Nitrogen use in cereal crops with Pivot Bio Proven$^{TM}$ inoculant

by

William G. Davis

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Approved by:

Major Professor
Charles W. Rice

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# Abstract

Nitrogen (N) fertilizer is a significant input of many cropping systems. Additionally, N losses pose environmental concerns and represent a loss of resources for the farmer. Proven™, an N-fixing bacterial inoculant for cereal crops is expected to fix ~20-33 kg N ha$^{-1}$ over a growing season. Bacterial inoculants that fix N reduce leaching, denitrification, and volatilization losses compared to fertilizer N, resulting in increased N use efficiency. Biological N fixation in cereal crops is novel and has the potential to increase N efficiency and decrease N loss. The objective of this study was to determine the N benefit provided by Proven™ and determine the effect of Proven™ on NUE in cereal crops. Field trials with corn for two years and sorghum for one year evaluated the efficacy of Proven™. The corn experiment was a split-plot design with four replications in 2019 and six replications in 2020. The main treatment was N fertilizer applied as urea at planting at 0, 56, 112, and 168 kg N ha$^{-1}$ in 2019, with an additional 140 and 154 kg N ha$^{-1}$ in 2020. The sub plot was the presence or absence of Proven™. Preplant and post harvest soil sampling to 90 cm depth quantified soil inorganic N (IN). In addition, the soil was sampled during selected growth stages to 30 cm for inorganic N. In-season plant measurements included NDVI, SPAD, and green leaf count.  Plant N uptake was determined at R6. At harvest, grain moisture, test weight, and yield were measured. Nitrogen use efficiency was calculated with and without Proven™ to determine the N benefit from Proven™ at the different N rates. The sorghum experiment was a split plot design with six replications planted in 2020. The main treatment was N fertilizer applied as urea ammonium nitrate at planting at 0, 34, 67, 101, and 135 kg N ha$^{-1}$, with and without Proven™ as the sub plot factor. Grain yield, moisture, test weight, and grain N were collected from the sorghum experiment at harvest. In 2019 there was a trend for greater average corn N uptake with Proven™ than without at all N rates, with a 10.9 kg

N $ha^{-1}$ benefit of Proven$^{TM}$ at 0 kg N $ha^{-1}$, although this difference was not statistically significant. Corn N uptake and yield were significantly affected by the N rate. No significant effects of Proven$^{TM}$ or an N rate and Proven$^{TM}$ interaction were found for yield or total plant N for corn in either year ($\alpha$= 0.05). Sorghum grain N and yield were significantly affected by N rate but not by Proven$^{TM}$ or a Proven$^{TM}$ by N rate interaction. Proven$^{TM}$ appeared to be a source of 18 kg N $ha^{-1}$ in 2019. Nitrogen Use Efficiency (NUE) calculations generally followed the expected N rate response. NUE calculations were not significantly affected by Proven$^{TM}$ in 2019. In 2020, N apparent recovery efficiency (NARE), total N balance index (TNBI), and N recovery efficiency (NRE) were significantly affected by a Proven$^{TM}$ by N rate interaction. There was little evidence that Proven$^{TM}$ provided a significant amount of N or increased NUE in corn or sorghum.

# Table of Contents

# List of Figures

# List of Tables

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# Dedication

This thesis is dedicated to Catherine Davis.

# Chapter 1 - Proven™ as a source of nitrogen in corn

## Introduction

### General Agronomic N description

Nitrogen (N) is an essential nutrient required for plant growth (Robertson & Groffman, 2015). Nitrogen limits plant productivity in most ecosystems (Robertson & Groffman, 2015), requiring supplemental N to optimize crop productivity (Rice et al., 1995). Biologically fixed N from the atmosphere is the primary natural source of biological N (Robertson & Groffman, 2015). Synthetic fertilizer N is also produced by atmospheric N fixation through the Haber-Bosch process, in which atmospheric N and hydrogen react under high pressure and temperature in the presence of an iron catalyst to produce ammonia (Erisman et al., 2008). The Haber-Bosch process is responsible for increasing global crop productivity and food security (Erisman et al., 2008). Synthetically produced N has several drawbacks. Synthetic N is energetically costly to produce. Secondly, more than half of the synthetic N fertilizer applied is lost to the atmosphere or water resources, thus contributing to water pollution, greenhouse gas emission, and loss of biodiversity in ecosystems (Bloch et al., 2020b; Ribaudo et al., 2011). Fertilizer N also represents a high annual cost for crop production (Mus et al., 2016).

### Mineralization and the N cycle/plant N availability

Nitrogen occurs in various forms and pools on earth (Robertson & Groffman, 2015; Bottomley and Myrold, 2015). Nitrogen is subject to several transformations between forms and pools, referred to as the N cycle (Robertson & Groffman, 2015). N movement to and from various pools is mainly controlled by microbes (Ward, 2012; Robertson & Groffman, 2015). These transformations regulate plant N availability (Rice et al. 1995). The major N transformations in the soil are N immobilization, N mineralization, nitrification, and denitrification (Robertson & Groffman, 2015). Nitrogen immobilization is the consumption of inorganic N by microbes which convert it to organic N. Nitrogen mineralization is the conversion of organic N to inorganic N by microbes. Nitrification is the conversion of ammonium to nitrate. Denitrification is the conversion of nitrate to nitrous oxide and diatomic N (Robertson & Groffman, 2015). Nitrogen mineralization is particularly important in crop production because it represents a source of N, which is sometimes overlooked and needs to be factored into efficient N recommendations (Rice et al., 1995).

The largest N pool in the biosphere is the atmosphere, which contains $3.9 \times 10^{21}$ g of the $4 \times 10^{21}$ g of biosphere N (Bottomley & Myrold, 2015). More than 99% of biosphere N is $N_2$ (Bottomley & Myrold, 2015). Most organisms, however, are unable to utilize $N_2$ (Bottomley & Myrold, 2015; Robertson & Groffman, 2015). The natural processes by which atmospheric $N_2$ is converted to soil N are by lighting strikes (5 Tg N $yr^{-1}$) and biological N fixation (100-140 Tg N $yr^{-1}$) (Bottomley & Myrold, 2015). Anthropomorphic N production via the Haber-Bosch process also represents a significant source of atmospheric N deposition, estimated to be ~110 Tg N $yr^{-1}$ ( Bottomley & Myrold, 2015). Anthropomorphic N has been recently estimated to constitute more than half of all atmospheric N deposition (Morris, 2018).

Nitrogen exists in many forms in soil. In agronomic situations, the primary form of N deposition is N fertilizer, of which there are also multiple forms. The application of urea results in the hydrolysis of urea to ammonia. This reaction also produces protons, which react with the ammonia to form ammonium.

The two primary forms of plant-available N are $NO_3^-$ and $NH_4^+$. Nitrate is mobile in most soils (Robertson & Groffman, 2015). Due to its mobile nature, $NO_3^-$ can be easily lost via leaching when precipitation is greater than evapotranspiration, resulting in $NO_3^-$ deposition in surface and groundwaters (Robertson & Groffman, 2015; Ribaudo et al., 2011). Nitrate accumulation in water bodies poses health and environmental concerns (Davidson et al., 2015; Ribaudo et al., 2011). More than 1.5 million Americans rely on ground water with concentrations of $NO_3^-$ near or above the EPA-regulated maximums (Davidson et al., 2015). High N concentrations in the ocean can also cause eutrophication in aquatic ecosystems (Ribaudo et al., 2011). Two-thirds of coastal systems in the United States are moderately or severely impaired by high N concentrations (Davidson et al., 2012). Ammonium is cationic, meaning it is less mobile than nitrate in the soil and less subject to leaching losses (Robertson & Groffman, 2015).

Another important aspect of the N cycle that impacts climate change is the production of nitrous oxide. Nitrous oxide is a gas present in the atmosphere at much lower concentrations than carbon dioxide but represents an important greenhouse gas with 296x the warming potential of carbon dioxide (Ehhalt et al., 2001). Approximately 73% of $N_2O$ emissions in the U.S. in 2009 were from agricultural sources, and emissions from N fertilization of agricultural soils made up more than 85% of U.S. $N_2O$ emissions from agriculture ("Emissions of Greenhouse Gasses" 2011). Improved N fertilization and water management practices have been identified to mitigate $N_2O$ emissions and promote sustainable agriculture (Bloch et al., 2020b; Roy, Finck, Blair, & Tandon, 2006).

## Biological N Fixation

Biologically fixed N represents a source of N that is less costly and less subject to loss, and therefore more efficient than fertilizer N (Bloch et al., 2020b). Biological N fixation is the conversion of atmospheric $N_2$ to $NH_3$, which is quickly converted to $NH_4^+$ in the soil and available for plant uptake (Mus et al., 2016). Biological N fixation can only be performed by diazotrophs, a group of prokaryotes and archaea (Mus et al., 2016; Bloch et al., 2020b). The enzyme responsible for biological N fixation is nitrogenase (Bloch et al., 2020b; Bottomley & Myrold, 2015). The Nif genes are responsible for nitrogenase production and the N fixation process. Biological N fixation is an anaerobic process (Bloch et al., 2020b), and the nif gene proteins are denatured by oxygen (Bottomley & Myrold, 2015).

Symbiotic biological N fixation in crop production is primarily limited to legumes (Mus et al., 2016). Rhizobia bacteria have a symbiotic relationship with legumes in which the rhizobia infects the legume roots, which produce nodules that house the rhizobia, exclude oxygen, and supply carbohydrates to the bacteria (Provorov, 1997). Cereal crops have signaling pathways similar to those responsible for nodulation in legumes. However, nodule-like symbioses of cereal crops and rhizobia have yet to be produced (Bloch et al., 2020b).

There are many diazotrophs that associate with plant roots besides rhizobia, including *Azospirillium, Gluconacetobacter, Herbaspirillium, and Burkholderia* (Bottomley & Myrold, 2015). These diazotrophs colonize the rhizosphere and intercellular spaces of roots (Bottomley & Myrold, 2015). Root-associated diazotrophs are especially prevalent in sugarcane, which excretes root mucilage with high carbohydrate concentrations that act as a food source for soil microorganisms (Bottomley & Myrold, 2015). Estimates of N supplied by root-associated diazotrophs in sugarcane range from 13 to >60% of total plant N (Bottomley & Myrold, 2015).

Biological N fixation is a very energetically intensive process, requiring 16 ATP to fix one $N_2$ molecule into two ammonia molecules (Bottomley & Myrold, 2015; Bloch et al., 2020b). Because this process is so energetically costly, most biological N fixers stop fixing atmospheric N in the presence of high inorganic N (IN) in the soil and assimilate the available N (Bloch et al., 2020a). The Nif gene expression typically decreases in the presence of high reactive N (Bottomley & Myrold, 2015).

## History of Biological N Fixation in Agronomy:

Biologically fixed N has been the primary form of plant available N in agricultural systems for most of history (Goyal et al., 2021). Since the advent of the Haber-Bosch process, however, anthropomorphically fixed N has increased to the point where BNF is often overlooked in many cropping systems.

### N fixation in legumes:

Biological N fixation is most established in legume crop production (de Bruijn, 2015). Legumes have a symbiotic relationship with rhizobia bacteria, which colonize Legume roots, consume plant carbohydrates, and fix N, which is then assimilated by the plant. Legume-rhizobia symbiosis is believed to fix approximately $2.00 \times 10^{8}$ tons N $yr^{-1}$ (de Bruijn, 2015) cited from (Ferguson et al., 2010). Biological N fixation outside of legume production is limited in agricultural systems (Mus et al., 2016). de Bruijn (2015) identified the development of BNF in cereal crops as the “holy grail” of nitrogen fixation research.

### N fixation in Cereal Crops:

Although BNF is well established in legume production, BNF in cereal crops is less robust (Soumare et al., 2020). Only a few soil bacteria had been identified as fixing N by 1960 (de Bruijn, 2015). Inoculants intended to fix N in cereal crops were already being marketed at this point, however, particularly *Azotobacter* spp., which were most available in Russia, and were also were researched in Brazil, but had little commercial success (Baldani & Baldani, 2005; Olivares et al., 2013). The effectiveness of biologically fixed N from *Azotobacter* was disputed in the late 60s and 70s, with some attributing benefits in plant growth associated with *Azotobacter* to plant hormone production rather than N fixation (Olivares et al., 2013).

Significant research into BNF by diazotrophs in cereal crops has been conducted in Brazil (de Bruijn, 2015), particularly by Johanna Dobereiner (Baldani & Baldani, 2005). In the 1970s and 80s, BNF research focused primarily on *Azospirillium*, a bacteria demonstrated to significantly reduce crop N fertilizer needs (Baldani & Baldani, 2005; Olivares et al., 2013). Estimates of N fixation by *Azospirrilium* vary widely, however, with some characterizing the contribution of N as minimal and others finding significant N contributions (Olivares et al., 2013). Isolates of nitrogen-fixing bacteria in the 1980s also led to the discovery of new N-fixing bacteria in sugarcane (Baldani & Baldani, 2005). Long-term cultivation of sugarcane with little or no N fertilization in Brazil has been theorized to have led to plant-microbe interactions that fix plant-available N, which led to the development of inoculants intended to fix N in sugarcane (Olivares et al., 2013). Sugarcane is advantageous for N fixing plant-microbe associations. N fixation by microbes is limited in many systems by shortages of carbohydrates in the soil that limit microbe activity, particularly because N fixation is an energetically costly process (Olivares et al., 2013). Sugarcane produces high-carbohydrate exudates, which provide energy to rhizosphere microbes and allows for greater N fixation (Olivares et al., 2013).

Today, due to better genomic technology, we can better identify microbes with the potential to fix N and are aware of more bacteria capable of BNF (de Bruijn, 2015). Genomic technology may also be critical in producing genetically edited plants and microbes to fix greater amounts of N (Bloch et al. 2020b).

## Proven$^{TM}$

Proven$^{TM}$ is a gene-edited bacterial inoculant produced by Pivot Bio derived from *Klebsiella variicola*, intended to fix N in cereal crops. *Klebsiella variicola* naturally fixes N on sugarcane roots (Lin et al., 2015).

Bloch et al. (2020b) identify two main pathways to achieve biological N fixation in cereal crops; through plant engineering or the use of bacterial inoculants. Within the plant engineering option, there are two main options, expression of nitrogenase in plant tissues and engineering cereal crops to exhibit nodule-like symbiosis with rhizobia (Bloch et al., 2020b). Producing nitrogenase in plant tissues poses issues because nitrogenase is denatured by oxygen, which is produced by plant cells (Bloch et al., 2020b). The option of producing nodulation in cereal crops has been demonstrated to be a promising method of achieving biological N fixation in cereal crops (Bloch et al., 2020b). The signaling mechanism responsible for nodulation is conducted by plant hormones ubiquitous in plants, meaning that the production of nodules in cereal crops may be possible (Bloch et al., 2020b). Methods to achieve nodulation for symbiotic N fixation are still not understood, however (Bloch et al., 2020b).

The second pathway identified by Bloch et al. (2020) to achieve biological N fixation is through bacterial inoculants. Many bacteria have the ability to biologically fix N, and many of these bacteria also have been observed to have associative or endophytic relationships with plants (Bottomley and Myrold, 2015; Bloch et al., 2020b). Thus, there is potential for a non-rhizobia diazotroph to associatively or endophytically fix N. Bloch et al. (2020b) divided bacterial inoculants into three subgroups; natural, transgenic, and gene-edited. Natural bacterial inoculants are native bacteria used as an inoculant intended to fix N in cereal crops. Natural bacterial inoculants can be problematic due to their inconsistent performance and lack of ability to increase yields in high N environments (Bottomley & Myrold, 2015; Bloch et al. 2020b). Transgenic bacterial inoculants have shown potential as biological N fixers in cereal crops, but Bloch et al. (2020b) claim that the transgenic microbe may face strict regulatory hurdles because transgenic organisms or genetically modified organisms (GMOs) are subject to stricter regulation under United States law than natural or gene-edited organisms. The efficacy of transgenic bacterial inoculants on large-scale applications has also not been tested. Proven™ has been gene-edited to continue to fix N in the presence of high soil N. Proven™ has specifically been gene-edited to manipulate negative and positive nif regulators to increase nif expression (Bloch et al., 2020b). Increased nif expression increases a diazotrophs ability to fix N. Proven™ has also been gene-edited to decrease the production and activity of glutamine synthetase. Glutamine synthetase is an enzyme that is essential for ammonium assimilation in diazotrophs, so limiting its activity allows diazotophs to fix N in the presence of high soil inorganic N, a situation where native diazotrophs would decrease N fixation in favor of assimilating available N (Bloch et al., 2020b).

## Nitrogen Use Efficiency

Nitrogen use efficiency (NUE) is a method of measuring crop productivity per unit of N fertilizer. Nitrogen efficient systems provide adequate plant N with minimal N loss (Robertson, 1997). NUE is affected by several factors, including climate, soil characteristics, crop and soil management, N application timing, form of N applied, and placement of N (Rice, Havlin, & Schepers, 1995). Crop and soil management, N application timing, form of N applied, and placement are producer-controlled factors that determine the proclivity for N loss from a system.

**NUE Estimates:**

Rice et al. (1995) reported NUEs from 12-74% for arable crops in the tropics. Nitrogen use efficiency is negatively impacted in high precipitation regions resulting in greater N loss in these systems (Rice et al., 1995). Sharma & Bali (2017) cited Raun & Johnson 1999 reporting estimates of NUE for global cereal production at 33%.

**N Management and NUE:**

Although many of the factors affecting NUE are outside of human control, such as climate and soil properties, some management decisions can be made to alter NUE (Rice et al., 1995). Nitrogen rate is the primary factor affecting N use efficiency (Meisinger et al., 2008). As the amount of fertilizer N applied increases, the NUE generally decreases, following the law of diminishing returns, which states that each unit of input added yields a diminishingly greater output (Roy et al., 2006). In other words, The greatest N efficiency is achieved at lower N rates, and as N rate increases, NUE decreases (Rice et al., 1995). N rates greater than the capacity of a crop to assimilate N result in greater rates of N loss and decreases in NUE (Meisinger et al., 2008; Rice et al., 1995). Determining an appropriate N rate for a crop is essential to maximizing NUE and requires knowledge about N inputs, pools, the cycles between these pools, and expected N removal rates of a crop (Meisinger et al. 2008; Rice et al., 1995). Some potential N sources are difficult to predict when planning N fertilizer rates, such as the amount of mineralized N, which is influenced by several confounding factors (Rice et al., 1995). Source, timing, and placement are also important factors affecting NUE (Meisinger et al., 2008).

The N fertilizer source selected impacts environmental interactions with applied N that affect plant availability and loss, and therefore NUE (Sharma & Bali, 2017; Abbasi et al., 2013).

