ENHANCING MINERAL NUTRIENT AVAILABILITY AND CORN PRODUCTIVITY WITH BIOSTIMULANTS

BY

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THESIS

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ABSTRACT

Corn (*Zea mays* L.) production has greatly increased since corn was first domesticated some 7,000 years ago (Beadle, 1980). Generating improved cultivars with novel breeding schemes and genetics in combination with enhanced management factors and technologies has resulted in the highest corn grain yields to date. Some of the most important management factors that have been utilized are hybrid, planting population, nitrogen fertility, additional nutrient fertility, and foliar protection (Ruffo et al., 2015). In recent years, a new technology has been discussed, namely, biological products. These products have a wide variety of uses but are typically intended to increase crop growth, relieve crop stresses, enhance the availability of soil mineral nutrients, improve the accumulation of mineral nutrients, and ultimately increase yields. In an effort to better understand biological products and their best fit in an agronomic management system, the objective of this research was to evaluate the effects of two biological products regarding their optimal application to provide increased soil nutrient availability and enhanced fertilizer use in corn production. This research involved the following two areas:

Utilizing a Microbial Enhancer to Improve Nitrogen Use and Corn Productivity

Multiple application methods and timings of a microbial enhancer were applied in combination with differing rates of nitrogen (N) fertilizer to evaluate the responses in N availability and use by corn. Certain application methods resulted in improvements in N uptake and use efficiency corresponding with grain yield increases. When applied earlier in the growing season, the yield trajectory was enhanced, evidenced by more kernel production, while later application timings resulted in heavier kernels.

Improving Fertilizer Use and Corn Productivity with a Phosphorus Solubilizing Bacteria

Differing rates of N and phosphorus (P) fertilizer were used in combination with applications of a phosphorus solubilizing bacteria (PSB) to determine the effects of a PSB on accumulation of essential nutrients by corn plants. Phosphorus solubilizing bacteria applications enhanced the amount of available phosphorus in the soil, thereby increasing the amount of phosphorus accumulated in the plants. This enhancement in available P corresponded with increases in grain yield due to a higher production of kernels.

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CHAPTER 1. UTILIZING A MICROBIAL ENHANCER TO IMPROVE NITROGEN USE AND CORN PRODUCTIVITY

ABSTRACT

Biological products are a diverse part of the agricultural market that is booming with new products every year. There are many categories of biological products with a large variety of uses; however, in general these product alter the biology of plants and soils in order to improve plant growth and production. Most of these products were originally used in high-value food and ornamental crops, but recently have begun to be applied to row crops like corn to increase grain yield. Due to the sheer number of different biologicals that are available, it is important to research their impacts in a corn management system. The objectives of this research were to study the effects of applications of a microbial enhancer called *Source* from Sound Agriculture on corn grain yield and to determine the basis of the yield responses. In 2018, corn was grown at three pre-plant nitrogen (N) fertilizer rates (broadcast, incorporated urea) of 0, 60, and 220 lbs N acre^{-1} in combination with three *Source* treatments, either an untreated control, an in-furrow application at planting, or a foliar application at the V4 growth stage. Additionally, in 2019, corn was grown at four pre-plant nitrogen (N) fertilizer rates (broadcast, incorporated urea) of 0, 60, 120, and 220 lbs N acre^{-1} and received one of four *Source* treatments: an untreated control, a foliar application at either V4 or VT, or with both V4 plus VT growth stage applications. These studies were conducted at three sites in southern, central, and northern Illinois to determine the product efficacy at multiple sites with differing soil fertility and weather patterns. In 2018, when averaged across all locations and N rates, *Source* applied in-furrow at planting and foliar at V4 increased grain yield by 5 and 6 bushels acre^{-1}, respectively. Similarly, in 2019 when averaged across locations and N rates, *Source* applied foliar at both the V4 plus VT growth stages increased grain yield by 3 bushels acre^{-1}. In both years, the yield responses from *Source* were most apparent at the southern Illinois site, which has the lowest inherent soil fertility. These yield increases were driven by a higher production of kernels and nitrogen accumulation. In 2018, in-furrow and foliar applications of *Source* resulted in an average increase of 267 kernels meter^{-2} at southern Illinois, while in 2019, foliar treatments of *Source* at the combination of V4 plus VT led to an increase of 115 kernels meter^{-2} compared to the control at the southern site. The same Source applications also increased total N accumulation at physiological maturity by 8 pounds of N acre^{-1} compared to the untreated control. The results of these studies show the impact of *Source* applications on corn grain yield, yield potential, and N use.

