
				superactive, mesic Typic Argiudolls	119
2015	Monmouth	40.932318, -90.722304	Muscatune sil	Fine-silty, mixed, superactive, mesic Aquic Hapludolls	147
2015	Urbana	40.047765, -88.230357	Flanagan sil	Fine, smectitic, mesic Aquic Argiudolls	144
2016	Chillicothe	40.915367, -89.515574	Cresent 1	Fine-loamy, mixed, superactive, mesic Typic Argiudolls	103
2016	Urbana	40.046583, -88.230843	Flanagan sil	Fine, smectitic, mesic Aquic Argiudolls	144
2017	Chillicothe	40.894703, -89.557978	Dickinson sl	Coarse-loamy, mixed, superactive, mesic Typic Hapludolls	103
2017	Urbana	40.047730, -88.232491	Flanagan sil	Fine, smectitic, mesic Aquic Argiudolls	144

†Optimum Crop Productivity (Index) Ratings for Illinois (Olson, 2000).

Year	Location	Planting Date	Harvest Date	Variety	Seeding Rate		Application Date		
						Planting	R1	R3	R5
					seeds ha-1				
2014	Urbana	27-May	22-Oct	Asgrow 3634	359,000	27-May	10-Jul	28-Jul	13-Aug
2015	Brownstown	22-May	25-Sep	Pioneer 93Y92	346,000	22-May	12-Jul	28-Jul	17-Aug
2015	Chillicothe	7-May	28-Sep	Hi Soy 33A32	371,000	7-May	24-Jun	13-Jul	10-Aug
2015	Monmouth	12-May	5-Oct	Asgrow 3832	359,000	13-May	30-Jun	13-Jul	10-Aug
2015	Urbana	6-May	1-Oct	Asgrow 3832	371,000	11-May	22-Jun	6-Jul	23-Jul
2016	Chillicothe	6-May	19-Sep	HiSoy 28A42	346,000	6-May	25-Jun	12-Jul	2-Aug
2016	Urbana	6-May	7-Oct	Asgrow 4135	356,000	9-May	28-Jun	15-Jul	10-Aug
2017	Chillicothe	15-May	9-Oct	Asgrow 36X6 <sup>+</sup>	339,000	15-May	7-Jul	13-Jul	10-Aug
2017	Urbana	16-May	28-Sep	Asgrow 38X6	384,000	17-May	6-Jul	17-Jul	11-Aug

Table 3.2. Planting date, harvest date, soybean variety, seeding rate, and application dates by year and location in Illinois.

†ILeVo® (Bayer CropScience AG) was used in addition to fungicide and insecticide seed treatment

	Brown	stown		Chillicothe*				<u>Monmouth</u>			<u>Urbana</u>			
Month	2015†	Ave‡	2015	2016	2017	Ave	2015	Ave	2014	2015	2016	2017	Ave	
							-mm						-	
May	150	138	106	80	80	104	120	121	172	154	119	143	124	
June	273	105	295	94	70	125	207	114	229	229	145	65	110	
July	89	101	176	165	86	99	230	104	203	106	112	57	119	
August	89	76	47	232	53	91	88	120	36	80	105	56	100	
September	167	81	73	136	15	83	71	95	89	164	140	21	80	
Total	769	502	697	708	304	502	716	555	662	733	620	342	533	

Table 3.3. Monthly rainfall by year and location in Illinois along with 30-year averages.

†Monthly data (Illinois State Water Survey, 2018)

\$30-year average (1981-2010) (Illinois State Water Survey, 2017)

\*Supplemental irrigation at Chillicothe in 2015 (15 mm), 2016 (53 mm), and 2017 (122 mm).