Plant uptake of $NO_3^-$ requires 20 ATP $mol^{-1}$ $NO_3^-$, whereas the uptake of $NH_4^+$ requires 5 ATP $mol^{-1}$ $NH_4^+$, meaning that plants supplied with more $NH_4^+$ than $NO_3^-$ expend less energy taking up equivalent amounts of N (Raun & Schepers, 2008). $NH_4^+$ is also less subject to leaching and denitrification in most soils than $NO_3^-$, resulting in less loss and greater late-season uptake (Raun & Johnson, 1999; Raun & Schepers, 2008). For both of these reasons, sources of N with greater $NH_4^+$ content will positively affect NUE compared to those with greater $NO_3^-$ content (Raun & Schepers, 2008). This highlights the importance of limiting nitrification of applied fertilizers. Coating of fertilizers can slow the release of N or inhibit nitrification (the conversion of $NH_4^+$ to $NO_3^-$) (Randall et al., 2008; Shoji et al., 2001). Nitrification inhibitors can decrease $NO_3^-$ leaching, denitrification, and $N_2O$ emission, and increase plant N uptake (Randall et al., 2008), although their effect on NUE are controversial (Alonso-Ayuso et al., 2016). Slow-release N fertilizers have been demonstrated to increase NUE in corn (*Zea mays*) (Shoji et al., 2001). One reason why slow-release fertilizers improve NUE is the delayed N release improves the synchrony of available N with crop growth (Randall et al., 2008)

Timing of N applications should be such that available N coincides with crop N demand to maximize NUE (Rice et al., 1995). Nitrogen fertilizer is frequently applied once a growing season at high rates intended to meet crop demand for the entire growing season (Raun & Schepers, 2008). Split-applications of N can improve NUE but have not been widely adopted (Raun & Schepers, 2008). This is primarily due to the difficulties posed by applying N fertilizer after full crop canopy is established, except via fertigation (Raun & Schepers, 2008; Rice et al., 1995). In most cases, synchronization of available N and crop N demand is difficult (Rice et al., 1995).

Placement of N fertilizer can also affect N loss and NUE (Rice et al., 1995). Placement of fertilizers should be such that they maximize availability to the crop and minimize potential losses to maximize NUE (Sharma & Bali, 2017). Banded applications of fertilizer can increase N recovery (Rice et al., 1995). Subsurface N applications also increase N recovery and NUE (Rice et al., 1995; Sharma & Bali, 2017). Biologically fixed N from plant-associated diazotrophs, such as Proven™, is more efficient than fertilizer N in part because it is generated in a plant-available form in the rhizosphere, where it is easily assimilated by the plant (Bloch et al., 2020a).

**Methods of Calculating NUE and N balance:**

There are many methods for evaluating NUE, each having distinct advantages and disadvantages (Congreves et al., 2021). The methods of calculating NUE discussed here include N apparent recovery efficiency (NARE), N recovery efficiency (NRE), N agronomic efficiency (NAE), Partial factor productivity (PFP), N real use efficiency (NRUE), N crop use efficiency (NUEcrop). Total N balance index (TNBI) is also discussed here as a model method of calculating N balance.

Nitrogen apparent recovery efficiency (NARE) is plant N with N application minus plant N without N application divided by N rate (Chen et al., 2016). This is also sometimes called Apparent crop recovery efficiency of applied N (Halvorson & Bartolo, 2014) or fertilizer-N recovery efficiency (REfertN) (Congreves et al., 2021). However, for this paper, it will be referred to as NARE. The advantage of this method of determining NUE is that it considers soil N uptake and is valuable for determining crop response to fertilizer N (Congreves et al., 2021). The disadvantages of NARE are that it requires the maintenance of unfertilized plots, which producers may avoid in favor of greater yield. It may not be suitable for long-term experiments where soil N in the unfertilized plots may become depleted (Congreves et al., 2021). Cassman et al. (2002) reported an average NARE of 0.37 for maize in the United States. Roberts et al. (2010) reported NAREs ranging from 0.14 to 0.66 for corn in Mississippi.

Nitrogen recovery efficiency (NRE) is plant N divided by N rate (Chen et al., 2016). (Also called Partial N Balance (PNB) (Congreves et al., 2021). The advantage of NRE is that the values determine if soil N is being removed (NRE >1) or if excess fertilizer N is being applied (NRE <1) (Congreves et al., 2021). The main disadvantage of this method of calculating NUE is that it does not differentiate between fertilizer N and soil N contributions to plant N (Congreves et al., 2021). Roberts et al. (2016) reported NREs ranging from 0.61 to 0.91 for corn in Arkansas.

Nitrogen agronomic efficiency (NAE) is yield with N application minus yield without N application, all divided by N rate (Chen et al., 2016). NAE is useful because it focuses on the economic advantage of N fertilizer use and considers soil N contributions (Congreves et al., 2021). The disadvantages of NAE are that it requires unfertilized plots to be maintained and that some trials could underestimate residual fertilizer N in the soil, affecting results (Congreves et al., 2021). Thompson et al. (2015) reported NAEs ranging from 1.15 to 77.7 kg grain N kg $N^{-1}$ for corn in the midwestern United States. Roberts et al. (2010) reported NAE ranging from 20 to 50 kg grain N kg $N^{-1}$ at optimal fertilizer N rates for corn in Mississippi.

Partial factor productivity (PFP) is yield divided by N rate (Chen et al., 2016). The advantage of PFP as a measure of NUE is that it is simple to calculate and requires little information (Congreves et al., 2021). The disadvantages of PFP are that it does not distinguish between soil N and fertilizer N so comparisons between sites are limited, and variability in soil N could skew results (Congreves et al., 2021). Thompson et al. (2015) reported PFPs ranging from 20.1 to 187.4 kg grain kg $N^{-1}$ for corn in the midwestern United States. Cassman et al. (2002)

reported that the average PFP for corn in the United States increased from 42 kg grain kg $N^{-1}$ in 1980 to 57 kg grain kg $N^{-1}$ in 2000. Halvorson & Bartolo (2014) identify 40-80 as a typical range for PFP for corn in the United States.

Nitrogen real use efficiency (NRUE) is plant N of the fertilized treatments divided by N rate minus residual soil N (Chen et al., 2016). The advantage of NRUE is that it considers N loss, making it suitable for monitoring the impacts of N management on the environment (Chen et al., 2016). The main disadvantage of NRUE is that it does not consider soil N before fertilization (Congreves et al., 2021).

NUEcrop: Yield N/Fert N (Congreves et al., 2021). The advantage of NUEcrop is that it determines the removal or surplus of N in a system (Congreves et al., 2021). A NUEcrop of >1 indicates soil N consumption, a NUEcrop of <1 indicates a surplus of fertilizer N (Congreves et al., 2021). The disadvantage of NUEcrop is that it does not differentiate between soil N and fertilizer N (Congreves et al., 2021). Halvorson & Bartolo (2014) reported NUEcrop values ranging from 1.21 to 1.99 with 0 kg N $ha^{-1}$, 0.51 to 0.66 with 56 kg N $ha^{-1}$, 0.44 to 0.54 with 112 kg N $ha^{-1}$, and 0.38 to 0.45 with 168 kg N $ha^{-1}$ applied for corn in the Arkansas River Valley.

Total N balance Index (TNBI) is a method of calculating N balance which was developed for this study and is a modified system N balance index from Congreves et al. (2021). The advantage of TNBI is it considers preplant soil N, plant N uptake at 0 N $ha^{-1}$, and residual soil N to better account for N sources and sinks. The greatest disadvantage of TNBI is that there are currently no estimates or averages for TNBI to compare the results of this study to those in the literature.

Because of the significant advantages of achieving biological N fixation in cereal crops and the novelty of Proven™ as an early gene-edited diazotrophic inoculant, it is important to understand the efficacy of Proven$^{TM}$ as a N source. The objectives of this study were to (i) investigate the N benefit derived from Proven$^{TM}$ for corn, and (ii) investigate how the presence of Proven$^{TM}$ affects NUE for corn. It is expected that Proven$^{TM}$ would contribute ~20-33 kg N $ha^{-1}$ $yr^{-1}$ (as reported by Pivot Bio) and that the presence of Proven$^{TM}$ will increase NUE.

# Materials and Methods

## Site Description and Experimental Design

This experiment took place over two years at the Kansas State University Agronomy North Farm (39°12'19.1"N, 96°35'43.9"W). The experimental design in both years was a split-plot randomized complete block design with four replications.

### 2019:

In 2019 the dominant soil series were Ivan and Kennebec silt-loams (both fine-silty, mixed, superactive, mesic Cumulic Hapludolls). The previous crop was no-till continuous corn for no less than 5 years before establishing this experiment. The average pH of the field was 6.65. Pre-plant P, K, and pH data are available in Table A.1. in the appendix. The experiment had four N rates (0, 56, 112, and 168 kg N $ha^{-1}$) applied as urea. Plots were 3 m by 14 m. The corn variety, Dekalb DKC64-35RIB (Bayer CropScience, St. Louis, MO), was planted at 74,131 seeds $ha^{-1}$ using a John Deere 1705 integral planter (Deere & Company, Moline, IL).

Urea was surface broadcast by hand on the day of planting. Proven$^{TM}$ was applied in-furrow at planting at 4.942 L $ha^{-1}$ (as directed by Pivot Bio). In 2019 the formulation of Proven$^{TM}$ provided needed to be activated one week to a month before application, which was done on 2 May 2019. Planting occurred on 17 May 2019. In 2019, 33 kg $ha^{-1}$ of $P_2O_5$ as Triple Super Phosphate (TSP) was applied on 4 June 2019. Weeds were controlled with an application of Atrazine 4L (2,338 mL $ha^{-1}$), Base Camp LV6 (2,4-D) (877 mL $ha^{-1}$), Buccaneer Plus (glyphosate) (2,388 mL $ha^{-1}$), and Dicamba DMA (585 mL $ha^{-1}$) on 26 April 2019. A second application of Explorer (mesotrione) (394.6 mL $ha^{-1}$), Atrazine 4L (3,069.3 mL $ha^{-1}$), Brawl II (s-metolachlor) (1,607.7 mL $ha^{-1}$), and Buccaneer Plus (glyphosate) (2,923.1 mL $ha^{-1}$) on May 31, 2019.

**2020:**

In 2020 the dominant soil series was a Kahola silt-loam (a fine-silty, mixed, superactive, mesic Cumulic Hapludoll). The previous crop was double-cropped wheat and soybean. The average pH of the field was 6.37. Pre-plant P, K, and pH data are available in Table A.2. in the appendix. For 2020, two additional N rates were added (140 and 154 kg N $ha^{-1}$) to the four original N rates in 2019. The experiment had six replications to have a more robust dataset for mean separation. Plot size was 6.10 m by 11.3 m. The corn variety Pioneer P1257AM (Pioneer Hi Bred International, Johnston, IA) was planted at 74,131 seeds $ha^{-1}$ using a John Deere 1705 integral planter (Deere & Company, Moline, IL).

Urea was applied by hand on the day of planting. Proven™ was applied in-furrow at planting at a rate of 0.935 L $ha^{-1}$ (as directed by Pivot Bio). The rates of Proven™ were different between years due to a change in the formulation of the product. Planting occurred 30 April 2020. In 2020, 77 kg $ha^{-1}$ of $P_2O_5$ as TSP was applied on 12 May 2020. Herbicide application included AMS, (394.6 mL $ha^{-1}$) Callisto (Mesotrione), (2,046.2 mL $ha^{-1}$) Brawl II (S-Metalachlor), (1,461.6 mL $ha^{-1}$) Atrazine 4L, and (4,677.0 mL $ha^{-1}$) glyphosate on 10 May 2020, and an application of Interline (glufosinate) on 15 June 2020 to control Palmer pigweeds (*Amaranthus palmeri*).

## Sampling

Several sampling events occurred before and during the growing season. Pre-plant and post-harvest soil samples were taken with a Giddings GSRTS hydraulic probe (Giddings Machine Co. Inc., Fort Collins, CO) and separated into depths of 0-15, 15-30, 30-60, and 60-90 cm to determine P, K, and IN concentrations as well as pH. Other soil sampling dates were conducted by hand to a depth of 30 cm and separated into depths of 0-15 and 15-30 cm to determine IN content. Plant populations were determined once in the spring and in the fall by counting the number of plants in 14 m of two rows from each plot. Vigor was assessed for general health visually on a scale from 1 to 10, with 1 being least healthy and 10 being the most. Factors such as height, greenness, and shoot size were considered when determining vigor. Whole plant samples were collected by cutting a predetermined number of plants (five at V6-V8, 3 at VT, and 10 at R6) from two rows of each plot at ground level. Plant samples were weighed, dried, and ground for N content analysis. For the R6 whole plant sample, the ears were separated from the vegetative biomass prior to weighing and grinding for separate analysis. Normalized difference vegetative index (NDVI), an index of plant greenness and biomass, both of which should be positively correlated with N uptake, was collected by using a GreenSeeker handheld crop sensor (Trimble, Sunnyvale, CA) above two data rows in each plot. Soil plant analysis development (SPAD) readings, a measure of leaf greenness, which should be positively correlated with N uptake, were collected using a SPAD meter (Minolta chlorophyll meter SPAD-502DL, Ramsey, NJ). Halfway between the leaf tip and stalk and halfway between the midrib and leaf edge on the ear leaf of 20 plants in two rows of each plot. SPAD readings were collected three times between R1 and R3. The number of green leaves were counted on ten plants in two rows of each plot three times between R1 and R6. Plants with greater N uptake are expected to have a greater number of green leaves during grain fill. Yield was determined at harvest by taking the ears from 6 m of row (3 m from two rows) of each plot. Ears were weighed, shelled, and the grain weighed for yield. A Dickey-John Grain Analysis Computer (Dickey-John Corporation, Auburn, IL) was used to determine grain moisture and test weight. Yield was reported on a 15.5% moisture basis. In 2019 the only whole plant sample taken was at R6, whereas whole plant samples were taken at V6-V8, VT, and R6 in 2020. Extended leaf plant height at V6 and ear height during grain fill were also collected in 2020. Extended leaf plant height was collected by measuring the height of the tallest leaf on 10 plants from each plot. Ear height was collected by measuring the height of ear insertion from 10 plants in each plot.

## Lab Procedures

Soil inorganic N content was determined by KCl extraction (Keeney & Nelson, 1982). Briefly, field moist soil (25 g) was placed in an Erlenmeyer flask with 100mL 1 *M* KCl, placed on an orbital shaker at 300rpm for 1 h. The Erlenmeyer flasks were then left to settle for 10 min, and the solution filtered through Whatman #42 filter paper. The samples were then submitted to the Kansas State University Soil Testing lab for $NH_4$-N and $NO_3$-N determined colorimetrically (Gelderman & Beegle, 1998). Gravimetric soil water content was determined by drying at 105$^{o}$ C until they reached a constant mass. All soil N values were reported on a dry weight basis. Pre-plant soil samples were measured for total C and N by dry combustion (Carbon and Nitrogen in Soil and Sediment, 2005). Plant N was determined by combustion by the Kansas State University Soil Testing Lab (Carbon, Hydrogen, and Nitrogen in Flour and Plant Tissue, 2005).

## Calculations

The calculations utilized in this study include an estimation of soil OM, N mineralized over the growing season (kg ha$^{-1}$), several methods of calculating NUE, and a novel method of calculating N balance. Methods of NUE calculated include NARE (kg N kg N$^{-1}$), NRE (kg N kg N$^{-1}$), NRUE (kg N kg N$^{-1}$), NAE (kg kg N$^{-1}$), PFP (kg kg N$^{-1}$), NUEcrop (kg N kg N$^{-1}$). The novel method of N balance calculated was TNBI (kg N). The equations for these calculations are below.

1. $OM = Total\ soil\ C * 1.72$ (Springob & Kirchmann, 2003).
2. $N\ mineralization = Residual\ soil\ N + Plant\ N - Preliminary\ soil\ N$ (Modified from Rice & Havlin, 1994).
3. $NARE = (Plant\ N_f - Plant\ N_0)/N\ rate$ (Chen et al., 2016).
4. $NRE = Plant\ N_f/N\ rate$ (Chen et al., 2016).
5. $NRUE = Plant\ N_f/(N\ rate - Residual\ soil\ N)$ (Chen et al., 2016).
6. $NAE = (Yield_f - Yield_0)/N\ rate$ (Chen et al., 2016).
7. $PFP = Yield_f/N\ rate$ (Congreves et al., 2021).
8. $NUEcrop = Grain\ N_f/N\ rate$ (Congreves et al., 2021).
9. $TNBI = (Preliminary\ soil\ N + Plant\ N_0 + N\ rate) - (Plant\ N + Residual\ soil\ N)$ (Modified from Congreves et al., 2021).

Variable descriptions: Subscript *f* indicates the fertilized treatments. Subscript 0 indicates the unfertilized and uninoculated treatments.

## Statistical Analysis

Type three ANOVA and regression analyses of the data were conducted using Rstudio to determine statistical significance (α=0.05) and analyze trends. Pearson Standardized Residuals were examined to determine independence of residuals and test for normality. Quadratic regression analyses were performed using the linear regression model lm function and a squared parameter in R. Pairwise comparisons were constructed using Fishers Least Significant Difference.

# Results

## 2019

### Growing conditions

The 30-year average precipitation for Riley County, Kansas (1981-2010) was 852 mm (Precipitation in Kansas, 2021). Annual precipitation in Riley County, Kansas in 2019 was 1163 mm (Precipitation in Kansas, 2021). Precipitation in the 2019 season was much greater than average. 2019 also had several rain events with high intensity, such as the 90.7 mm rainfall of July 4th (Fig. 1.1). Saturated soils delayed planting and corn development. However, it is uncertain if the delay in corn development resulted in decreased yields or crop growth. Excessive precipitation in 2019 may have contributed to N loss via leaching and denitrification (Cregger et al., 2014).

The 30-year average temperature for Southern Riley County, Kansas (1981-2010) was 12.7 °C, with average monthly maximum and minimum temperatures in Riley County of 19.5 and 5.89, respectively (U.S. Climate Normals Quick Access, 2021). The average daily maximum and minimum temperatures in Riley County, Kansas in 2019 were 18.3 and 6.6 °C (Kansas

Mesonet, 2021). In 2019, average maximums were slightly lower than the 30-year average, and average minimums were slightly higher than the 30-year average. Overall maximum and minimum averages during the growing season were unlikely to reduce plant growth and development compared to the 30-year average (Fig. 1.2).