INTRODUCTION

Currently, the world is anticipating a shortage of food supply in the near future. With the world population at an all-time high of 7.7 billion and expected to reach 9.7 billion people by 2050 (United Nations, 2019), it is crucial for crop yield improvements to combat this food demand. With the transition of rural areas becoming more urbanized, it is projected that only 10% of the increased production could come from arable land expansion while the remaining 90% needs to originate from increased cropping intensity (Alexandratos and Bruinsma, 2012). Corn (*Zea mays*, L.) is one of the world's major food crops, especially in the United States, where 91.7 million acres were planted in 2019 (USDA NASS, 2019). Also, corn is a feasible solution to the world's food demand crisis due to plant's higher yield potential compared to other cereal crops (Abebe and Feyisa, 2017). However, to reach this yield potential, producers must use proper agronomic management. There are many different management strategies, but five have been noted to have the largest impact on corn grain yield: hybrid, planting population, N fertility, additional nutrient fertility, and foliar protection (Ruffo et al., 2015). Of these management factors, nitrogen fertility is often the most limiting to corn grain yields because of the high levels accumulated by the crop and the uncertainty associated with N availability (Below, 2002)

There are seventeen essential mineral nutrients needed for plant growth and development. A nutrient must fulfill the following criteria to be considered "essential": (a) a deficiency of the element hinders the plant to complete its life cycle; (b) a deficiency is specific for the element and supplying another element cannot correct the deficiency; and (c) the element is directly involved in the metabolism of the plant (Arnon and Stout, 1939). Nitrogen is one of the most important mineral nutrients for corn because, of the seventeen essential elements excluding those obtained from the air (carbon, hydrogen, and oxygen), nitrogen is the most abundant element in the plant (Fernandez, Ebelhar, Nafziger, & Hoeft, 2009). A corn crop that yields 230 bushels acre^{-1} can uptake 256 lbs N acre^{-1} (Bender et al., 2013). Nitrogen is a key component in organic compounds such as proteins, nucleic acids, chlorophyll, and growth regulators. These components have a large impact on corn growth due to their large role in establishing and maintaining the photosynthetic apparatus and sink capacity (Below, 2002).

Plants accumulate N in two forms, nitrate (NO_3^-) and ammonium (NH_4^+), which become plant-available as a result of natural processes due to living soil microbes. Nitrogen in the soil pertains to either organic or inorganic forms, with organic N corresponding with 90% of the total N in soils. A portion of N in organic matter can be converted into NH_4^+ through mineralization, but this process is slow and usually cannot support the amount of N needed for crop growth (Below, 2002; Scharf, 2015a). Microbes in the soil can also take NH_4^+ and convert it into organic forms of N, which is called immobilization. These microbes, as well as other organisms like plants and animals, will decompose following death, in which case NH_4^+ is recycled back into the inorganic N pool. Ammonia also undergoes a process called nitrification, resulting in the plant-available N form of nitrate. Nitrate is accumulated into plants through the cell membranes and can either be reduced to NH_4^+ in the root or shoot, or stored in the vacuole for later assimilation, when needed (Below, 2002). Nitrate must be reduced to NH_4^+ before amino acid synthesis, and is an energy-expensive process (Below, 2002). Due to the negatively charged soil particles, NO_3^- is highly susceptible to loss through leaching or runoff of water. Additionally, NO_3^- is vulnerable to loss to the atmosphere through denitrification under anaerobic conditions, where soil microbes convert NO_3^- into nitrous oxide (N_2O) as well as nitrogen gas (N_2). While plants may directly utilize NH_4^+ accumulated from the soil, NH_3 is toxic inside the plant, meaning it must be assimilated into an amino acid immediately following uptake. Another aspect of the nitrogen cycle is biological fixation of nitrogen. The atmosphere mainly consists of 80% nitrogen gas (N_2) and with sufficient energy this gaseous form of N can be reduced or fixed into ammonia (NH_3). However, this process of N-fixation is complex due to the high stability of the triple bond in N_2 gas, and only certain organisms can carry this process out with an enzyme called nitrogenase (Bernhard, 2010). Nitrogen fixation is an energy-demanding process, requiring at least 16 molecules of ATP per molecule N_2 reduced (Marschner, 2011).