	Brownstown	Chillicothe			Monmouth	Urbana			
Application Timing	2015*‡	2015	2016	2017	2015	2014	2015	2016	2017
					kg ha <sup>-1</sup>				-
Check	4106 bc	4298 b	3442 c	5288 b	5348 b	4976 bc	6121 b	5683 NS†	4688 NS
Planting (PLT)	3805 c	5804 a	4766 a	5318 b	5372 b	5024 bc	5770 c	5718	4902
R1	3923 bc	4830 b	4048 b	5343 b	5320 b	4917 c	6189 ab	5713	4704
R3	4514 a	4815 b	4123 b	5323 b	5315 b	5156 bc	6215 ab	5785	4856
R5	4187 ab	4793 b	4264 b	5192 b	5230 b	5014 bc	6150 ab	5844	4760
PLT+R1+R3+R5	3777 с	6128 a	4792 a	5867 a	5585 a	5412 a	6459 a	5484	5067

Table 3.4. Yield response of soybean to nitrogen application timings at nine site-years in Illinois.

\* Different letters indicate significantly different values (alpha=0.10) within location and year

 $^{+}NS = not significant at alpha=0.10$  $^{+}Nitrogen rate in 2015 was 112 kg N ha<sup>-1</sup> and in 2014, 2016, and 2017 was 52 kg N ha<sup>-1</sup>$ 

Brownstown					Chillicothe	<u>)</u>	<u>Monmouth</u>			<u>Urbana</u>		
Application Timing	2015‡		20	015	2016	2017	2015	20	15	2016	20	17
							kg N ha-1					
Check	51	cd*	37	c	46 NS	42 NS	34 b	c 78	NS 3	5 b	48	c
Planting (PLT)	34	d	35	с	45	45	27 c	197	30	5 b	50	с
R1	44	cd	40	с	56	48	45 b	145	38	8 b	46	с
R3	83	с	35	с	64	41	42 b	89	40	) b	83	bc
R5	201	В	88	b	54	47	213 a	115	5	7 a	134	b
PLT+R1+R3+R5	335	a	143	a	85	49	213 a	248	62	2 a	234	a

Table 3.5. Nitrogen in the top 61cm of the soil profile at R6 by application timing in eight environments.

\* Different letters indicate significantly different values (alpha=0.10) within location and year

†NS = not significant at alpha=0.10

<sup>±</sup>Nitrogen rate in 2015 was 112 kg N ha<sup>-1</sup> and in 2014, 2016, and 2017 was 52 kg N ha<sup>-1</sup>

## **3.7 FIGURES**



Figure 3.1. Soybean yield response to nitrogen rates applied at planting in the form of urea treated with Agrotain® Ultra (Koch Agronomic Services, LLC) at Chillicothe on loam (2016) and sandy loam soils (2017).


Figure 3.2. Normalized Difference Vegetation Index (NDVI) measurements and Sudden Death Syndrome (SDS) index ratings vs. soybean yield (kg ha<sup>-1</sup>) at Chillicothe on loam (2015, 2016) and sandy loam (2017) soils. SDS Index ratings were equal to (rating, 1-9) x (percent incidence)/9. The coefficients of determination (R<sup>2</sup>) for NDVI in 2015, 2016, and 2017 were .56 (<.0001), .71 (<.0001), and .20 (.0265), respectively. R<sup>2</sup> for SDS index in 2016 was .68 (<.0001).</li>



Figure 3.3. Linear regression between yield and plant N at R6 accounting for environmental differences (A) and yield response to N for each plot and corresponding check yield not accounting for environment (B). Environments included 2014 Urbana (–), 2015
Brownstown (◊), 2015 Chillicothe (□), 2015 Monmouth (≭), 2015 Urbana (+), 2016
Chillicothe (△), 2016 Urbana (-), 2017 Chillicothe (X), and 2017 Urbana (○).



Figure 3.4. Total soil nitrogen by timing in the top 61 cm of soil when sampled at R6 averaged across sites corresponding to lower (52) or higher (112 kg N ha<sup>-1</sup>) rates. Also included were the control and treatment with "Repeated" (Planting+R1+R3+R5) applications. Significant differences (P=.10) within each rate were indicated by differences in letters.

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