### Soil inorganic N

At the time of planting, approximately 4 mg N $kg^{-1}$ of IN was present in the soil profile to 90 cm, with ~14 mg N $kg^{-1}$ in the top 15 cm (Fig. 1.3). At the V6 growth stage, IN reflected the N fertilizer increments. Most of the IN was in the 0-15 cm (Table 1.1). At the R1 growth stage, IN was at low levels for both depths even at the 168 kg N $ha^{-1}$ rate (Table 1.1). At harvest, approximately 2 mg N $kg^{-1}$ of IN was present in the soil profile, with 4 mg N $kg^{-1}$ in the top 0-15 cm (Fig. 1.3).

### Plant Population

The average plant population in the spring was 60,690 plants $ha^{-1}$ (Table 1.2). Plant population at harvest averaged 58,256 plants $ha^{-1}$ (Table 1.2). Some lodging losses occurred but lodging rates were not collected in 2019. Plant population was not significantly different between treatments at either sampling date.

### NDVI

Normalized Difference Vegetative Index is an index of plant reflectance that is indicative of plant biomass and greenness and can be used to make inferences about plant health. Plants with greater N uptake should have greater biomass and greater greenness resulting in higher

NDVI values. Three NDVI readings were taken in 2019 (at V6, V7, and V8) (Table 1.3). NDVI at V6 was not significantly affected by Proven$^{TM}$ (Pr=0.129), N rate (Pr=0.272), or a Proven$^{TM}$ by N rate interaction (Pr=0.731). NDVI at V7 was significantly affected by Proven$^{TM}$ (Pr=0.008) and N rate (Pr=0.004), but not a Proven$^{TM}$ by N rate interaction (Pr=0.276). NDVI  at V7 generally followed the expected response to N fertilizer. NDVI was greatest at the 112 and 168 kg N ha$^{-1}$, which were significantly greater than NDVI for 0 kg N ha$^{-1}$, but not for 56 kg N ha$^{-1}$ (Table 1.4). The NDVI values for the 0 and 56 kg N ha$^{-1}$ were not significantly different (Table 1.4). NDVI at V7 was significantly greater in plots not treated with Proven$^{TM}$ than in plots treated with Proven$^{TM}$ (Table 1.5). NDVI at V8 was significantly affected by Proven$^{TM}$ (Pr<0.001) and N rate (Pr=<0.001), but not a Proven$^{TM}$ by N rate interaction (Pr=0.063). NDVI at V8 generally followed the expected N rate response but with limited resolution of significance. NDVI was greatest for 56, 112, and 168 kg N ha$^{-1}$, which were significantly greater than NDVI at 0 N ha$^{-1}$ (Table 1.6). NDVI at V8 was significantly greater in plots not treated with Proven$^{TM}$ than plots treated with Proven$^{TM}$ (Table 1.7). For both V7 and V8 NDVI, the significant increase in NDVI without Proven$^{TM}$ suggests that Proven$^{TM}$ was decreasing NDVI rather than increasing NDVI.

**SPAD**

Soil plant analysis development (SPAD) is a light meter that uses the reflectance of plant leaves to determine greenness. Because N is essential for chlorophyll production, increased N uptake should increase leaf greenness and increase the SPAD value of the plant tissues. Three SPAD collections were taken during the 2019 season (at R1, R2, and R3) (Table 1.8). At R1, SPAD was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.941) or a

Proven$^{TM}$ by N rate interaction (Pr=0.571). SPAD values at R1 conformed to the expected N rate response, with 112 and 168 kg N ha$^{-1}$ having the greatest SPAD values, which were significantly greater than 56 kg N ha$^{-1}$, which was in turn significantly greater than 0 kg N ha$^{-1}$ (Table 1.9). At R2, SPAD was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.796) or a Proven$^{TM}$ by N rate interaction (Pr=0.746). SPAD values at R2 followed the same N response as seen at R1 (Table 1.10), although the average SPAD value decreased as the growing season progressed (Table 1.9) (Table 1.10) (Table 1.11). At R3, SPAD was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.140) or a Proven$^{TM}$ by N rate interaction (Pr=0.139). R3 SPAD values followed the expected N rate response (Table 1.11). R3 SPAD values were greatest at 168 kg N ha$^{-1}$, which was significantly greater than the SPAD values at 56 kg N ha$^{-1}$, but not 112 kg N ha$^{-1}$ (Table 1.11). SPAD at the 112 kg N ha$^{-1}$ rate was not significantly different from 56 or 168 kg N ha$^{-1}$ (Table 1.11). SPAD at the 0 N ha$^{-1}$ rate was significantly lower than all other SPAD values (Table 1.11).

### Green Leaf Count

Green leaf count (GLC) is a count of the number of green leaves on a plant. Because corn frequently cannibalizes vegetative biomass N during grain fill, plants with greater plant N should have a greater number of green leaves during grain fill. Three Green Leaf Counts (GLC) were collected during the 2019 season (two at R3, taken on 8/1/2019, and 8/9/2019, and one at R4) (Table 1.12). At the first R3 sampling date (8/1/2019), GLC was significantly affected by N rate (Pr<0.001) and a Proven$^{TM}$ by N rate interaction (Pr=0.014), but not by Proven$^{TM}$ (Pr=0.606). GLC at R3 followed the expected N rate response, with the higher N rates typically having greater GLCs. The 168 kg N R3 GLC was significantly greater without Proven$^{TM}$ than with

Proven$^{TM}$. At 56 kg N, GLC was significantly greater in plots treated with Proven$^{TM}$ than plots not treated with Proven$^{TM}$ (Table 1.13). Differences between with and without Proven$^{TM}$ were not significant within 0 and 112 kg N ha$^{-1}$ (Table 1.13). At the second R3 sampling date (8/9/2019) GLC was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.811) or a Proven$^{TM}$ by N rate interaction (Pr=0.220). GLC at R3-2 was greatest at 112 and 168 kg N ha$^{-1}$ (Table 1.14). GLC was significantly greater at 168 kg N ha$^{-1}$ than 56 kg N ha$^{-1}$. GLC at 112 kg N ha$^{-1}$ was greater than GLC at 0 kg N ha$^{-1}$ (Table 1.14). For 0 and 56 kg N ha$^{-1}$, 56 and 112 kg N ha$^{-1}$, and 112 and 168 kg N ha$^{-1}$ pairs were not significantly different (Table 1.14). At R4, GLC was significantly affected by N rate (Pr=0.004), but not by Proven$^{TM}$ (Pr=0.575) or a Proven$^{TM}$ by N rate interaction (Pr=0.266). GLC at R4 followed the same N rate response as GLC at R3-2 (Table 1.15).

### Yield

Corn grain yield (Fig. 1.4) was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.411) or a Proven$^{TM}$ by N rate interaction (Pr=0.490). Corn grain yield followed the N rate response expected with the greatest yield at the highest N rate and significantly lower yields at the lower N rates, although 0, 56, and 112 kg N ha$^{-1}$ were not significantly different. Yield was greater with Proven$^{TM}$ than without at the 0 and 168 kg N ha$^{-1}$ rates by 509 and 956 kg ha$^{-1}$ (Fig. 1.4).

### Harvest Whole Plant N

Whole plant N at harvest in 2019 was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.094) or a Proven$^{TM}$ by N rate interaction (Pr=0.966). Whole plant N followed the expected N rate response of greater plant N associated with greater N fertilizer application. N uptake was greatest at 112 and 168 kg N ha$^{-1}$, which were significantly greater than 56 kg N ha$^{-1}$ which was significantly greater than N uptake at 0 kg N ha$^{-1}$. There was a trend for greater Plant N in plots treated with Proven$^{TM}$ than plots not treated with Proven$^{TM}$ (Fig. 1.5). Nitrogen uptake was greater with Proven$^{TM}$ than without with a diminishing return with N rate: 10.9, 7.26, 8.72, and 4.30 kg ha$^{-1}$ at the 0, 56, 112, and 168 kg N ha$^{-1}$, respectively.

**Mineralized N**

In 2019 mineralized N (Table 1.16) was greater with Proven$^{TM}$ than without by 18.4 kg N ha$^{-1}$ but was not significantly affected by the presence of Proven$^{TM}$ (Pr=0.296). This may suggest that Proven$^{TM}$ is a source of approximately 18 kg N ha$^{-1}$ but is not statistically significant.

**NARE**

In 2019 NARE ((whole plant N – whole plant N without Proven$^{TM}$ with 0 kg N ha$^{-1}$) was not significantly affected by Proven$^{TM}$ (Pr=0.112), N rate (Pr=0.697), or a Proven$^{TM}$ by N rate interaction (Pr=0.685) (Table 1.17). NARE was generally greater with less N applied, conforming to expectations that NUE is greater at lower N rates. NARE was generally greater with Proven$^{TM}$, although not significant.

**TNBI**

TNBI is a novel method of N balance that is a modified form of NBI that includes plant N uptake parameters ((preplant soil N + whole plant N without Proven$^{TM}$ with 0 kg N ha$^{-1}$ + N rate) – (whole plant N + post-harvest soil N) (Table 1.18). TNBI was significantly affected by N rate (Pr<0.001), but not by Proven™ (Pr=0.671) or a Proven™ by N rate interaction (Pr=0.826) in 2019. TNBI in 2019 generally followed the expected N rate response and was significantly greater at 168 kg N $ha^{-1}$ than at the other rates (Table 1.19). No significant differences between the 0, 56, and 112 kg N rates were found (Table 1.19).

**NRE**

In 2019, NRE was significantly affected by N rate (Pr<0.001) but not by Proven™ (Pr=0.112) or a Proven™ by N rate interaction (Pr=0.685). NRE followed the expected N rate response (Table 1.20). NRE was greatest at the 56 kg N $ha^{-1}$ rate, followed by 112 and then 168 kg N $ha^{-1}$ (Table 1.20). NRE was significantly different at each N rate (Table 1.20).

**NRUE**

NRUE in 2019 was significantly affected by N rate (Pr<0.001) and by Proven™ (Pr=0.026), but not by a Proven™ by N rate interaction (Pr=0.592). NRUE generally followed the expected N rate response, although there were fewer N rates tested since NRUE cannot be calculated for the 0 kg N $ha^{-1}$ rate (Table 1.21). NRUE was greatest at the 56 kg N $ha^{-1}$ rate, which was significantly greater than NRUE at 112 and 168 kg N $ha^{-1}$ (Table 1.21). NRUE was not significantly different between 112 and 168 kg N $ha^{-1}$ (Table 1.21). There were no significant differences between N rates with and without Proven™ (Table 1.22).

**NAE**

In 2019 NAE was not significantly affected by N rate (Pr=0.090), Proven™ (Pr=0.963), or a Proven™ by N rate interaction (Pr=0.651) (Table 1.23).

**PFP**

PFP in 2019 was significantly affected by N rate (Pr<0.001) but not by Proven$^{TM}$ (Pr=0.963) or a Proven$^{TM}$ by N rate interaction (0.651). PFP followed the N rate response expected since PFP was greatest at 56 kg N ha$^{-1}$, followed by 112 and 168 kg N ha$^{-1}$ (Table 1.24). PFP was significantly different at each N rate (Table 1.24).

**NUEcrop**

NUEcrop in 2019 was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.874) or a Proven$^{TM}$ by N rate interaction (Pr=0.533). NUEcrop was greatest at 56 kg N ha$^{-1}$, followed by 112 and then 168 kg N ha$^{-1}$ (Table 1.25). NUEcrop was significantly different at each N rate (Table 1.25). The N rate response of NUEcrop was consistent with expected results.

## 2020

**Growing conditions**

The 30-year average precipitation for Riley County, Kansas (1981-2010) was 852 mm (Precipitation in Kansas, 2021). Annual precipitation in Riley County, Kansas in 2020 was 795 mm (Precipitation in Kansas, 2021). Precipitation in 2020 was slightly below average and had less extreme rain events (Fig. 1.6).

The 30-year average temperature for Southern Riley County, Kansas (1981-2010) was 12.7 °C, with average monthly maximum and minimum temperatures in Riley County of 19.5 and 5.89, respectively (U.S. Climate Normals Quick Access, 2021). Average daily maximum and minimum temperatures in Riley County, Kansas in 2020 were 19.2 and 6.8 °C, respectively

(Kansas Mesonet, 2021). In 2020 average maximums were slightly lower than the 30-year average and average minimums were slightly higher than the 30-year average. Overall maximum and minimum averages during the growing season were unlikely to significantly reduce plant growth and development (Fig. 1.7).

### Soil inorganic N

At the time of planting, approximately 4 mg N $kg^{-1}$ as inorganic N was present in the soil profile to 90 cm, with 5 mg N $kg^{-1}$ in the top 15 cm and approximately the same concentration in the 15-30 cm depth (Fig. 1.8). At V8 IN generally reflected the N fertilizer increments, with one exception being the 140 kg N $ha^{-1}$ plots having less IN than the 112 kg N $ha^{-1}$ plots (Table 1.26). The majority of the IN was in the 0-15 cm (Table 1.26). At harvest, approximately 4 mg N $kg^{-1}$ of IN was present in the soil profile, with 7 mg N $kg^{-1}$ in the top 15 cm (Fig. 1.8). The increase in soil IN in the 0-15 cm depth is most likely from residual fertilizer N.

### Plant Population

The average plant population in the spring was 70,757 plants $ha^{-1}$ (Table 1.27). Plant population at harvest averaged 64,673 plants $ha^{-1}$ (Table 1.27). Lodging losses in 2020 were negligible. Plant population was not significantly different between treatments at either sampling date.

### Extended Leaf Plant Height

Extended leaf plant height (ELPH) is the height of the most vertically extended leaf on a plant. Because N is essential for plant growth, plants with greater N applied should exhibit greater ELPHs. ELPH at V6 in 2020 was significantly affected by Proven™ (Pr=0.003), N rate (Pr<0.001), and a Proven™ by N rate interaction (Pr=0.001). ELPH generally followed the expected N rate response, with ELPH increasing as N rate increased. At the 0, 112, and 168 kg N $ha^{-1}$ ELPH was greater in plots not treated with Proven™ than plots treated with Proven™ (Table 1.28). ELPH with and without Proven™ was not significantly different at the 56, 140, and 154 kg N $ha^{-1}$ rates.

**NDVI**

Normalized Difference Vegetative Index is an index of plant reflectance indicative of plant biomass and greenness, which can be used to make inferences about plant health. Plants with greater N uptake should have greater biomass and greater greenness resulting in higher NDVI values. Two NDVI samples were taken in 2020 (at V7 and V9) (Table 1.29). NDVI at V7 was significantly affected by N rate (Pr<0.001), but not by Proven™ (Pr=0.550) or a Proven™ by N rate interaction (Pr=0.492). NDVI at V7 somewhat followed the expected N rate response, with the 0 N rate having significantly lower NDVI than the other rates, which were not significantly different from each other, although significant differences among 56-168 kg N $ha^{-1}$ would be expected (Table 1.30). NDVI at V9 was also significantly affected by N rate (Pr=<0.001), but not by Proven™ (Pr=0.227) or a Proven™ by N rate interaction (Pr=0.247). NDVI at V9 followed the same N rate response as NDVI at V7 (Table 1.31).

**SPAD**

SPAD is an index based on reflectance of plant leaves to determine greenness, which is performed by a SPAD meter. Because N is essential for the production of the green pigment chlorophyll, increased N uptake should increase leaf greenness and increase the SPAD value of the plant tissues. Three SPAD collections were taken during the 2020 season (at R1, R2, and R4) (Table 1.32). At R1, SPAD was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.561) or a Proven$^{TM}$ by N rate interaction (Pr=0.600). SPAD generally followed the expected N rate response (Table 1.33). SPAD was greatest at 112, 140, 154, and 168 kg N ha$^{-1}$, which were not significantly different, and 56 kg N ha$^{-1}$ was significantly greater than the SPAD of 0 kg N ha$^{-1}$ (Table 1.33). At R2, SPAD was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.057) or a Proven$^{TM}$ by N rate interaction (Pr=0.601). SPAD at R2 followed the same N rate response as with R1 SPAD values (Table 1.34). At R4, SPAD was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.607) or a Proven$^{TM}$ by N rate interaction (Pr=0.183). The N rate response exhibited by the SPAD values at R4 was similar to those exhibited at R1 and R2, except 154 kg N ha$^{-1}$, which was not significantly different from the SPAD of 56 kg N ha$^{-1}$. This deviation does not correspond with expectations about SPAD response to N rate, although the rest of the N rate response does (Table 1.35).

### Green Leaf Count

Green leaf count (GLC) is a count of the number of green leaves on a plant. Because corn frequently cannibalizes vegetative biomass N during grain fill, plants with greater plant N should have a greater number of green leaves during grain fill. Three GLCs were taken during the 2020 growing season at R2, R4, and R5 (Table 1.36). GLC at R2 was significantly affected by Proven$^{TM}$ (Pr=0.028), N rate (Pr=0.006), and Proven$^{TM}$ by N rate interaction (Pr<0.001). GLC at R2 did not follow the expected N rate response, as several of the higher N rates had significantly lower GLCs than the lower N rates (Table 1.37). At 168 kg N ha$^{-1}$, GLC was significantly greater with Proven$^{TM}$ than without Proven$^{TM}$ (Table 1.37). At 0 and 154 kg N ha$^{-1}$ GLC was significantly greater without Proven$^{TM}$ than plots treated with Proven$^{TM}$. Differences between with and without Proven$^{TM}$ were not significant within N rate at 56, 112, and 140 kg N ha$^{-1}$. A consistent benefit from Proven$^{TM}$ was not observed in GLC at R2 (Table 1.38). GLC at R4 was significantly affected by a Proven$^{TM}$ by N rate interaction (Pr=0.003), but not by Proven$^{TM}$ (Pr=0.575) or N rate (Pr=0.242). A consistent N rate response by GLC at R4 was not observed, although in general, the lowest N rates had lower GLCs than higher N rates (Table 1.38). GLC was significantly greater at 112 kg N ha$^{-1}$ in plots treated with Proven$^{TM}$ than in plots not treated with Proven$^{TM}$ (Table 1.38). Differences between with and without Proven$^{TM}$ were not significant within N rates at 0, 56, 140, 154, and 168 kg N ha$^{-1}$. GLC at R5 was not significantly affected by Proven$^{TM}$ (Pr=0.164), N rate (Pr=0.247), or a Proven$^{TM}$ by N rate interaction (Pr=0.253).

### Ear Height

Ear Height (EH) is a measure of the height of ear insertion on a corn plant. Since N is required for crop growth, corn plants with greater N available should be taller and have a greater EH. EH at R5 was only collected in 2020 (Table 1.39). EH was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.513) or a Proven$^{TM}$ by N rate interaction (Pr=0.114). EH somewhat followed the expected N rate response, in that the EH of 0 kg N ha$^{-1}$ was significantly lower than the other rates, although there were no significant differences between the EHs of the rest of the N rates (Table 1.40).