Due to the future food demand and the susceptibility of nitrogen loss, it is important to discover ways to sustainably increase corn grain yields. In 2017, the average corn grain yield in the US was 176.6 bushels acre^{-1}, setting a new record (USDA-NASS, 2017). Grain yields have been trending upwards, and as growers learn to better manage their crops, yield barriers continue to be exceeded. In recent years, there has been a large increase in the number and diversity of biological products available in the market with many of these technologies purported to improve nutrient availability, reduce stress, increase growth, and subsequently increase yields. There are many different categories and types of products that can be classified as biological management factors. For a number of these products, the modes of action are not completely understood, showing the need for further research. One grouping of these biological products is known as biostimulants, and these types of products were referenced for the first time in the 2018 Farm Bill (Agriculture Improvement Act, 2018). In this Farm Bill, biostimulants were defined as "a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield". Biostimulants vary in their formulations as well as their constituents; however, the vast majority of them contain, or are derived from, humic substances, seaweed extracts, beneficial bacteria, beneficial fungi, protein hydrolysates, and many other substances (Albrecht, 2018). The product evaluated in this study (*Source*, Sound Agriculture, Emeryville, CA) has an active ingredient of maltol lactone, but there is little published information about this compound. *Source* is referred to as a microbial enhancer because upon entering plants, it sends a cascading signal to corn plant roots that stimulate N-fixing and phosphorus-solubilizing microbes in the soil to improve nutrient availability.

There are several agronomic management application techniques that have made a large impact on agriculture. Placing fertilizer and biological products directly under the seed in-furrow has led to improvements in both crop growth and yield. When urea-ammonium nitrate (UAN) was knife-injected into the soil in a band, corn grain yield and N accumulation were increased compared to surface-broadcast applied UAN indicative of more efficient uptake of N (Gordon, 1992). In-furrow applications are an important management technique because the product being utilized is concentrated directly where the plant roots will eventually be located. This placement allows the plant to utilize the newly-available nutrients that were generated by the microbes that were enhanced by the biostimulant.

Another common application method of some biologicals is foliar sprays. The ease and flexibility of foliar applications make this method attractive for farmers. Not all growers have an in-furrow system on their planter to apply biologicals; however, many farmers will make multiple foliar applications throughout the growing season. For fields that have problematic weeds, herbicide applications by foliar spray will be made pre-plant and as well as early in the crop vegetative growth stages. If insects or diseases reach a critical crop-impact threshold, some farmers will also apply an insecticide and/or fungicide during the crop reproductive stages. Many foliar application possibilities make it easy for a farmer to include a biological product along with the pesticide as long as they are compatible in a tank mix.

To have the greatest effect of providing a biological product, understanding the crop's growth habit and physiology during the season are important. Corn growth and development is divided into stages to denote the progression in growth as well as the physiological processes in the plant. The most common staging system is the collar method, which is divided into vegetative (V) and reproductive (R) phases (Abendroth et al., 2011; Nielsen, 2002). Vegetative stages are defined by the leaves up to and including the uppermost leaf with a fully defined collar. For a leaf to be fully collared, the leaf blade and leaf sheath must fully wrap around the stem and meet on each side. Corn plants continuously exhibit leaves until the plant reaches the tassel stage (VT). When a plant reaches VT, vegetative growth ceases and reproductive development begins. Even though early plant growth is denoted as vegetative growth, there is still critical reproductive development happening during the vegetative stages that determines the grain yield potential. At the V5-V6 (five to six leaf) growth stages, all leaves have been initiated and the kernel row number on the ears has been determined. Additionally, by the V15-V16 (15 to 16 leaf) growth stages, the potential kernel number per row has been established. Any stress, such as nutrient deficiencies, during vegetative growth stages can have a large impact on the plant's yield potential (Abendroth et al., 2011; Fageria er al., 2006).

While nutrient stress during vegetative growth can affect yields, stress during the reproductive stages can also influence kernel growth and final grain yield. Reproductive growth stages are focused on the developmental progress of the corn grain and determined by analyzing mid-length down the primary ear. The stages range from silking (R1) to physiological maturity (R6). The R1 growth stage begins when the silks emerge from the husks, receiving pollen from the tassel and subsequently, initiating ovule fertilization. Incomplete fertilization of the ear will result in a severe impact on yield potential; therefore, this stage in reproductive development is the most sensitive to stresses such as drought. Lack of moisture during flowering and pollination can lead to a significant reduction in yield due to loss of kernel set (Setter, 2001). Dry matter is accumulated in the grain mainly during the R3-R5 grain filling stages, also known as milk, dough, and dent stages, respectively. In the dough and dent stages, corn kernels accrue more than half of the final kernel dry weight; therefore, stresses such as drought, heat, and nutrient deficiencies during this time frame can greatly decrease the weight of each kernel, corresponding to lower grain yields. (Wilhelm, 1999). Additionally, the total number of kernels per ear can also affect the final average kernel weight, in a mechanism known as yield component compensation (Adams, 1967).

The objective of this research was to determine the effects of a microbial enhancer, trade name *Source*, on soil N availability and N fertilizer use in corn production. With an increase in N use efficiency, growers would benefit from improved grain yields and profits, while also reducing the loss of N to the environment.

MATERIALS AND METHODS

Field Characteristics

The microbial enhancer experiment was implemented in the 2018 and 2019 growing seasons at three locations across the state of Illinois. In 2018, this study was conducted at the Crop Science Research and Education Center (CSREC) located at the University of Illinois Urbana-Champaign and two off-site locations: at Harrisburg, IL, in the southern part of the state and Yorkville, IL, in the northern part of the state. Soil types differed between locations with the Harrisburg location consisting of a Harco silt loam, the Champaign location consisted of a Catlin silt loam, and the Yorkville location consisting of a Drummer silty clay loam soil type. In 2019, the study was implemented at Champaign and Yorkville, as well as an alternate southern location at Ewing, IL. The soil types in 2019 were a Flanagan silt loam at Champaign, a Drummer silty loam at Yorkville, and a Cisne silt loam at Ewing.

Pesticide Applications

In 2018, all locations were maintained weed-free with a pre-emergence application of S-metolachlor (2-chloro-N-[2-ethyl-6-methylphenyl]-N-[2-methoxy-1-methylethyl] acetamide) + atrazine (1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) + mesotrione (2-[4-[methylsulfonyl]-2-nitrobenzoyl] cyclohexane-1,3-dione) known as Lumax EZ (Syngenta, Basel, Switzerland) at a rate of 3.25 qt acre^{-1} at Harrisburg, IL; bicyclopyrone (bicyclo[3.2.1]oct-3-en-2-one, 4-hydroxy-3-[[2-[(2-methoxyethoxy)methyl]-6-[trifluoromethyl]-3-pyridinyl]carbonyl]) + mesotrione + S-metolachlor + atrazine known as Acuron (Syngenta, Basel, Switzerland) at a rate of 3 qt acre^{-1} at Champaign, IL; and pyroxasulfone (3-[[[5-[difluoromethoxy]-1-methyl-3-[trifluoromethyl]-1H-pyrazol-4-yl]methyl]sulfonyl]-4,5-dihydro-5,5-dimethylisoxazole) known as Zidua (BASF Corporation, Ludwigshafen, Germany) at a rate of 3 oz acre^{-1}, flumioxazin (2-[7-

fluoro-3,4-dihydro-3-oxo-4-[2-propynyl]-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3[2H]-dione) + pyroxasulfone known as Fierce (Valent, Walnut Creek, CA) at a rate of 0.5 oz acre^{-1}, and atrazine known as AAtrex 4L (Syngenta, Basel, Switzerland) at a rate of 1 qt acre^{-1} at Yorkville, IL.