### In-Season Whole Plant Samples

In-season whole plant N (WPN) analysis was only conducted during the 2020 season (Table 1.31). WPN was expected to be greater with greater available N. In-season whole plant samples (WPS) were collected at V8 and VT (Table 1.41). At V8, WPN was significantly affected by N rate (Pr<0.001), but not by Proven™ (Pr=0.243) or a Proven™ by N rate interaction (Pr=0.591). WPN at V8 somewhat followed the expected N rate response, although 154 kg N ha$^{-1}$ was not significantly higher than 56 kg N ha$^{-1}$, despite 112, 154, and 168 kg N ha$^{-1}$ being significantly greater than 56 kg N ha$^{-1}$.  At VT, WPN was significantly affected by N rate (Pr<0.001), but not by Proven™ (Pr=0.259) or a Proven™ by N rate interaction (Pr=0.220). WPN at VT followed the expected N rate response, with 112, 140, 154, and 158 kg N ha$^{-1}$ having the greatest WPN, which were significantly greater than the WPN of 56 kg N ha$^{-1}$, which was significantly greater than the WPN of 0 kg N ha$^{-1}$ (Table 1.43).

**Yield**

Yield in 2020 (Fig. 1.9) was significantly affected by N rate (Pr<0.001), but not by Proven™ (Pr=0.510) or a Proven™ by N rate interaction (Pr=0.901). Yield followed the expected N rate response, with the greatest N rate having the greatest yield (Fig. 1.9). Yield also increased at a decreasing rate per unit of N applied (Fig. 1.9). Yield at 0 kg N ha$^{-1}$ was significantly lower than that of 56 kg N ha$^{-1}$, which was not significantly different from 112 kg N ha$^{-1}$. There were no significant differences between 140, 154, and 168 kg N ha$^{-1}$.

**Harvest Whole Plant N**

Whole plant N at harvest in 2020 (Fig. 1.10) was significantly affected by N rate (Pr<0.001), but not by Proven™ (Pr=0.151), or a Proven™ by N rate interaction (Pr=0.335). The

N rate response of WPN was generally expected (Fig. 1.10). WPN at 112, 140, 154, and 168 kg N ha$^{-1}$ were significantly greater than WPN at 56 kg N ha$^{-1}$, which was significantly greater than WPN at 0 kg N ha$^{-1}$. WPN also increased at a decreasing rate per unit of N applied (Fig. 1.10). Generally, there was a trend for greater N uptake without Proven$^{TM}$, rather than with, although this was not statistically significant.

### N Mineralization

In 2020, mineralized N was not significantly affected by the presence of Proven$^{TM}$ (Pr=0.327). Mineralized N without Proven$^{TM}$ was greater than with by 11.9 kg ha$^{-1}$ (Table 1.44). This suggests that Proven$^{TM}$ may not have been acting as a source of N and background mineralization rates had a 11.9 kg ha$^{-1}$ discrepancy between treatments, or that Proven$^{TM}$ was acting as a source of N but the amount of N that Proven$^{TM}$ fixed was offset by an amount of N mineralization 11.9 kg ha$^{-1}$ greater than the amount fixed by Proven$^{TM}$.

### NARE

NARE in 2020 was not significantly affected by N rate (Pr=0.727) but was significantly affected by Proven$^{TM}$ (Pr=0.022), and a Proven$^{TM}$ by N rate interaction (Pr=0.003). The N rate exhibited by NARE in 2020 did not follow the expected N response (Table 1.45). Few significant differences between N rates were observed. NARE was greater at 56 kg N ha$^{-1}$ without Proven$^{TM}$ than with Proven$^{TM}$ (Table 1.45). No other significant differences between with and without Proven$^{TM}$ within N rates were observed (Table 1.45).

### TNBI

TNBI in 2020 was significantly affected by N rate (Pr<0.001) and a Proven$^{TM}$ by N rate interaction (Pr=0.009), but not by Proven$^{TM}$ (Pr=0.913). There was a general trend for greater TNBI. Values at the higher N rates, which would be expected, but significant differences between rates were few. TNBI was significantly greater at 168 kg N ha$^{-1}$ without Proven$^{TM}$ than at 168 kg N ha$^{-1}$ with Proven$^{TM}$ (Table 1.46). There were no other significant differences within any rate with and without Proven$^{TM}$ (Table 1.46).

**NRE**

In 2020 NRE was significantly affected by N rate (Pr<0.001), Proven$^{TM}$ (Pr=0.022), and a N rate by Proven$^{TM}$ interaction (Pr=0.003). NRE generally followed the expected N rate response, with the lower N rates having greater NRE values (Table 1.47). Generally, as N rate increased NUE decreased (Table 1.47). The NREs of 56 kg N ha$^{-1}$ were significantly greater than the NRE of 112 kg N ha$^{-1}$ (Table 1.47). There were no significant differences between 140, 154, and 168 kg N ha$^{-1}$ (Table 1.47), although this was not unexpected since the differences in N rate between these rates were small. For 56 kg N ha$^{-1}$, the treatment without Proven$^{TM}$ had a significantly greater NRE than with (Table 1.47). No other significant differences with and without Proven$^{TM}$ were detected within N rates (Table 1.47). 112 kg N ha$^{-1}$ with Proven$^{TM}$ was not significantly different from 112 kg N ha$^{-1}$ without Proven$^{TM}$ (Table 1.47).

**NRUE**

NRUE in 2020 was not significantly affected by N rate (Pr=0.559), Proven$^{TM}$ (Pr=0.366), or a Proven$^{TM}$ by N rate interaction (Pr=0.452) (Table 1.48).

**NAE**

NAE in 2020 was significantly affected by N rate (Pr<0.001), but not by Proven™ (Pr=0.267) or a Proven™ by N rate interaction (Pr=0.394). NAE followed the expected N rate response, with greater NAE associated with lower N rates (Table 1.49). NAE at 56 kg N $ha^{-1}$ was significantly greater than the NAE of 112, 140, 154, and 168 kg N $ha^{-1}$ (Table 1.49). No significant differences were found between 112, 140, 154, and 168 kg N $ha^{-1}$ (Table 1.49).

**PFP**

In 2020 PFP was significantly affected by N rate (Pr<0.001), but not by Proven™ (Pr=0.267) or a Proven™ by N rate interaction (Pr=0.394). PFP followed the expected N rate response of greater PFP at lower N rates (Table 1.50). PFP was greatest at 56 kg N $ha^{-1}$, which was significantly greater than the PFP at 112 kg N $ha^{-1}$, which was significantly greater than the PFP of 140, 154, and 168 kg N $ha^{-1}$ (Table 1.50). No significant differences were found between 140, 154, and 168 kg N $ha^{-1}$ (Table 1.50).

**NUEcrop**

NUEcrop was significantly affected by N rate (Pr<0.001), but not by Proven™ (Pr=0.803) or a Proven™ by N rate interaction (Pr=0.742). NUEcrop followed the expected N rate response of greater NUEcrop at lower N rates (Table 1.51). NUEcrop was significantly greater at 56 kg N $ha^{-1}$ than at 112 kg N $ha^{-1}$ (Table 1.51). NUEcrop at 112 kg N $ha^{-1}$ was significantly greater than NUEcrop at 154, and 168 kg N $ha^{-1}$, but not at 140 kg N $ha^{-1}$ (Table 1.51). There were no significant differences between 140, 154, and 168 kg N $ha^{-1}$ (Table 1.51).

# Discussion

Average grain yields in 2019 and 2020 were 10,420 and 10,332 kg $ha^{-1}$ respectively. Average corn yield in Riley County, Kansas was estimated to be 10,000 kg $ha^{-1}$ (National Agricultural Statistics Service, 2020), meaning that yields in this experiment were similar to the county average. Yields in both years were not significantly affected by Proven™ or a Proven™ by N rate interaction. No consistent trend in yield with and without Proven™ was observed either.

Soil N mineralization was 76 to 94 kg N $ha^{-1}$ for 2019 and 202, respectively. This estimate is much higher than the 25 kg N $ha^{-1}$ reported by Mikha et al. (2006) at the same 2019 site. This discrepancy could be due to the accumulation of OM from sustained no-tillage between 2006 and 2019. In 2019, N uptake with Proven™ from the 0 kg N $ha^{-1}$ treatment was 18.4 kg N $ha^{-1}$ greater, suggesting Proven™ fixed this amount. In 2020, mineralized N was greater without Proven™ by 11.9 kg N $ha^{-1}$, although this difference was not statistically significant.

In-season plant measurements responded to N rate but not Proven™. Results from NDVI were often not consistent between years or dates. NDVI values generally followed expected N rate responses. Values for NDVI were consistent with Garcia-Martinez et al. (2020), who reported results of 0.46 and 0.90, 47 and 79 days after planting, respectively. The SPAD values for this study consistently followed the expected N response. More significant differences between N rates were found for SPAD than NDVI. Kandel (2020) reported SPAD values ranging from ~35 at 60 days after sowing to ~60 at 140 days after sowing. SPAD values in this study fell within this range. Green leaf count generally followed the N rate response. Some GLC dates offered inconsistent N rate results as well. GLC, in particular, may be affected by a litany of confounding factors, making conclusions based on GLC limited. Comparisons between studies of GLC are limited because leaf number is heavily influenced by cultivar. In-season plant N uptake generally followed the expected N response. The lack of significant differences between N rates exhibited by these parameters highlights the importance of collecting high resolution data to detect the smaller N increments provided by Proven$^{TM}$.

There was little or no significant evidence that Proven$^{TM}$ increased NUE in either year of this experiment. NARE values in 2019 ranged from 0.47 to 0.73 in 2019 and 0.36 to 0.79 for 2020. NARE for each year were slightly higher than those reported by Roberts et al. (2010). The higher NARE is likely due to uptake of soil N (Congreves et al. 2021). NARE of most treatments exceeded the national average NARE of 0.37 reported by Cassman et al. (2002), indicating higher than average efficiency. Lack of significance of N rate contributing to NARE and high variability of NARE was also observed by Roberts et al. (2010).

NREs from this study (1.23 to 2.92 kg N kg N$^{-1}$. and 1.12 to 2.86 kg N kg N$^{-1}$. in 2019 and 2020, respectively) were much greater than NREs reported by Roberts et al. (2016), which ranged from 0.61 to 0.91 kg N kg N$^{-1}$. NREs >1 indicate that soil N was being removed and that fertilizer N was not in excess (Congreves et al., 2021

In 2019, NAE was not significantly affected by N rate, Proven$^{TM}$, or a Proven$^{TM}$ by N rate interaction. In 2020, NAE was significantly affected by N rate, but not by Proven$^{TM}$ or a Proven$^{TM}$ by N rate interaction. NAEs in 2020 ranged from 23.5 to 44.3 kg grain N kg N$^{-1}$, well within the range of 1.15 to 77.7 kg grain N kg N$^{-1}$ described in Thompson et al. (2015). NAE was greatest at 56 kg N ha$^{-1}$ (the lowest of the rates used that can be calculated), and 112, 140, 154, and 168 kg N ha$^{-1}$ were all significantly lower with no significant differences between NAEs. This adheres to the expectation that NUE decreases with increasing N fertilization (Rice et al., 1995; Roy et al., 2006). Differences between N rates at 112, 140, 154, and 168 kg N ha$^{-1}$

were not significant. This highlights the difficulty detect significant differences with and without Proven$^{TM}$, since the N provided by Proven$^{TM}$ was much less than 56 kg N ha$^{-1}$, which is the discrepancy between 112 and 168 kg N ha$^{-1}$ which were not significantly different.

PFP values from both years ranged from 67.4 to 187.2 kg grain kg N$^{-1}$ which falls within the range described in Thompson et al. (2015) of 20.1 to 187.4 kg grain kg N$^{-1}$. PFP was significantly affected by N rate for both years. Significant decreases in PFP from 56 to 112 to 168 kg N ha$^{-1}$ were congruent with previous findings that NUE decreases with increasing N fertilization (Rice et al., 1995; Roy et al., 2006).

NUEcrop values from this study were similar to those reported by Halvorson & Bartolo (2014). However, values reported by Halvorson & Bartolo (2014) were much lower at the 112 and 168 kg N ha$^{-1}$ rates than those found in this study. This may be again due to uptake of soil N contributing largely to grain N (Congreves et al., 2021). The NUEcrop at rates > 140 kg N ha$^{-1}$ were <1, indicating a surplus of N fertilizer (Congreves et al., 2021). For both years, NUEcrop values for < 112 kg N ha$^{-1}$ were >1, suggesting consumption of soil N (Congreves et al., 2021). In 2020, the trend for higher NUEcrop at lower N rates is congruent with previous findings that NUE decreases with increasing N fertilization (Rice et al., 1995; Roy et al., 2006).

The several NUE calculations performed for this study have distinct advantages and differing levels of utility. NRE and NUEcrop have the advantage of being able to be used as indexes to determine if fertilizer N was in excess or if soil N was being consumed (Congreves et al., 2021). NRE and NUEcrop do not consider soil N, so comparisons between sites are limited. NUEcrop was advantageous between the two methods because it considered whole plant N rather than just grain N, although if total plant N samples were taken similarly to the methods described in this study, then NRE can be calculated from a subset of the data required to calculate NUEcrop. NARE and NAE both have the advantage of considering soil N contributions to plant productivity (Congreves et al., 2021). NARE is a measure of plant N efficiency that considers unfertilized plant N uptake, which allows for differentiation between plant N derived from fertilizer N and plant N derived from soil N (Congreves et al., 2021). NAE is a measure of yield efficiency that considers an unfertilized treatment, which helps distinguish yield derived from N fertilizer and yield derived from soil N uptake (Congreves et al., 2021). Both NARE and NAE were advantageous as they focus on different plant productivities and both account for productivity of an unfertilized treatment for better determination of the benefit of applied N. NRUE also has its distinct advantage as a NUE metric. NRUE considers residual soil N to determine N loss throughout the season (Chen et al., 2016). This allows NRUE to estimate environmental impacts related to N loss from a system (Chen et al., 2016). NRUE does have significant disadvantages. First, there is limited data on NRUE in various crops, making comparisons to other studies and interpretation of results difficult. Second, NRUE does not consider pre-plant soil N or unfertilized N uptake so NRUE may not reflect soil contributions to plant N, and may underestimate N loss if soil N loss is significant (Chen et al., 2016). An improved method of NRUE may be possible to better address these issues. PFP has limited advantages as it does not consider soil N contributions and does not function as an index to determine N removal or excess (Congreves et al., 2021). The advantage of PFP is it is simple to calculate and does not require the maintenance of unfertilized plots, which may be beneficial for producers to estimate N efficiency without sacrificing time or land (Congreves et al., 2021). All of these NUE calculations have a specific value, but for the sake of scientific research PFP and NAE may not be as valuable and informative as the other measures. NRUE has potential as NUE measures but needs more robust datasets to improve interpretation.

TNBI was advantageous as a method of calculating N balance because it considers pre-plant N, residual N, and plant uptake without fertilization, allowing the user to more comprehensively account for N sources and sinks factors. The disadvantage of TNBI is that there are no estimates of TNBI outside of this paper, making interpretation of TNBI results difficult. In addition, TNBI may over estimate N sources, since some unfertilized plant N would assumedly be from the already accounted preliminary soil N, not just mineralized N.

Plant N was not significantly affected by Proven$^{TM}$ or a Proven$^{TM}$ by N rate interaction in either year. In 2019 Plant N was greater with than without Proven$^{TM}$ by 10.9, 7.26, 8.71, and 4.30 kg N ha$^{-1}$ in 0, 56, 112, and 168 kg N ha$^{-1}$, respectively. This suggests that Proven$^{TM}$ could be supplying N to the crop, but was not enough N to produce a statistically significant response in plant N. In 2020, Plant N was greater with than without Proven$^{TM}$ by 9.54 and 1.62 kg N ha$^{-1}$ at the 140 and 168 kg N ha$^{-1}$. There was no trend in 2020 that may suggest any plant N benefit from Proven$^{TM}$. Estimates of N provided by Proven$^{TM}$ by academic research is not currently available.

Proven$^{TM}$ did not produce a statistically significant effect on many of the factors tested, including our most indicative response variables, yield, plant N uptake, and NUE.

Evidence for the efficacy of Proven$^{TM}$ as a source of N in this study was limited. There is potential for greater N fixation by Proven$^{TM}$ in the future with future generations or formulations of Proven$^{TM}$ or new genetic editing techniques (Bloch et al., 2020b). The progress made in increasing N fixation through genetic editing has shown significant promise and further development could yield bacteria that are able to replace synthetic fertilizers (Bloch et al., 2020a).

## Conclusions

Proven$^{TM}$ did not significantly affect many of the factors tested, including our most indicative response variables, yield, plant N uptake, and NUE. The 2019 plant N uptake had a trend for greater plant N uptake when treated with Proven$^{TM}$, but this effect was not statistically significant and was not observed in 2020 plant N uptake. Most parameters responded as expected to N rate. It is important to consider, however, that Proven$^{TM}$ was only intended to act as a source of, at most, ~30 kg N $ha^{-1}$ $yr^{-1}$. Considering that the recommended N rate for corn in this region is 168 kg N $ha^{-1}$, it is possible that the contribution from Proven$^{TM}$ was not substantial enough to cause a significant effect on the parameters tested in this study.

### Future research

More research is needed to more accurately determine the efficacy of Proven$^{TM}$ as a source of N in corn. Research that can better differentiate N provided by Proven$^{TM}$ and N provided by fertilizer, such as $^{15}N$ studies, would be particularly valuable in determining the efficacy of Proven$^{TM}$. Research focusing on the ecological benefit of Proven$^{TM}$ compared to fertilizer N is also needed to better quantify the benefits provided by Proven$^{TM}$ versus conventional N sources. These needs for more research are highlighted by Pivot Bio's projected increases in N fixation from future generations and formulations of Proven$^{TM}$. More research focusing on Proven$^{TM}$ in cereal crops with lesser N requirements than corn, such as wheat or sorghum, would also be beneficial since the N expected to be fixed by Proven$^{TM}$ would contribute a greater portion of total plant N than in crops with higher N requirements.