In 2019, all locations were maintained weed-free with a pre-emergence herbicide application of Acuron at a rate of 2 qt acre^{-1} at Ewing, IL; Acuron at a rate of 3 qt acre^{-1} at Champaign, IL; and acetochlor (2-chloro-2'-methyl-6'-ethyl-Nethoxymethylacetanilide) + atrazine known as Breakfree ATZ (Corteva Agriscience, Wilmington, DE) at a rate of 2 qt acre^{-1} at Yorkville, IL.

In both years, all plots received an in-furrow soil insecticide application of tefluthrin ([2,3,5,6-tetrafluoro-4-methylphenyl]methyl-[1α,3α]-[Z]-[±]-3-[2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethylcyclopropanecarboxylate), known as Force 3G (Syngenta, Basel, Switzerland) at a rate of 4 oz acre^{-1}.

In-season weed control for all locations in 2018 was applied at the V5 to V6 growth stages with atrazine, known as AAtrex 4L (Syngenta, Basel, Switzerland) at a rate of 1 qt acre^{-1}, topramezone (3-[4,5-dihydro-isoxazolyl]-2-methyl-4-[methylsulfonyl]phenyl)[5-hydroxy-1-methyl-1H-pyrazol-4-yl]methanone, known as Armezon (BASF Corporation Ludwigshafen, Germany) at a rate of 0.75 oz acre^{-1}, glyphosate (N-phosphonomethyl glycine, in the form of a potassium salt), known as RoundUp PowerMax (Bayer, St. Louis, MO) at a rate of 32 oz acre^{-1}, and ammonium sulfate (AMS; 21-0-0-24S) at a rate of 0.2 gal acre^{-1}.

In 2019, in-season weed control at Ewing was applied at the V6 growth with tembotrione (2-[2-chloro-4-[methylsulfonyl]-3-[(2,2,2-trifluoroethoxy) methyl]benzoyl]-1,3-cyclohexanedione) known as Laudis (Bayer, St. Louis, MO) at a rate of 3 oz acre^{-1}; AAtrex 4L at a rate of 1 qt acre^{-1}; RoundUp PowerMax at a rate of 32 oz acre^{-1}; and AMS at a rate of 0.2 gal acre^{-1}. In-season weed control at Champaign was performed at the V6 growth stage with AAtrex 4L at a rate of 1 qt acre^{-1}; Armezon at a rate of 0.75 oz acre^{-1}; RoundUp PowerMax at a rate of 32 oz acre^{-1}; and AMS at a rate of 0.2 gal acre^{-1}. At Yorkville, the in-season weed control was applied at the V6 growth stage with S-Metolachlor + glyphosate + mesotrione, known as Halex GT (Syngenta, Basel, Switzerland) at a rate of 3.6 pint acre^{-1}; sodium salt of diflufenzopyr [2-(1-[([3,5-difluorophenylamino]carbonyl)-hydrazono]ethyl)-3-pyridinecarboxylic acid, sodium salt] + sodium salt of dicamba (3,6-dichloro-2-methoxybenzoic acid, sodium salt) also known as Status (BASF Corporation, Ludwigshafen, Germany) at 4 oz acre^{-1}; AAtrex 4L at a rate of 1 qt acre^{-1}; RoundUp PowerMax at a rate of 13 oz acre^{-1}; FS AquaSupreme (FS Growmark, Bloomington, IL) surfactant at a rate of 0.1 gal acre^{-1}; and AMS at a rate of 0.2 gal acre^{-1}

Agronomic Management

Soybean was the previous crop and conventional tillage was used in both seasons. In 2018, a hybrid previously shown to have a large response to N fertilizer was planted to achieve a population of 34,000 plants acre^{-1} and a population of 36,000 plants acre^{-1} in 2019. The hybrid differed between the two growing seasons with Croplan 6110SS (WinField United, St. Paul, MN) utilized in 2018 and Golden Harvest G10T63-3122 (Syngenta, Basel, Switzerland) used in 2019. Both of these hybrids have a 110-day relative maturity. Plots were planted with a Seed Pro 360 planter (ALMACO, Nevada, IA) on 1 May 2018 at Harrisburg, 7 May 2018 at Champaign, and 18 May 2018 at Yorkville. In the following year, plots were planted on 4 June 2019 at Ewing, 2 June 2019 at Champaign, and 9 June 2019 at Yorkville.

Treatment Applications

Applications were designed to evaluate a microbial enhancer, known as *Source* (Sound Agriculture, Emeryville, CA), for its role in nitrogen use and productivity of corn. In 2018, this product was supplied to the corn plants either in-furrow at planting or as a foliar application at the V4 growth stage (four fully collared leaves) and combined with differing rates of N from zero, limiting, to typically sufficient (Table 1.1). Nitrogen was broadcast-applied at 0, 60, and 220 lbs acre^{-1} as urea (46-0-0) at pre-plant then incorporated into the soil. All treatments of the microbial enhancer were applied at a rate of 17 oz acre^{-1}. The in-furrow treatments of *Source* were applied at planting to all plot rows with a planter-attached liquid starter applicator system (Surefire Ag Systems, Atwood, KS) and at a total volume rate of 8 gal acre^{-1} with water as a carrier. Foliar treatments of *Source* at V4 and VT were applied with MasterLock (WinField United, St. Paul, MN) surfactant at a rate of 6.4 oz acre^{-1}. A pressurized CO_2 backpack sprayer was used with water as a carrier for a total spray volume of 15 gal acre^{-1} application rate. The boom consisted of flat fan nozzles (TeeJet XR1002) with a 110° spray pattern to provide even and full coverage across the center two plot rows. In 2018, the V4 applications of *Source* occurred on 23 May 2018, 29 May 2018, and 5 June 2018 at Harrisburg, Champaign, and Yorkville, respectively.

In 2019, some treatments differed compared to the 2018 growing season. *Source* was applied to the foliage at 17 oz acre^{-1} at the V4, VT (tasseling/beginning of reproductive stages), or at both the V4 and VT growth stages (*Source* was applied at 17 oz acre^{-1} at each timing) and each of these treatments were combined with differing rates of applied N (Table 1.2). Nitrogen was broadcast-applied at 0, 60, 120, and 220 lbs acre^{-1} as urea at pre-plant and incorporated into the soil. Foliar applications at Ewing occurred on 28 June 2019 (V4) and 9 August 2019 (VT). Plots at Champaign were treated with *Source* on 24 June 2019 (V4) and 30 July 2019 (VT). Lastly, the *Source* applications were completed at Yorkville on 2 July 2019 (V4) and 12 August 2019 (VT).

Experimental Design and Statistical Analysis

Treatments were arranged in a randomized complete block design with six replications and nine treatments for a total of 54 plots at each location (grand total of 162 plots) in 2018 and 16 treatments for a total of 96 plots at each location (grand total of 288 plots) in 2019. Each plot was four rows wide and 37.5 ft in length with 30 in row spacing. Statistical analysis was conducted using PROC MIXED in SAS (version 9.4; SAS Institute, Cary, NC). *Source* treatment and N fertilizer rate were considered fixed effects, with location as a random factor in the model. Significance was declared at $P \leq 0.10$ due to the fastidious nature of biological products to be certain we would not miss differences that may exist. PROC GLM of SAS was utilized to conduct the Brown-Forsythe test of the Levene test for homogeneity of variance on the errors and significance was declared at $P \leq 0.05$. PROC UNIVARIATE of SAS was used to determine possible outliers and assess the normality of the errors, with significance declared at $P \leq 0.01$. In addition to the Shaprio-Wilk test, QQ plots and histograms were studied to determine normality of the errors, when the Shapiro-Wilk tests were significant. With homogeneity of variance and normality assumptions met, the data were analyzed separately by year due to differing treatments.