## Conflicts

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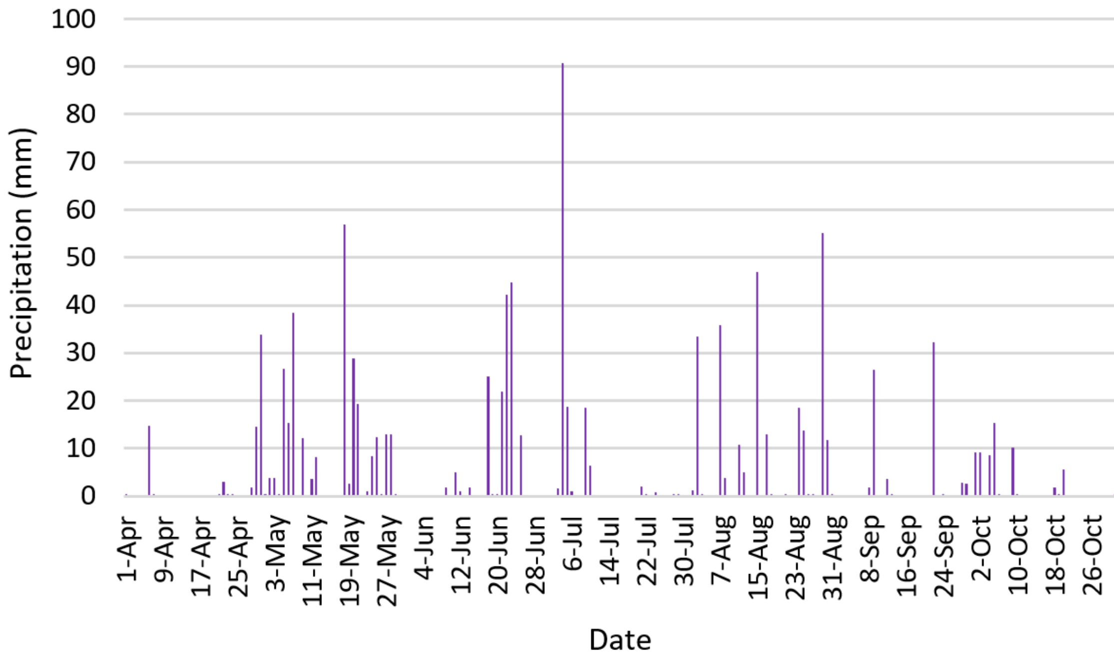


**Figure 1.1. Growing season daily precipitation, 2019.**

Compiled from Mesonet, 2021: Kansas Mesonet Historical Data. Accessed 11 August, 2021.

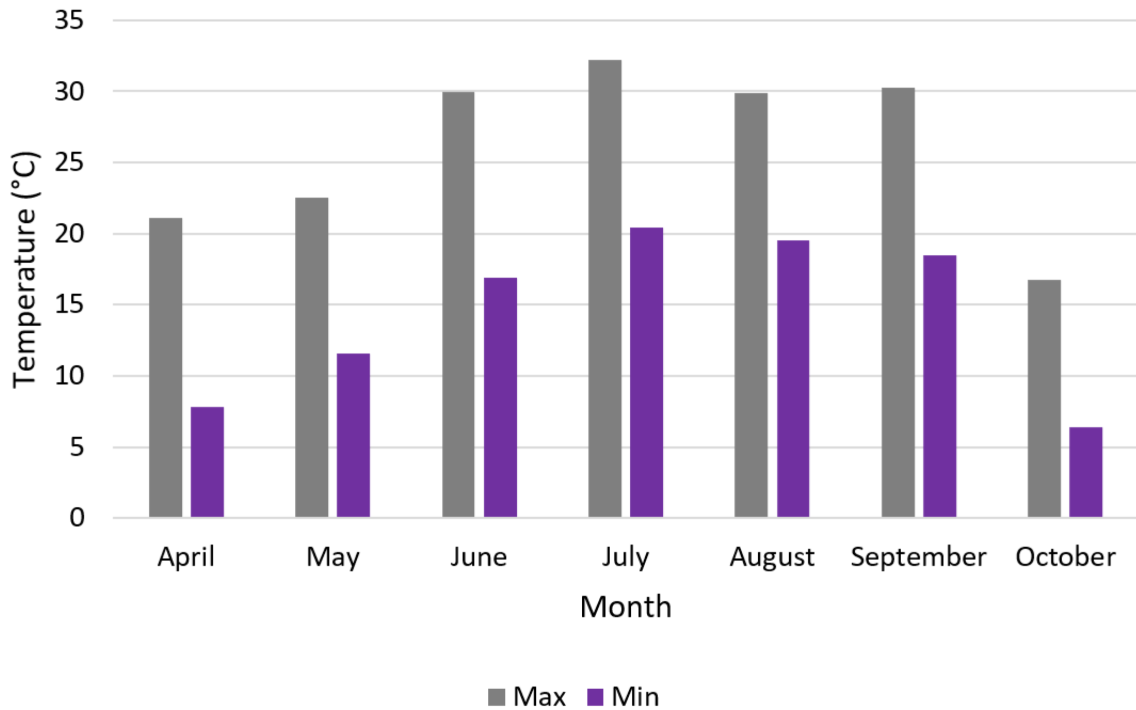


**Figure 1.2. Growing season monthly average maximum and minimum temperature, 2019.** Compiled from Mesonet, 2021: Kansas Mesonet Historical Data. Accessed 11 August, 2021.

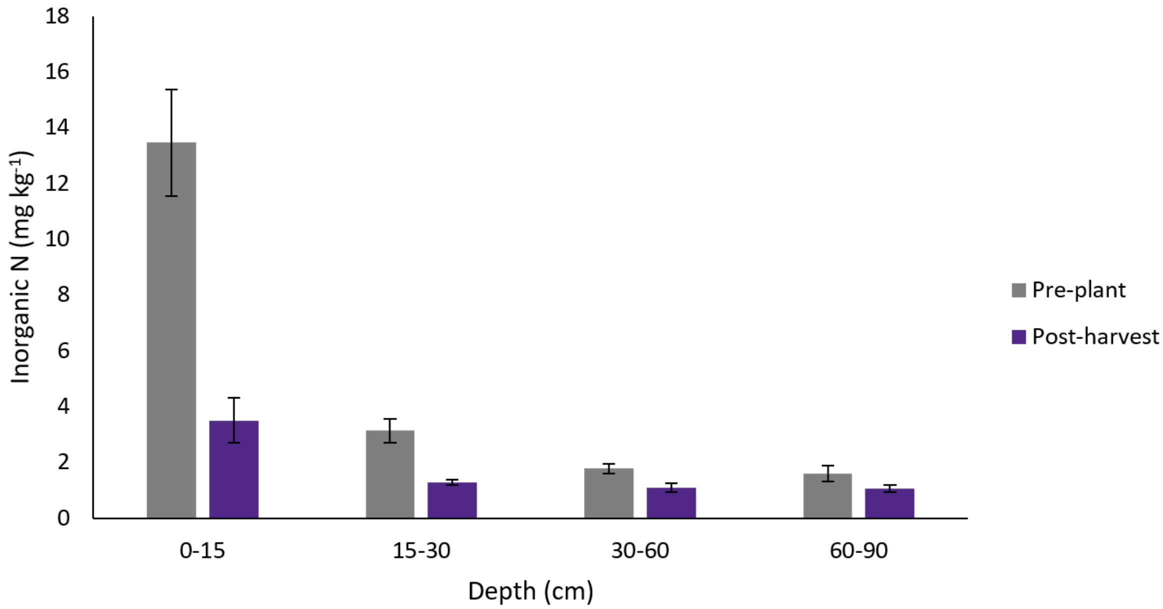


**Figure 1.3. Pre-plant and post-harvest soil inorganic N for 2019.**

Error bars represent standard error.

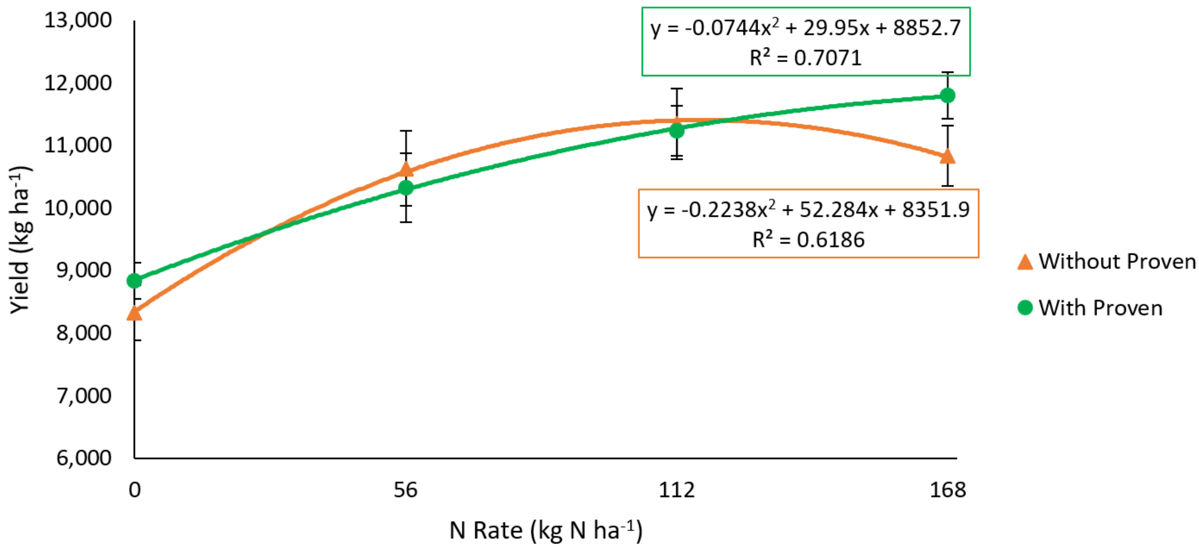


**Figure 1.4. Corn grain yield vs. N rate with and without Proven™ for 2019**

Error bars represent standard error.

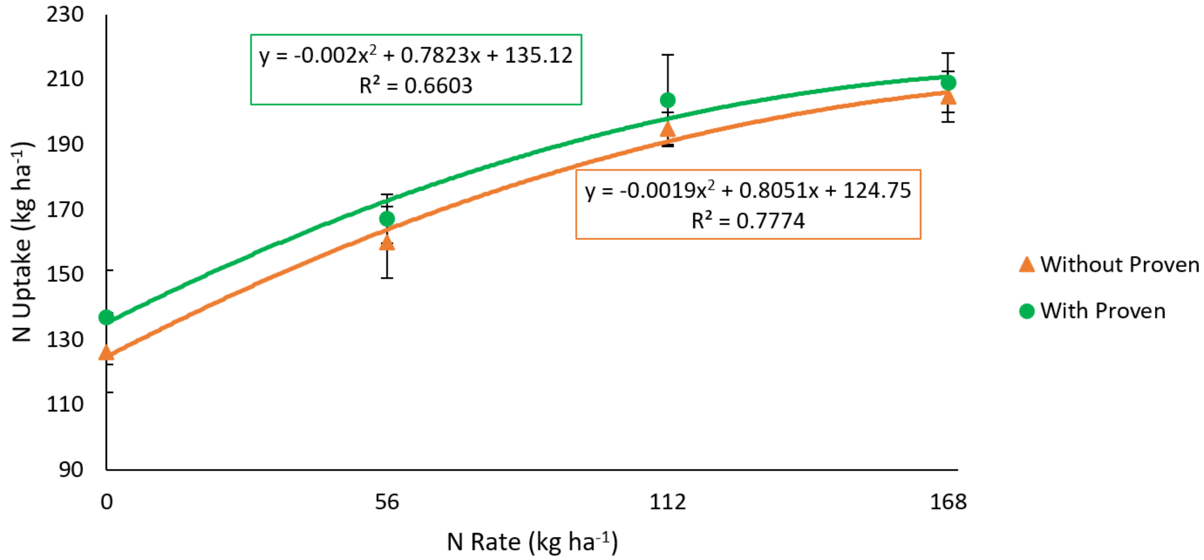


**Figure** 1.5**. Whole plant N at R6 growth stage vs. N rate with and without Proven™, 2019.** Error bars represent standard error.

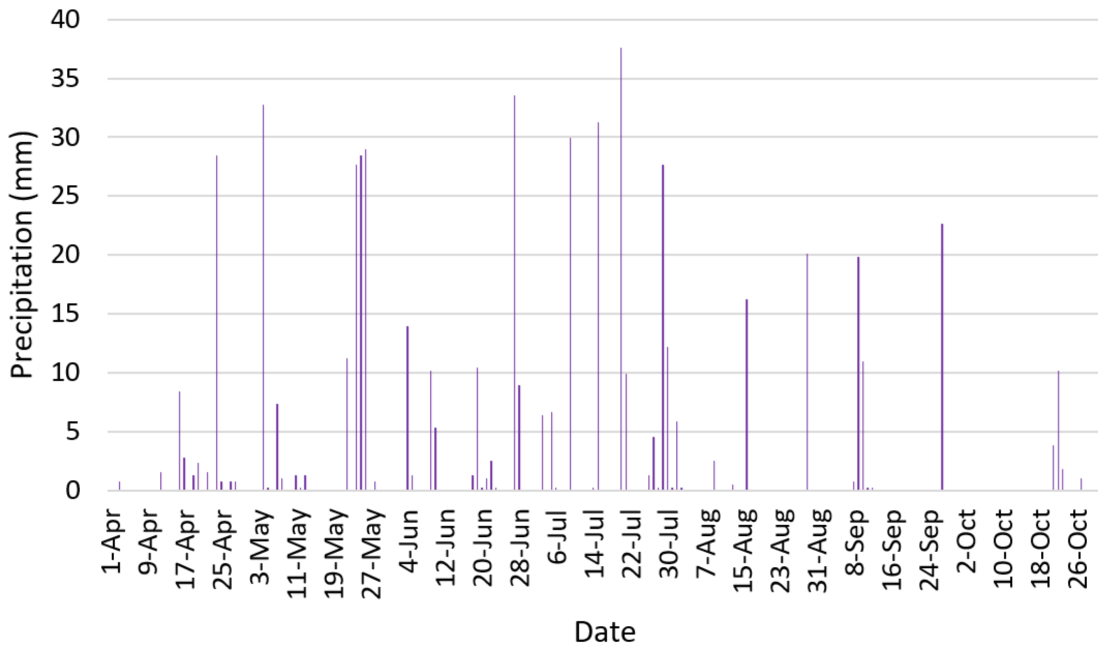


**Figure** 1.6**. Growing season daily precipitation, 2020.**

Compiled from Mesonet, 2021: Kansas Mesonet Historical Data. Accessed 11 August, 2021.

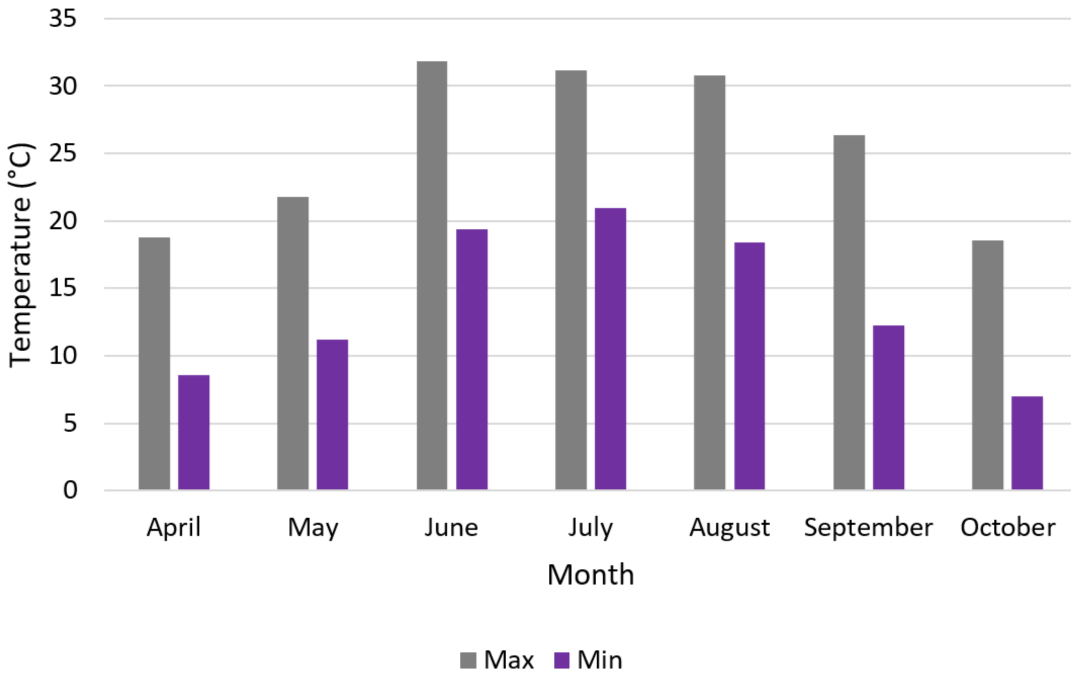


**Figure 1.7. Monthly growing season average maximum and minimum temperature, 2020.** Compiled from Mesonet, 2021: Kansas Mesonet Historical Data. Accessed 11 August, 2021.

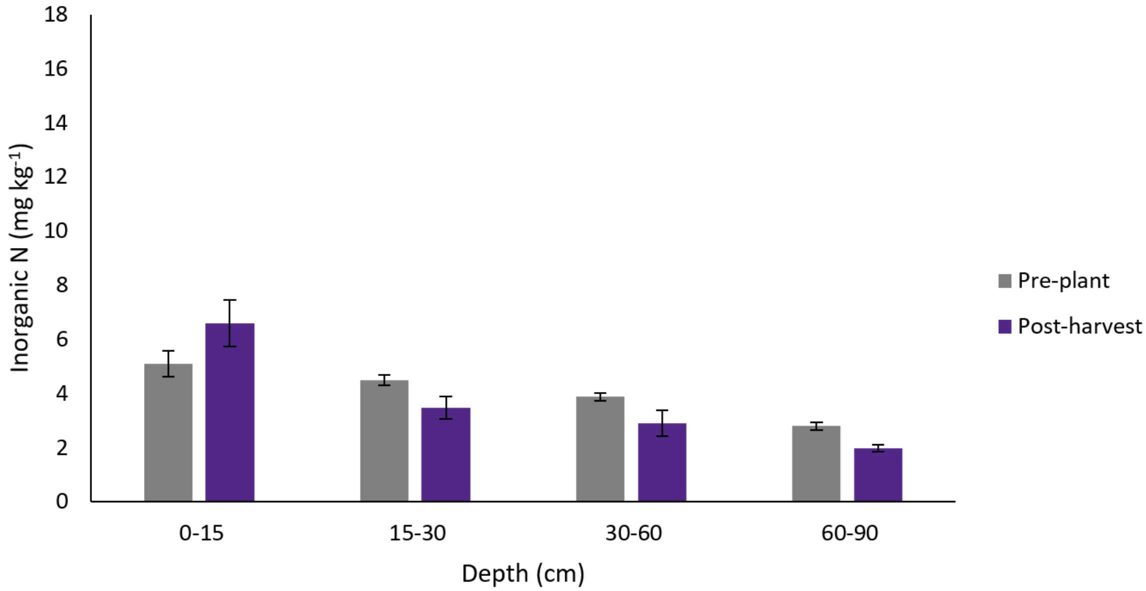


**Figure 1.8. Pre-plant and post-harvest soil inorganic N, 2020.**

Error bars represent standard error.