Measured Parameters

Pre-plant soil samples (0-12 in deep) were obtained from plot areas prior to planting and analyzed (A & L Great Lakes Laboratories, Fort Wayne, IN) to confirm soil fertility levels.

Following physiological maturity, the middle two rows of each plot were harvested with a SPC40 combine (ALMACO, Nevada, IA) to determine grain yield, with values adjusted to 15.5% moisture. In both years, the center two rows of each plot were mechanically harvested for determination of grain yield and harvest moisture, and the yield subsequently standardized to bushels/acre at 15.5% moisture. The harvest dates in 2018 were 9 September, 21 September, and 12 October 2018, at Harrisburg, Champaign, and Yorkville, respectively. In the second year of this study, the harvest dates were 15 October, 13 November, and 18 November 2019, at Ewing, Champaign, and Yorkville, respectively. The combine also collected subsamples of the harvested grain that were evaluated for grain quality (protein, oil, and starch concentrations at 0% grain moisture) by utilizing near-infrared transmittance spectroscopy (NIT) with an Infratec 1241 Grain Analyzer (Foss, Eden Prairie, MN). The grain quality data is presented in supplemental tables A1.1 to A1.4. Average kernel weights were evaluated based on a representative subsample of 300 kernels and adjusted to 0% moisture. Kernel number on a per-acre basis was obtained from dividing total grain weight by the average kernel weight.

In 2018, grain, stover, and total uptake of nitrogen in the plant was estimated based on the grain protein concentrations and final yields. Grain N concentration was calculated algebraically by dividing grain protein concentration obtained by NIT by the constant 6.25 (Jones, 1932). Following calculation of the grain N concentration, the final yield was used to determine total grain N content. Using a harvest index estimate of 0.7, total N uptake was calculated based on grain nitrogen content. Subtracting the grain N content value from the total N content value provided an estimation of the stover N content.

In 2019, grain N content was determined through the same technique as 2018; however, stover N was measured from plant samples that were obtained at the R6 growth stage (physiological maturity). Two random plants were manually sampled at the soil surface from each of the center two rows on 29 September 2019 at Ewing, 16 October 2019 in Champaign, and 24 October 2019 in Yorkville. The plants were partitioned into grain and stover (including husk) components. Stover biomass accumulation was obtained by weighing the fresh plant stover and then passed through a BC600XL chipper (Vermeer Corporation, Pella, IA) to attain representative subsamples. The stover subsamples were immediately weighed to measure the fresh weight, and later weighed again when dried to 0% moisture in a forced air oven at 167 ºF to determine dry weight. The stover dry weight was then calculated by multiplying the total fresh weight by the quotient of the dry and fresh weight of the subsample. The corn ears that were partitioned were then dried, the grain was removed from the ear by a corn sheller (AEC Group, St. Charles, IA), and analyzed for moisture content using a Dickey John moisture reader (GSF, Ankeny, IA). Cob weight was calculated by difference, and dry stover and cob weights were added to obtain total R6 stover biomass. Dried stover subsamples were ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) to pass through a 2 mm mesh screen. Representative subsamples were evaluated for N concentration using a combustion-based analyzer (EA1112, CE Elantech, Lakewood, NJ). This N concentration was multiplied by the total dry stover biomass on a per acre basis to get total N accumulated acre^{-1} in the stover tissue.

Additionally, nitrogen recovery efficiency was determined by calculating the difference between the plant total N uptake of an individual fertilized treatment with the total N uptake of the check plot (zero N applied treatment) and then dividing by the amount of N that was applied. Nitrogen recovery efficiency represents the percentage of fertilizer-applied N that was taken up by the plant.