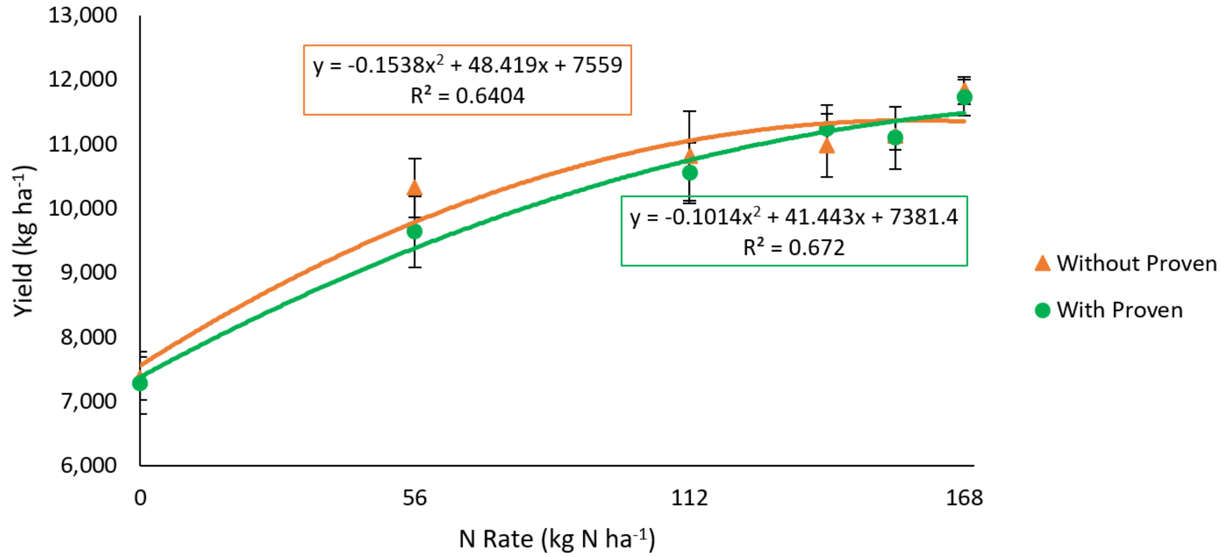


**Figure 1.9. Corn grain yield vs. N rate with and without Proven™ for 2020**

Error bars represent standard error.

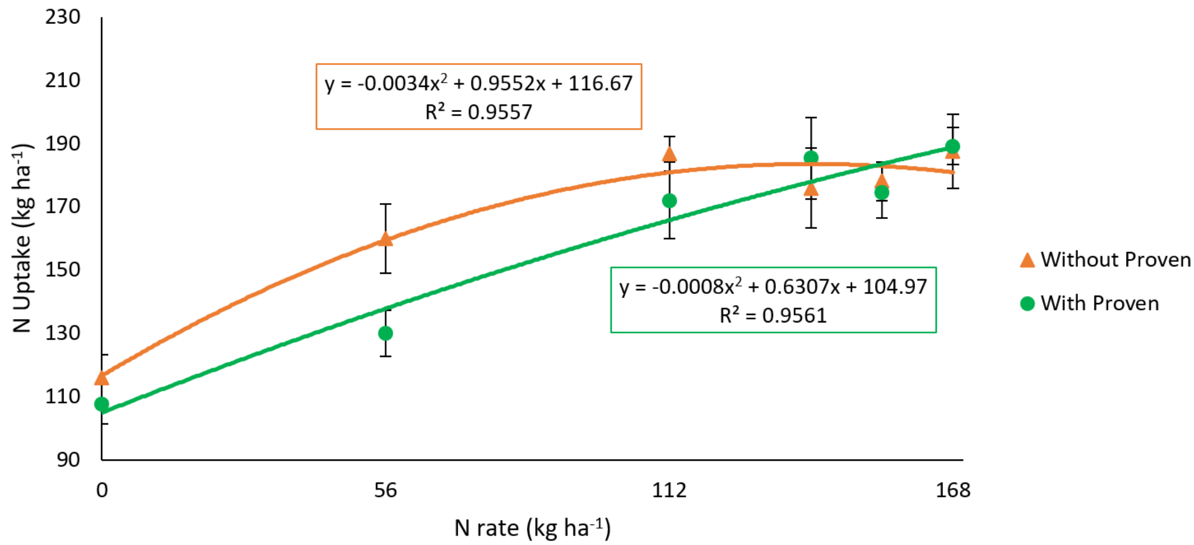


**Figure 1.10. Whole plant N at R6 with and without Proven™ in 2020.**

Error bars represent standard error.

**Table 1.1. Inorganic soil N (IN) for 0-15 and 15-30 cm at V6 and VT corn growth stages in 2019.**

| | V6 | | | | R1 | | | |
|---|---|---|---|---|---|---|---|---|
| | 0-15 cm | | 15-30 cm | | 0-15 cm | | 15-30 cm | |
| N rate (kg ha$^{-1}$) | IN (mg kg$^{-1}$) | SE | IN (mg kg$^{-1}$) | SE | IN (mg kg$^{-1}$) | SE | IN (mg kg$^{-1}$) | SE |
| 0 | 16.2 | 1.60 | 11.2 | 1.48 | 1.87 | 0.23 | 1.25 | 0.14 |
| 56 | 31.2 | 3.19 | 14.6 | 1.14 | 1.75 | 0.17 | 1.43 | 0.18 |
| 112 | 40.8 | 2.88 | 15.4 | 0.97 | 2.19 | 0.22 | 1.96 | 0.26 |
| 168 | 74.8 | 11.1 | 18.5 | 0.74 | 6.04 | 1.64 | 8.53 | 2.20 |

SE represents standard error.

**Table 1.2. 2019 Plant population by treatment.**

| N rate (kg ha$^{-1}$) | Proven™ | Spring Population (plants ha$^{-1}$) | Spring SE | Harvest Population (plants ha$^{-1}$) | Harvest SE |
|---|---|---|---|---|---|
| 0 | N | 64011 | 2135 | 61148 | 1846 |
| 0 | Y | 59087 | 2516 | 55652 | 2347 |
| 56 | N | 62980 | 1033 | 60461 | 1309 |
| 56 | Y | 55881 | 2658 | 53591 | 2951 |
| 112 | N | 63209 | 771 | 59881 | 1096 |
| 112 | Y | 57026 | 4451 | 57255 | 3402 |
| 168 | N | 61492 | 1580 | 58285 | 1128 |
| 168 | Y | 61835 | 2267 | 59774 | 2547 |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.3. NDVI by treatment for 2019.**

| | | V6 | | V7 | | V8 | |
|---|---|---|---|---|---|---|---|
| N rate (kg ha$^{-1}$) | Proven$^{TM}$ | NDVI | SE | NDVI | SE | NDVI | SE |
| 0 | N | 0.50 | 0.01 | 0.66 | 0.01 | 0.69 | 0.01 |
| 0 | Y | 0.49 | 0.02 | 0.64 | 0.02 | 0.66 | 0.03 |
| 56 | N | 0.54 | 0.01 | 0.71 | 0.01 | 0.78 | 0.01 |
| 56 | Y | 0.51 | 0.02 | 0.66 | 0.02 | 0.70 | 0.02 |
| 112 | N | 0.52 | 0.01 | 0.71 | 0.00 | 0.77 | 0.01 |
| 112 | Y | 0.50 | 0.02 | 0.68 | 0.02 | 0.73 | 0.02 |
| 168 | N | 0.51 | 0.01 | 0.70 | 0.01 | 0.76 | 0.01 |
| 168 | Y | 0.51 | 0.02 | 0.70 | 0.01 | 0.76 | 0.02 |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.4. NDVI at V7 growth stage by N rate with pairwise groups for 2019**

| N rate (kg $ha^{-1}$) | NDVI | SE | Group |
|---|---|---|---|
| 0 | 0.65 | 0.01 | b |
| 56 | 0.68 | 0.02 | ab |
| 112 | 0.69 | 0.01 | a |
| 168 | 0.70 | 0.01 | a |

SE represents standard error.

**Table 1.5. NDVI at V7 growth stage by Proven™ with pairwise groups for 2019.**

| Proven™ | NDVI | SE | Group |
|---|---|---|---|
| N | 0.70 | 0.005 | a |
| Y | 0.67 | 0.010 | b |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.6. NDVI at V8 growth stage by N rate with pairwise groups for 2019.**

| N rate (kg ha$^{-1}$) | NDVI | SE | Group |
|---|---|---|---|
| 0 | 0.68 | 0.02 | b |
| 56 | 0.74 | 0.02 | a |
| 112 | 0.75 | 0.02 | a |
| 168 | 0.76 | 0.01 | a |

SE represents standard error.

**Table 1.7. V8 NDVI at V8 growth stage by Proven$^{TM}$ with pairwise groups for 2019.**

| Proven$^{TM}$ | NDVI | SE | Group |
|---|---|---|---|
| N | 0.75 | 0.008 | a |
| Y | 0.71 | 0.012 | b |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.8. SPAD readings by treatment for 2019.**

| N rate (kg ha$^{-1}$) | Proven™ | SPAD R1 | SE | SPAD R2 | SE | SPAD R3 | SE |
|---|---|---|---|---|---|---|---|
| 0 | N | 54.0 | 1.60 | 51.0 | 1.98 | 50.4 | 2.68 |
| 0 | Y | 54.1 | 1.30 | 51.6 | 2.39 | 51.2 | 1.62 |
| 56 | N | 56.5 | 1.30 | 55.3 | 1.38 | 54.5 | 1.11 |
| 56 | Y | 57.7 | 0.98 | 56.5 | 1.60 | 56.3 | 1.81 |
| 112 | N | 59.4 | 0.83 | 58.8 | 0.85 | 58.9 | 0.59 |
| 112 | Y | 59.2 | 0.88 | 58.6 | 0.92 | 57.5 | 0.49 |
| 168 | N | 61.1 | 0.83 | 61.2 | 1.21 | 59.1 | 0.88 |
| 168 | Y | 60.0 | 0.23 | 60.3 | 1.44 | 61.6 | 1.17 |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.9. SPAD readings at R1 growth stage  by N rate with pairwise groups for 2019.**

| N rate (kg ha$^{-1}$) | SPAD | SE | Group |
|---|---|---|---|
| 0 | 54.0 | 0.96 | c |
| 56 | 57.1 | 0.79 | b |
| 112 | 59.3 | 0.56 | a |
| 168 | 60.5 | 0.45 | a |

SE represents standard error.

**Table 1.10. SPAD at R2 by N rate with pairwise groups for 2019.**

| N rate (kg ha$^{-1}$) | SPAD | SE | Group |
|---|---|---|---|
| 0 | 51.3 | 1.44 | c |
| 56 | 55.9 | 1.01 | b |
| 112 | 58.7 | 0.58 | a |
| 168 | 60.8 | 0.89 | a |

SE represents standard error.

**Table 1.11. SPAD at R3 by N rate with pairwise groups for 2019.**

| N rate (kg ha$^{-1}$) | SPAD | SE | Group |
|---|---|---|---|
| 0 | 50.8 | 1.46 | c |
| 56 | 55.4 | 1.04 | b |
| 112 | 58.2 | 0.45 | ab |
| 168 | 60.3 | 0.82 | a |

SE represents standard error.

**Table 1.12. GLC by treatment for 2019.**

| N rate (kg $ha^{-1}$) | Proven™ | GLC R3 | SE | GLC R3-2 | SE | GLC R4 | SE |
|---|---|---|---|---|---|---|---|
| 0 | N | 11.6 | 0.19 | 11.3 | 0.18 | 11.1 | 0.15 |
| 0 | Y | 11.8 | 0.18 | 11.5 | 0.18 | 11.4 | 0.17 |
| 56 | N | 12.1 | 0.13 | 11.6 | 0.17 | 11.5 | 0.19 |
| 56 | Y | 12.5 | 0.15 | 11.8 | 0.17 | 11.3 | 0.15 |
| 112 | N | 12.5 | 0.12 | 12.4 | 0.13 | 12.0 | 0.12 |
| 112 | Y | 12.4 | 0.12 | 12.0 | 0.15 | 12.0 | 0.12 |
| 168 | N | 13.0 | 0.12 | 12.5 | 0.14 | 12.2 | 0.11 |
| 168 | Y | 12.6 | 0.16 | 12.4 | 0.14 | 12.3 | 0.10 |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.13. GLC at R3 by treatment with pairwise groups for 2019.**

| N rate (kg $ha^{-1}$) | Proven™ | GLC | SE | Group |
|---|---|---|---|---|
| 0 | N | 11.6 | 0.19 | e |
| 0 | Y | 11.8 | 0.18 | de |
| 56 | N | 12.1 | 0.13 | cde |
| 56 | Y | 12.5 | 0.15 | ab |
| 112 | N | 12.5 | 0.12 | abcd |
| 112 | Y | 12.4 | 0.12 | abcd |
| 168 | N | 13.0 | 0.12 | a |
| 168 | Y | 12.6 | 0.16 | bc |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.14. GLC at R3-2 by N rate with pairwise groups for 2019.**

| N rate (kg $ha^{-1}$) | GLC | SE | Group |
|---|---|---|---|
| 0 | 11.4 | 0.13 | c |
| 56 | 11.7 | 0.12 | bc |
| 112 | 12.2 | 0.10 | ab |
| 168 | 12.5 | 0.10 | a |

SE represents standard error.

**Table 1.15. GLC at R4 by N rate with pairwise groups for 2019.**

| N rate (kg ha$^{-1}$) | GLC | SE | Group |
|---|---|---|---|
| 0 | 11.2 | 0.12 | c |
| 56 | 11.4 | 0.12 | bc |
| 112 | 12.0 | 0.09 | ab |
| 168 | 12.3 | 0.08 | a |

SE represents standard error.

**Table 1.16. Mineralized N by treatment for 2019.**

| Proven™ | Mineralized N (kg $ha^{-1}$) | SE |
|---|---|---|
| N | 76.1 | 16.1 |
| Y | 94.5 | 7.25 |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.17. NARE by treatment for 2019.**

| N rate (kg $ha^{-1}$) | Proven$^{TM}$ | NARE (kg N kg $N^{-1}$) | SE |
|---|---|---|---|
| 56 | N | 0.60 | 0.35 |
| 56 | Y | 0.73 | 0.32 |
| 112 | N | 0.61 | 0.08 |
| 112 | Y | 0.69 | 0.09 |
| 168 | N | 0.47 | 0.08 |
| 168 | Y | 0.49 | 0.09 |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.18. TNBI by treatment for 2019.**

| N rate (kg ha$^{-1}$) | Proven$^{TM}$ | TNBI (kg N) | SE |
|---|---|---|---|
| 0 | N | 50.0 | 18.3 |
| 0 | Y | 31.5 | 13.9 |
| 56 | N | 48.7 | 23.6 |
| 56 | Y | 51.4 | 24.2 |
| 112 | N | 58.6 | 12.0 |
| 112 | Y | 59.4 | 12.6 |
| 168 | N | 118 | 16.8 |
| 168 | Y | 117 | 12.7 |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.19. TNBI by N rate with pairwise groups for 2019.**

| N rate (kg ha$^{-1}$) | TNBI (kg N) | SE | Group |
|---|---|---|---|
| 0 | 40.8 | 11.2 | b |
| 56 | 50.1 | 15.7 | b |
| 112 | 59.0 | 8.05 | b |
| 168 | 117 | 9.72 | a |

SE represents standard error.

**Table 1.20. NRE by N rate with pairwise groups for 2019.**

| N rate (kg ha$^{-1}$) | NRE (kg N kg N$^{-1}$) | SE | group |
|---|---|---|---|
| 56 | 2.92 | 0.113 | a |
| 112 | 1.78 | 0.063 | b |
| 168 | 1.23 | 0.033 | c |

SE represents standard error.

**Table 1.21. NRUE by N rate with pairwise groups for 2019.**

| N rate (kg ha$^{-1}$) | NRUE (kg N kg N$^{-1}$) | SE | group |
|---|---|---|---|
| 56 | 4.17 | 0.30 | a |
| 112 | 2.46 | 0.30 | b |
| 168 | 1.47 | 0.11 | b |

SE represents standard error.

**Table 1.22. NRUE by Proven$^{TM}$ with pairwise groups for 2019.**

| Proven$^{TM}$ | NRUE (kg N kg $N^{-1}$) | SE | group |
|---|---|---|---|
| N | 2.92 | 0.31 | a |
| Y | 2.48 | 0.23 | a |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.23. NAE by treatment for 2019.**

| N rate (kg $ha^{-1}$) | Proven™ | NAE (kg kg $N^{-1}$) | SE |
|---|---|---|---|
| 56 | N | 41.1 | 18.0 |
| 56 | Y | 35.6 | 13.5 |
| 112 | N | 26.9 | 5.19 |
| 112 | Y | 26.0 | 6.18 |
| 168 | N | 14.9 | 5.28 |
| 168 | Y | 20.6 | 4.62 |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.24. PFP by N rate with Pairwise groups for 2019.**

| N rate (kg $ha^{-1}$) | PFP (kg kg $N^{-1}$) | SE | group |
|---|---|---|---|
| 56 | 187.1841 | 6.80946 | a |
| 112 | 100.8276 | 2.857356 | b |
| 168 | 67.36852 | 1.985557 | c |

SE represents standard error.

**Table 1.25. NUEcrop by N rate with pairwise groups for 2019.**

| N rate (kg $ha^{-1}$) | NUEcrop (kg N kg $N^{-1}$) | SE | group |
|---|---|---|---|
| 56 | 1.90 | 0.091 | a |
| 112 | 1.10 | 0.050 | b |
| 168 | 0.82 | 0.030 | c |

SE represents standard error.

**Table 1.26. Inorganic soil N for 0-15 and 15-30 cm at the V8 stage of corn growth for 2020.**

| | V8 | | | |
|---|---|---|---|---|
| | 0-15 cm | | 15-30 cm | |
| N rate ($kg\ ha^{-1}$) | IN ($mg\ kg^{-1}$) | SE | IN ($mg\ kg^{-1}$) | SE |
| 0 | 12.6 | 1.01 | 5.17 | 1.09 |
| 56 | 18.6 | 2.25 | 11.3 | 3.99 |
| 112 | 41.0 | 5.18 | 10.6 | 1.03 |
| 140 | 36.2 | 2.43 | 11.7 | 1.37 |
| 154 | 45.7 | 5.53 | 15.0 | 1.50 |
| 168 | 44.3 | 5.33 | 17.2 | 2.12 |

SE represents standard error.

**Table 1.27. Plant population by treatment for 2020.**

| N rate (kg ha$^{-1}$) | Proven™ | Spring population/ha | Spring SE | Harvest population/ha | Harvest SE |
|---|---|---|---|---|---|
| 0 | N | 69626 | 818 | 64763 | 818 |
| 0 | Y | 70887 | 436 | 63776 | 456 |
| 56 | N | 71565 | 300 | 65122 | 393 |
| 56 | Y | 71080 | 508 | 64494 | 490 |
| 112 | N | 70790 | 776 | 65032 | 292 |
| 112 | Y | 70111 | 360 | 64314 | 633 |
| 140 | N | 71081 | 461 | 64852 | 303 |
| 140 | Y | 71468 | 461 | 64583 | 393 |
| 154 | N | 70984 | 425 | 64583 | 652 |
| 154 | Y | 70693 | 515 | 64583 | 556 |
| 168 | N | 71081 | 461 | 64852 | 807 |
| 168 | Y | 69723 | 679 | 65122 | 481 |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.28. ELPH by treatment with pairwise groups for 2020.**

| N rate (kg $ha^{-1}$) | Proven™ | ELPH (cm) | SE | Group |
|---|---|---|---|---|
| 0 | N | 76.5 | 0.69 | f |
| 0 | Y | 72.8 | 0.66 | g |
| 56 | N | 78.5 | 0.58 | ef |
| 56 | Y | 78.7 | 0.71 | def |
| 112 | N | 81.3 | 0.78 | ab |
| 112 | Y | 79.5 | 0.60 | cde |
| 140 | N | 81.2 | 0.72 | abc |
| 140 | Y | 80.9 | 0.56 | abcd |
| 154 | N | 81.0 | 0.74 | abc |
| 154 | Y | 82.3 | 0.79 | ab |
| 168 | N | 83.2 | 0.69 | a |
| 168 | Y | 81.0 | 0.55 | bcd |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.29. NDVI by treatment for 2020.**

| N rate (kg $ha^{-1}$) | Proven$^{TM}$ | NDVI V7 | SE | NDVI V9 | SE |
|---|---|---|---|---|---|
| 0 | N | 0.67 | 0.011 | 0.78 | 0.005 |
| 0 | Y | 0.66 | 0.009 | 0.78 | 0.005 |
| 56 | N | 0.71 | 0.008 | 0.81 | 0.005 |
| 56 | Y | 0.70 | 0.009 | 0.80 | 0.004 |
| 112 | N | 0.70 | 0.007 | 0.80 | 0.004 |
| 112 | Y | 0.71 | 0.009 | 0.80 | 0.003 |
| 140 | N | 0.71 | 0.008 | 0.80 | 0.003 |
| 140 | Y | 0.72 | 0.008 | 0.81 | 0.004 |
| 154 | N | 0.71 | 0.005 | 0.80 | 0.003 |
| 154 | Y | 0.71 | 0.008 | 0.80 | 0.004 |
| 168 | N | 0.71 | 0.009 | 0.80 | 0.003 |
| 168 | Y | 0.72 | 0.008 | 0.81 | 0.003 |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.30. NDVI at V7 by N rate with pairwise groups for 2020.**

| N rate (kg ha$^{-1}$) | NDVI | SE | Group |
|---|---|---|---|
| 0 | 0.67 | 0.007 | b |
| 56 | 0.70 | 0.006 | a |
| 112 | 0.71 | 0.005 | a |
| 140 | 0.72 | 0.006 | a |
| 154 | 0.71 | 0.005 | a |
| 168 | 0.72 | 0.006 | a |

SE represents standard error.

**Table 1.31. NDVI at V9 by N rate with pairwise groups for 2020.**

| N rate (kg ha$^{-1}$) | NDVI | SE | Group |
|---|---|---|---|
| 0 | 0.78 | 0.004 | b |
| 56 | 0.80 | 0.003 | a |
| 112 | 0.80 | 0.002 | a |
| 140 | 0.81 | 0.003 | a |
| 154 | 0.80 | 0.002 | a |
| 168 | 0.81 | 0.002 | a |

SE represents standard error.

**Table 1.32. SPAD by treatment for 2020.**

| N rate (kg $ha^{-1}$) | Proven™ | SPAD R1 | SE | SPAD R2 | SE | SPAD R4 | SE |
|---|---|---|---|---|---|---|---|
| 0 | N | 51.9 | 1.07 | 52.7 | 1.35 | 53.4 | 1.06 |
| 0 | Y | 50.3 | 1.17 | 50.2 | 0.63 | 50.2 | 1.54 |
| 56 | N | 58.1 | 1.01 | 59.5 | 0.62 | 57.9 | 0.93 |
| 56 | Y | 57.0 | 1.01 | 57.4 | 1.12 | 56.2 | 1.02 |
| 112 | N | 61.1 | 1.51 | 61.2 | 1.03 | 58.5 | 0.53 |
| 112 | Y | 60.8 | 0.71 | 60.4 | 1.78 | 60.0 | 1.52 |
| 140 | N | 60.3 | 0.76 | 60.8 | 1.09 | 59.1 | 0.45 |
| 140 | Y | 60.1 | 1.28 | 61.2 | 0.82 | 59.8 | 0.90 |
| 154 | N | 61.3 | 0.99 | 61.1 | 1.36 | 58.7 | 0.99 |
| 154 | Y | 61.2 | 1.40 | 61.4 | 1.52 | 58.7 | 0.86 |
| 168 | N | 59.4 | 0.93 | 62.8 | 1.24 | 59.4 | 1.06 |
| 168 | Y | 60.9 | 0.53 | 60.2 | 0.62 | 60.2 | 0.82 |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.33. SPAD at R1 by N rate with pairwise groups for 2020.**

| N rate (kg $ha^{-1}$) | SPAD | SE | Group |
|---|---|---|---|
| 0 | 51.1 | 0.80 | c |
| 56 | 57.6 | 0.70 | b |
| 112 | 60.9 | 0.80 | a |
| 140 | 60.2 | 0.71 | a |
| 154 | 61.3 | 0.82 | a |
| 168 | 60.2 | 0.56 | a |

SE represents standard error.

**Table 1.34. SPAD at R2 by N rate with pairwise groups for 2020.**

| N rate (kg ha$^{-1}$) | SPAD | SE | Group |
|---|---|---|---|
| 0 | 51.5 | 0.80 | c |
| 56 | 58.5 | 0.69 | b |
| 112 | 60.8 | 0.99 | a |
| 140 | 61.0 | 0.65 | a |
| 154 | 61.2 | 0.97 | a |
| 168 | 61.5 | 0.77 | a |

SE represents standard error.

**Table 1.35. SPAD at R4 by N rate with pairwise groups for 2020.**

| N rate (kg $ha^{-1}$) | SPAD | SE | Group |
|---|---|---|---|
| 0 | 51.8 | 1.01 | c |
| 56 | 57.1 | 0.70 | b |
| 112 | 59.3 | 0.80 | a |
| 140 | 59.4 | 0.49 | a |
| 154 | 58.7 | 0.62 | ab |
| 168 | 59.8 | 0.65 | a |

SE represents standard error.

**Table 1.36. GLC by treatment for 2020.**

| N rate (kg ha$^{-1}$) | Proven™ | GLC R2 | SE | GLC R4 | SE | GLC R5 | SE |
|---|---|---|---|---|---|---|---|
| 0 | N | 10.8 | 0.14 | 9.78 | 0.14 | 9.83 | 0.10 |
| 0 | Y | 10.5 | 0.14 | 9.73 | 0.13 | 9.50 | 0.10 |
| 56 | N | 11.4 | 0.14 | 9.95 | 0.13 | 9.90 | 0.10 |
| 56 | Y | 11.6 | 0.12 | 10.3 | 0.13 | 9.75 | 0.10 |
| 112 | N | 11.8 | 0.13 | 10.1 | 0.14 | 9.82 | 0.10 |
| 112 | Y | 11.8 | 0.10 | 10.6 | 0.15 | 9.80 | 0.09 |
| 140 | N | 12.0 | 0.10 | 10.1 | 0.23 | 9.72 | 0.11 |
| 140 | Y | 11.7 | 0.13 | 10.2 | 0.16 | 9.68 | 0.11 |
| 154 | N | 12.1 | 0.12 | 10.4 | 0.15 | 9.87 | 0.08 |
| 154 | Y | 11.6 | 0.16 | 10.1 | 0.15 | 9.82 | 0.11 |
| 168 | N | 11.8 | 0.17 | 10.4 | 0.14 | 9.90 | 0.10 |
| 168 | Y | 12.1 | 0.13 | 10.3 | 0.13 | 10.0 | 0.09 |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.37. GLC at R2 by treatment with pairwise groups for 2020.**

| N rate (kg $ha^{-1}$) | Proven$^{TM}$ | GLC R2 | SE | Group |
|---|---|---|---|---|
| 0 | N | 10.8 | 0.14 | e |
| 0 | Y | 10.4 | 0.14 | f |
| 56 | N | 11.4 | 0.14 | cde |
| 56 | Y | 11.6 | 0.12 | abcde |
| 112 | N | 11.8 | 0.13 | abcd |
| 112 | Y | 11.8 | 0.10 | abcd |
| 140 | N | 12.0 | 0.10 | abcd |
| 140 | Y | 11.7 | 0.13 | abcde |
| 154 | N | 12.1 | 0.12 | ab |
| 154 | Y | 11.6 | 0.16 | cde |
| 168 | N | 11.8 | 0.17 | b de |
| 168 | Y | 12.1 | 0.13 | a c |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.38. GLC at R4 by treatment with pairwise groups for 2020.**

| N rate (kg $ha^{-1}$) | Proven™ | GLC R4 | SE | Group |
|---|---|---|---|---|
| 0 | N | 9.78 | 0.14 | bc |
| 0 | Y | 9.73 | 0.13 | c |
| 56 | N | 9.95 | 0.13 | bc |
| 56 | Y | 10.3 | 0.13 | ab |
| 112 | N | 10.1 | 0.14 | bc |
| 112 | Y | 10.6 | 0.15 | a |
| 140 | N | 10.1 | 0.23 | abc |
| 140 | Y | 10.2 | 0.16 | abc |
| 154 | N | 10.4 | 0.15 | ab |
| 154 | Y | 10.1 | 0.15 | bc |
| 168 | N | 10.4 | 0.14 | ab |
| 168 | Y | 10.3 | 0.13 | ab |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.39. EH by treatment for 2020.**

| N rate (kg ha$^{-1}$) | Proven$^{TM}$ | EH (cm) | SE |
|---|---|---|---|
| 0 | N | 77.5 | 0.99 |
| 0 | Y | 75.8 | 0.98 |
| 56 | N | 82.4 | 1.10 |
| 56 | Y | 80.6 | 0.96 |
| 112 | N | 81.6 | 1.04 |
| 112 | Y | 84.2 | 1.04 |
| 140 | N | 83.8 | 0.99 |
| 140 | Y | 85.3 | 1.14 |
| 154 | N | 85.4 | 0.92 |
| 154 | Y | 83.5 | 1.26 |
| 168 | N | 85.0 | 1.06 |
| 168 | Y | 84.0 | 1.37 |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.40. EH by N rate with pairwise groups for 2020.**

| N rate (kg $ha^{-1}$) | EH | SE | Group |
|---|---|---|---|
| 0 | 76.7 | 0.70 | b |
| 56 | 81.5 | 0.73 | a |
| 112 | 82.9 | 0.74 | a |
| 140 | 84.5 | 0.75 | a |
| 154 | 84.5 | 0.78 | a |
| 168 | 84.5 | 0.86 | a |

SE represents standard error.

**Table 1.41. In-season WPN by treatment for 2020.**

| N rate (kg ha$^{-1}$) | Proven$^{TM}$ | WPN V8 (kg ha$^{-1}$) | SE | WPN VT (kg ha$^{-1}$) | SE |
|---|---|---|---|---|---|
| 0 | N | 42.6 | 2.75 | 73.4 | 4.94 |
| 0 | Y | 37.7 | 2.20 | 69.9 | 4.78 |
| 56 | N | 53.0 | 4.39 | 107 | 7.76 |
| 56 | Y | 46.2 | 2.78 | 83.8 | 6.18 |
| 112 | N | 57.9 | 3.83 | 129 | 5.87 |
| 112 | Y | 59.5 | 2.26 | 112 | 10.8 |
| 140 | N | 57.9 | 3.49 | 118 | 12.4 |
| 140 | Y | 61.1 | 4.77 | 124 | 8.82 |
| 154 | N | 57.3 | 3.68 | 121 | 2.81 |
| 154 | Y | 56.8 | 4.86 | 118 | 7.76 |
| 168 | N | 66.4 | 6.66 | 121 | 9.97 |
| 168 | Y | 58.2 | 1.48 | 131 | 4.86 |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.42. WPN at V8 by N rate with pairwise groups for 2020.**

| N rate (kg $ha^{-1}$) | WPN (kg $ha^{-1}$) | SE | Group |
|---|---|---|---|
| 0 | 40.2 | 1.83 | c |
| 56 | 49.6 | 2.68 | b |
| 112 | 58.7 | 2.13 | a |
| 140 | 59.5 | 2.86 | a |
| 154 | 57.0 | 2.91 | ab |
| 168 | 62.2 | 3.48 | a |

SE represents standard error.

**Table 1.43. WPN at VT by N rate with pairwise groups for 2020.**

| N rate (kg $ha^{-1}$) | WPN (kg $ha^{-1}$) | SE | Group |
|---|---|---|---|
| 0 | 71.7 | 3.32 | c |
| 56 | 95.4 | 5.88 | b |
| 112 | 121 | 6.34 | a |
| 140 | 121 | 7.30 | a |
| 154 | 119 | 3.96 | a |
| 168 | 126 | 5.49 | a |

SE represents standard error.

**Table 1.44. Mineralized N by treatment for 2020.**

| Proven$^{TM}$ | Mineralized N (kg ha$^{-1}$) | SE |
|---|---|---|
| N | 94.8 | 8.09 |
| Y | 82.9 | 9.88 |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.45. NARE by treatment with pairwise groups for 2020.**

| N rate (kg $ha^{-1}$) | Proven$^{TM}$ | NARE (kg N kg $N^{-1}$) | SE | Group |
|---|---|---|---|---|
| 56 | N | 0.79 | 0.30 | a |
| 56 | Y | 0.25 | 0.18 | c |
| 112 | N | 0.63 | 0.07 | ab |
| 112 | Y | 0.50 | 0.07 | abc |
| 140 | N | 0.43 | 0.11 | bc |
| 140 | Y | 0.50 | 0.12 | abc |
| 154 | N | 0.40 | 0.07 | bc |
| 154 | Y | 0.38 | 0.03 | bc |
| 168 | N | 0.43 | 0.05 | bc |
| 168 | Y | 0.44 | 0.05 | bc |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.46. TNBI by treatment with pairwise groups for 2020.**

| N rate (kg ha$^{-1}$) | Proven™ | TNBI (kg N) | SE | Group |
|---|---|---|---|---|
| 0 | N | 21.2 | 5.90 | e |
| 0 | Y | 33.1 | 9.07 | de |
| 56 | N | 30.5 | 17.2 | de |
| 56 | Y | 62.5 | 14.3 | bcde |
| 112 | N | 59.9 | 9.81 | bcde |
| 112 | Y | 69.7 | 5.50 | abcd |
| 140 | N | 93.0 | 12.6 | ab |
| 140 | Y | 93.9 | 17.6 | ab |
| 154 | N | 82.6 | 18.7 | abc |
| 154 | Y | 92.0 | 9.40 | ab |
| 168 | N | 111 | 11.4 | a |
| 168 | Y | 41.7 | 37.6 | cde |

Y indicates the presence of Proven™ and N indicates that no Proven™ was applied. SE represents standard error.

**Table 1.47. NRE by N rate and Proven$^{TM}$ interaction with pairwise groups for 2020.**

| N rate (kg ha$^{-1}$) | Proven$^{TM}$ | NRE (kg N kg N$^{-1}$) | SE | group |
|---|---|---|---|---|
| 56 | N | 2.86 | 0.20 | a |
| 56 | Y | 2.32 | 0.13 | b |
| 112 | N | 1.67 | 0.05 | c |
| 112 | Y | 1.54 | 0.11 | cd |
| 140 | N | 1.26 | 0.09 | e |
| 140 | Y | 1.32 | 0.09 | de |
| 154 | N | 1.16 | 0.04 | e |
| 154 | Y | 1.13 | 0.05 | e |
| 168 | N | 1.12 | 0.07 | e |
| 168 | Y | 1.13 | 0.03 | e |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.48. NRUE by treatment for 2020.**

| N rate (kg ha$^{-1}$) | Proven$^{TM}$ | NRUE (kg N kg N$^{-1}$) | SE |
|---|---|---|---|
| 56 | N | -51.3 | 59.2 |
| 56 | Y | 4.84 | 0.55 |
| 112 | N | 2.39 | 0.14 |
| 112 | Y | 2.22 | 0.14 |
| 140 | N | 1.79 | 0.06 |
| 140 | Y | 1.75 | 0.14 |
| 154 | N | 5.22 | 3.62 |
| 154 | Y | 1.57 | 0.04 |
| 168 | N | 1.51 | 0.15 |
| 168 | Y | 2.89 | 3.59 |

Y indicates the presence of Proven$^{TM}$ and N indicates that no Proven$^{TM}$ was applied. SE represents standard error.

**Table 1.49. NAE by N rate with pairwise groups for 2020.**

| N rate (kg $ha^{-1}$) | NAE (kg kg $N^{-1}$) | SE | group |
|---|---|---|---|
| 56 | 44.3 | 5.33 | a |
| 112 | 28.4 | 3.21 | b |
| 140 | 25.8 | 2.28 | b |
| 154 | 23.5 | 2.31 | b |
| 168 | 25.5 | 1.45 | b |

SE represents standard error.

**Table 1.50. PFP by N rate with pairwise groups for 2020.**

| N rate (kg ha$^{-1}$) | PFP (kg kg N$^{-1}$) | SE | group |
|---|---|---|---|
| 56 | 178 | 6.36 | a |
| 112 | 95.4 | 3.60 | b |
| 140 | 79.3 | 2.10 | c |
| 154 | 72.2 | 1.65 | c |
| 168 | 70.1 | 0.99 | c |

SE represents standard error.

**Table 1.51. NUEcrop by N rate with pairwise groups for 2020.**

| N rate (kg ha$^{-1}$) | NUEcrop (kg N kg N$^{-1}$) | SE | group |
|---|---|---|---|
| 56 | 1.87 | 0.09 | a |
| 112 | 1.074 | 0.06 | b |
| 140 | 0.91 | 0.03 | bc |
| 154 | 0.88 | 0.02 | c |
| 168 | 0.87 | 0.02 | c |

SE represents standard error.

# Chapter 2 - Proven™ as a nitrogen source in sorghum

## Introduction

### Role of Nitrogen in Agronomy

Nitrogen (N) is an element that is essential to the growth and development of all plant life (Robertson & Groffman, 2015). Because of its importance to plant growth, supplemental N is required for the optimization of crop productivity in most agronomic situations (Rice, Havlin, & Schepers, 1995). This supplemental N, most often in the form of N fertilizer, is subject to loss via several pathways (leaching, denitrification, volatilization) (Bloch et al., 2020b; Ribaudo et al., 2011). The reason N is subject to such great losses is that it is usually applied in large quantities at the beginning of the growing season, which the plant is not immediately able to consume. This means there is soil inorganic N that is not assimilated for ~6 weeks which is subject to losses (Rice et al., 1995; Robertson & Groffman, 2015). This loss of N fertilizer is not only a loss of costly resources for the farmer (Mus et al., 2016), but also pose environmental risks to water, the atmosphere, and non-agricultural ecosystems (Bloch et al., 2020b; Ribaudo et al., 2011).

### Biological N Fixation

One alternative source of N, which was the dominant form of N deposition until the advent of industrial N production, is biological N fixation (Bottomley & Myrold, 2015; Morris, 2018). This is a process by which microbes convert atmospheric $N_2$ to $NH_3$, which is then rapidly converted to $NH_4^+$ in the soil and available for plant uptake (Mus et al., 2016). Biological N fixation is used in legume production (Mus et al., 2016). Legumes have a symbiotic relationship with a bacteria known as Rhizobia, which colonizes legume roots and fix N, which is then available to the plant (Provorov, 1997). The advantage of biologically fixed N is that it is supplied throughout the growing season near or on crop roots, meaning that N is taken up by the plant with little residual N (Bloch et al., 2020a). Because of this decreased risk of loss, biologically fixed N represents a more efficient use of N  (Bloch et al., 2020a). Cereal crops do not have a well-established symbiotic relationship with an N fixing bacteria as legumes (Bloch et al. 2020b). There are some naturally occurring root-associated N fixing bacteria that colonize the rhizosphere of cereal crops. However, these microbes tend to stop fixing N in the presence of high inorganic N in the soil, limiting their efficacy in agronomic situations (Bloch et al., 2020a; Bottomley & Myrold, 2015). Finding a microbe that can fix atmospheric N in cereal crop roots has been proposed as a way of mitigating N loss and promoting NUE in agronomic systems (Bloch et al., 2020b).

## Proven™

Proven™ is a gene-edited bacterial inoculant derived from *Kelbsiella variicola* which is intended to fix atmospheric N on cereal crop roots. It is reported to fix approximately 30 kg N $ha^{-1}$ $yr^{-1}$. Because Proven™ is only expected to fix 30 kg N $ha^{-1}$ $yr^{-1}$, supplemental N from other sources will be required to maximize plant productivity for most cereal crops. The N provided by Proven™ is biologically fixed during the growing season, thus less subject to loss and increased NUE in cereal crops (Bloch et al., 2020b).

Because of the significant advantages of achieving biological N fixation in cereal crops and the novelty of Proven™ as an early gene-edited diazotrophic inoculant, it is important to understand the efficacy of Proven™ as a N source. The objectives of this study were to (i) investigate the N benefit derived from Proven$^{TM}$ in sorghum, and (ii) investigate the affect of Proven$^{TM}$ on NUE in sorghum. Proven$^{TM}$ is expected to contribute ~20-33 kg N ha$^{-1}$ yr$^{-1}$ (as reported by Pivot Bio) and that the presence of Proven$^{TM}$ will increase NUE.

## Materials and Methods

### Site Description and Experimental Design

This experiment occurred during the 2020 growing season at the Kansas State University Agronomy Farm at Ashland Bottoms. The experimental design was a split-plot randomized complete block design. The dominant soil series was a Wymore silty clay loam (Soil classification). The previous crop was soybean. In 2020 five N rates (0, 34, 67, 101, and 135 kg N ha$^{-1}$) and six replications were included in the study. The individual plot size was 3.05 m by 29.9 m. The sorghum variety Pioneer 84P68 was planted at 148,262 seeds ha$^{-1}$.

### Management

N was applied via tractor in the form of urea ammonium-nitrate (UAN) before Proven$^{TM}$ was applied in-furrow at planting at a rate of 0.935 L ha$^{-1}$ (as directed). No P or K was applied. Weeds were controlled with a pre-emergence application of 1,462 mL ha$^{-1}$ Atrazine 4L, 394.6 mL ha$^{-1}$ Explorer (mesotrione), and 2,046 mL ha$^{-1}$ Brawl II (s-metolachlor). Chinch bugs were controlled using zeta-cypermethrin (Mustang Maxx) at a rate of 292.3 mL ha$^{-1}$.

### Sampling

Several samples were taken both before and during the experiment. A preliminary soil sample to a depth of 60 cm was taken from the 0 kg N plots and separated into depths of 0-15, 15-30, and 30-60 cm. One NDVI was collected on 17 July 2020. Yield was collected at harvest by a combine, harvesting the grain from 30 m of the two center rows in each plot. Grain yield was determined at harvest, as well as grain moisture and test weight. Yield was reported on a 12.5% moisture basis.

## Lab Procedures

Soil inorganic N was determined by KCl extraction. Briefly, 25g of field moist soil was placed in an Erlenmeyer flask with 100mL KCl. The flasks were then covered and placed on an orbital shaker at 300rpm for 1 hour. The Erlenmeyer flasks were left for 10 minutes to settle, and the solution filtered through Whatman #42 filter paper. The samples were submitted to the Kansas State University Soil Testing lab for NH4-N and NO3-N analysis. Soil moisture content was determined via gravimetric water content by drying at 105$^{o}$ C. All soil N measurements were reported on a dry weight basis. Grain N content was determined by combustion by the Kansas State University Soil Testing Lab.

## Efficiency Calculations

NUEcrop (kg N kg N$^{-1}$), PFP (kg kg N$^{-1}$), and NAE (kg kg N$^{-1}$) were the three NUE calculations used in this chapter. The equations for these three efficiencies are below.

1. $NUEcrop = Grain\ N_f / N\ rate$ (Congreves et al., 2021)
2. $PFP = Yield_f / N\ rate$ (Congreves et al., 2021)
3. $NAE = (Yield_f - Yield_0) / N\ rate$ (Chen et al., 2016)

# Results

## NDVI

Sorghum NDVI was collected on 17 July 2020. NDVI was significantly affected by N rate (Pr(>Chisq)=0.003), but not by Proven$^{TM}$ (Pr(>Chisq)=0.730) or a Proven$^{TM}$ by N rate interaction (Pr(>Chisq)=0.330). Sorghum NDVI followed the expected N rate response but with few significant differences (Table 2.1). Sorghum NDVI was greatest with N applied, which were not significantly different between N rates, but were significantly greater than NDVI at 0 kg N ha$^{-1}$ (Table 2.1).

## Yield

Yield was significantly affected by N rate (Pr(>Chisq)<0.001), but not by Proven$^{TM}$ (Pr(>Chisq)=0.151) or a Proven$^{TM}$ by N rate interaction (Pr(>Chisq)=0.833). Yield generally followed the expected N rate response, with yield being greatest at 67, 101, and 135 kg N ha$^{-1}$, which were significantly greater than yield at 34 kg N ha$^{-1}$, which was greater than yield at 0 kg N ha$^{-1}$ (Table 2.2).

## Grain N

Grain N was significantly affected by N rate (Pr(>Chisq)<0.001), but not by Proven$^{TM}$ (Pr(>Chisq)=0.515) or a Proven$^{TM}$ by N rate interaction (Pr(>Chisq)=0.259). Grain N followed the expected N rate response (Table 2.3). Grain N uptake increased as N rate increased (Table 2.3).

## NUEcrop

NUEcrop was significantly affected by N rate (Pr<0.001), but not by Proven$^{TM}$ (Pr=0.938) or a Proven$^{TM}$ by N rate interaction (Pr=0.606). The N rate response of NUEcrop was as expected, with NUEcrop decreasing as N rate increases (Table 2.4). NUEcrops at each N rate was significantly different (Table 2.4).

### PFP

PFP was significantly affected by N rate ($Pr<0.001$), but not by Proven$^{TM}$ ($Pr=0.223$), or a Proven$^{TM}$ by N rate interaction ($Pr=0.904$). PFP followed the expected N rate response with PFP decreasing as N rate increased (Table 2.5). PFPs for each N rate was significantly different (Table 2.5).

### NAE

NAE was not significantly affected by N rate ($Pr=0.370$), Proven$^{TM}$ ($Pr=0.977$), or a Proven$^{TM}$ by N rate interaction ($Pr=0.670$) (Table 2.6).

## Discussion

NUEcrop values of 0.50 and 0.33 kg N kg $N^{-1}$ at N rates of 80 and 170 kg N $ha^{-1}$ respectively were reported by Sigua et al. (2018), which were 30 to 50% of the NUEcrop values obtained in this study. NUEcrop could have been increased by greater availability of soil N than by Sigura et al. (2018). NUEcrop in this study followed the expected N rate response.

PFPs in this study ranged from 74.8 to 278 kg kg $N^{-1}$. Abunyewa et al. (2017) reported PFPs ranging from ~60 to ~180 kg kg $N^{-1}$. PFP values from this study were greater than those reported by Abunyewa et al. (2017). This may have been due to differences in climate conditions and cultivars between studies.

NDVI, yield, grain N, and NUEcrop were all significantly affected by N rate and generally followed expected N rate responses. Proven$^{TM}$ did not produce any statistically significant responses in any of these parameters.

## Conclusions

Proven$^{TM}$ did not significantly affect NDVI, grain yield, grain N, or NUEcrop in sorghum. The N rate responses by all parameters met expectations, with the exception of NAE, which showed no N rate response. Further research on the effects of Proven$^{TM}$ on grain sorghum with more sampling dates and greater replication would help in understanding N contributions from Proven$^{TM}$ and their effects on yield, whole plant N, and NUE.

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**Table 2.1. Sorghum NDVI by N rate with groups.**

| N rate (kg $ha^{-1}$) | NDVI | SE | group |
|---|---|---|---|
| 0 | 0.83 | 0.0020 | b |
| 34 | 0.84 | 0.0042 | a |
| 67 | 0.84 | 0.0017 | a |
| 101 | 0.84 | 0.0016 | a |
| 135 | 0.84 | 0.0015 | a |

SE represents standard error.

**Table 2.2. Yield by N rate with pairwise groups**

| N rate (kg ha$^{-1}$) | Yield (kg ha$^{-1}$) | SE | group |
|---|---|---|---|
| 0 | 7846 | 131 | c |
| 34 | 8390 | 108 | b |
| 67 | 8902 | 154 | a |
| 101 | 8946 | 135 | a |
| 135 | 8906 | 144 | a |

SE represents standard error.

**Table 2.3. Grain N by N rate with pairwise groups.**

| N rate ($kg\ ha^{-1}$) | Grain N ($kg\ ha^{-1}$) | SE | group |
|---|---|---|---|
| 0 | 82.0 | 3.93 | d |
| 34 | 90.6 | 3.23 | c |
| 67 | 107 | 3.28 | b |
| 101 | 114 | 2.95 | ab |
| 135 | 116 | 3.35 | a |

SE represents standard error.

**Table 2.4. NUEcrop by N rate with pairwise groups.**

| N rate (kg ha$^{-1}$) | NUEcrop (kg N kg N$^{-1}$) | SE | group |
|---|---|---|---|
| 30 | 3.02 | 0.11 | a |
| 60 | 1.78 | 0.05 | b |
| 90 | 1.27 | 0.03 | c |
| 120 | 0.97 | 0.03 | d |

SE represents standard error.

**Table 2.5. PFP by N rate with pairwise groups.**

| N rate (kg $ha^{-1}$) | PFP (kg kg $N^{-1}$) | SE | group |
|---|---|---|---|
| 30 | 279 | 3.69 | a |
| 60 | 149 | 2.96 | b |
| 90 | 99.8 | 1.72 | c |
| 120 | 74.8 | 1.07 | d |

SE represents standard error.

**Table 2.6. NAE by treatment.**

| N rate (kg ha$^{-1}$) | Proven$^{TM}$ | NAE (kg kg N$^{-1}$) | SE |
|---|---|---|---|
| 30 | N | 0.41 | 0.09 |
| 30 | Y | 0.35 | 0.22 |
| 60 | N | 0.45 | 0.10 |
| 60 | Y | 0.43 | 0.06 |
| 90 | N | 0.32 | 0.04 |
| 90 | Y | 0.44 | 0.03 |
| 120 | N | 0.32 | 0.05 |
| 120 | Y | 0.28 | 0.03 |

SE represents standard error.

# Appendix A - Supplemental Data

**Table A.1. Pre-plant P, K, and pH by depth for 2019**

| N rate (kg ha$^{-1}$) | Proven™ | Depth (cm) | P (ppm) | SE | K (ppm) | SE | pH | SE |
|---|---|---|---|---|---|---|---|---|
| 0 | N | 0-15 | 12.2 | 4.48 | 298 | 14.3 | 6.85 | 0.29 |
| 0 | N | 15-30 | 5.95 | 1.73 | 208 | 11.8 | 6.78 | 0.17 |
| 0 | Y | 0-15 | 7.88 | 1.23 | 241 | 17.7 | 6.70 | 0.40 |
| 0 | Y | 15-30 | 5.18 | 0.66 | 192 | 9.84 | 6.70 | 0.18 |
| 56 | N | 0-15 | 9.93 | 4.03 | 248 | 26.6 | 6.63 | 0.13 |
| 56 | N | 15-30 | 7.80 | 3.47 | 193 | 10.8 | 6.50 | 0.07 |
| 56 | Y | 0-15 | 8.60 | 1.47 | 261 | 17.4 | 6.73 | 0.34 |
| 56 | Y | 15-30 | 5.50 | 0.81 | 203 | 7.96 | 6.65 | 0.24 |
| 112 | N | 0-15 | 7.03 | 1.24 | 235 | 16.6 | 6.60 | 0.11 |
| 112 | N | 15-30 | 8.18 | 0.84 | 230 | 32.9 | 6.63 | 0.16 |
| 112 | Y | 0-15 | 9.50 | 3.10 | 266 | 12.0 | 6.73 | 0.19 |
| 112 | Y | 15-30 | 7.28 | 2.74 | 182 | 9.98 | 6.53 | 0.07 |
| 168 | N | 0-15 | 10.6 | 1.08 | 247 | 18.2 | 6.55 | 0.27 |
| 168 | N | 15-30 | 8.38 | 3.24 | 182 | 7.94 | 6.58 | 0.12 |
| 168 | Y | 0-15 | 7.28 | 1.07 | 258 | 11.9 | 6.60 | 0.21 |
| 168 | Y | 15-30 | 8.25 | 1.79 | 190 | 3.70 | 6.60 | 0.12 |

Y indicates treatments designated to have Proven™ applied and N indicates treatments designated to have no Proven™ was applied. SE represents standard error.

**Table A.2. Pre-plant P, K, and pH by depth for 2020**

| N rate (kg $ha^{-1}$) | Proven™ | Depth (cm) | P (ppm) | SE | K (ppm) | SE | pH | SE |
|---|---|---|---|---|---|---|---|---|
| 0 | N | 0-15 | 17.8 | 5.58 | 195 | 11.9 | 5.98 | 0.17 |
| 0 | N | 15-30 | 5.67 | 1.42 | 174 | 6.85 | 6.28 | 0.10 |
| 0 | Y | 0-15 | 25.2 | 4.10 | 216 | 10.3 | 6.37 | 0.25 |
| 0 | Y | 15-30 | 7.28 | 1.12 | 192 | 7.29 | 6.42 | 0.22 |
| 56 | N | 0-15 | 24.0 | 4.36 | 205 | 9.84 | 6.17 | 0.32 |
| 56 | N | 15-30 | 6.23 | 1.35 | 191 | 3.98 | 6.33 | 0.27 |
| 56 | Y | 0-15 | 24.6 | 7.47 | 219 | 10.7 | 6.47 | 0.25 |
| 56 | Y | 15-30 | 6.70 | 1.08 | 189 | 10.6 | 6.50 | 0.35 |
| 112 | N | 0-15 | 31.0 | 8.07 | 211 | 11.8 | 6.03 | 0.18 |
| 112 | N | 15-30 | 5.60 | 1.20 | 192 | 10.4 | 6.18 | 0.26 |
| 112 | Y | 0-15 | 16.9 | 4.36 | 203 | 12.1 | 6.25 | 0.37 |
| 112 | Y | 15-30 | 5.80 | 1.30 | 205 | 10.7 | 6.45 | 0.30 |
| 140 | N | 0-15 | 27.6 | 5.62 | 212 | 4.88 | 6.07 | 0.27 |
| 140 | N | 15-30 | 9.86 | 2.33 | 184 | 3.62 | 6.42 | 0.32 |
| 140 | Y | 0-15 | 19.9 | 3.37 | 203 | 6.03 | 6.18 | 0.30 |
| 140 | Y | 15-30 | 5.95 | 0.56 | 188 | 6.77 | 6.28 | 0.33 |
| 154 | N | 0-15 | 18.7 | 4.84 | 203 | 12.0 | 6.38 | 0.38 |
| 154 | N | 15-30 | 6.52 | 1.41 | 175 | 8.94 | 6.58 | 0.35 |
| 154 | Y | 0-15 | 15.1 | 1.52 | 196 | 9.08 | 6.68 | 0.18 |
| 154 | Y | 15-30 | 4.88 | 1.19 | 172 | 9.92 | 6.30 | 0.24 |
| 168 | N | 0-15 | 18.2 | 3.61 | 205 | 13.8 | 6.35 | 0.35 |
| 168 | N | 15-30 | 5.38 | 0.87 | 181 | 7.50 | 6.55 | 0.31 |
| 168 | Y | 0-15 | 18.8 | 3.21 | 214 | 8.91 | 6.83 | 0.33 |
| 168 | Y | 15-30 | 5.68 | 0.96 | 182 | 10.2 | 6.75 | 0.38 |

Y indicates treatments designated to have Proven™ applied and N indicates treatments designated to have no Proven™ was applied. SE represents standard error.