RESULTS AND DISCUSSION

Effects of Application Methods of Source on Nitrogen Uptake and Use, 2018

Soil Characteristics

Preplant composite soil test values varied across the locations (Table 1.3). In general, native soil organic matter and CEC levels trended to increase moving from southern to northern Illinois. Notably, Yorkville soils contained the highest inherent soil fertility levels in combination with a slightly lower pH (Table 1.3).

Weather

The 2018 production year across the state of Illinois experienced above-average temperatures in May and June along with timely rains, resulting in adequate crop emergence and rapid vegetative growth (Table 1.4). Field sites in Harrisburg and Champaign received little weather-induced heat or moisture stress throughout the growing season. However, Yorkville received 5.0 inches more precipitation than the 30 year average in May and June, but a deficit of 4.1 inches of rain in July and August compared to the 30-year average (Table 1.4).

Grain Yield and Yield Components

At Harrisburg, grain yield and kernel number were affected by both N rate and *Source* application; however, there was no interaction between the fixed sources of variation, while kernel weight was only affected by N fertilizer rate (Table 1.5). Plots that received no N or *Source* treatment (check plot) produced the lowest grain yields at Harrisburg compared to the other two locations, corresponding with the site's characteristically lower soil fertility (Tables 1.3 and 1.6). Grain yield increased with each increasing N rate as observed in a number of past studies demonstrating that higher levels of applied pre-plant N can increase grain yields (Stevenson and Baldwin, 1969; Ahmad et al., 2009; Bushong et al., 2016). Within an N fertilizer rate, plots treated with *Source* either in-furrow or foliarly tended to have higher grain yields, especially in combination with the lower N rates of 0 and 60 lbs N acre^{-1}. When averaged over the N fertilizer rates, in-furrow at planting and V4 foliar applications of *Source* increased grain yield by 12 and 10 bushels acre^{-1}, respectively. (Table 1.6). These grain yield responses were a result of greater production of kernels (Table 1.6). This finding was consistent with studies involving several in-furrow applied biostimulants that fostered a greater number of corn kernels (Sible, 2019) and (Harmon, 2017). Due to yield component compensation, in many cases, improvements in one yield component can affect the other. However, even with the greater production of kernels from adding *Source*, average kernel weight remained the same, indicating that *Source*-treated corn plants were able to maintain the improved yield potential (Table 1.6).

In the central part of the state at Champaign, the check plot yield and soil organic matter were greater in comparison to Harrisburg, showing the soil's inherent ability to provide more N through mineralization as well as the availability of other essential nutrients such as phosphorus and sulfur provided by soil organic matter (Table 1.6). At this site, N fertilizer rate affected grain yield, kernel number, and average kernel weight; but *Source* treatment and the interaction of N rate and *Source* did not affect these three parameters (Table 1.5). Previous studies have documented that N-induced yield increases of cereal crops are largely due to more grains per plant because N aids in their initiation as well as decreasing kernel abortion (Below, 2002). However, N is also a large constituent in chlorophyll, and when active later in the season, rates of photosynthesis are improved, in turn increasing kernel grain fill (Below, 2002). There were some trends of increased grain yield when *Source* was applied either in-furrow at planting or foliarly at the V4 growth stage in combination with 60 lbs N acre^{-1}. Similar to the results at Harrisburg, both

Source treatments tended to increase kernel number regardless of N fertilizer rate at Champaign (Table 1.6).

At Yorkville, the highest yielding environment, grain yield and kernel number were affected by N fertilizer rate, but were not affected by *Source* treatment or the interaction of *Source* and N rate (Table 1.5). Also, average kernel weight responded to N fertilizer rate and the interaction of N rate and *Source* treatment. Either *Source* application tended to increase grain yield in combination with all N fertilizer rates, except for the foliar application at 60 lbs N acre^{-1} (Table 1.6). The largest *Source*-driven yield response occurred from *Source* applied foliarly at the V4 growth stage in combination with 220 lbs N acre^{-1}, resulting in a 16 bushel acre^{-1} numerical increase, and this yield difference was due to an increase in kernel weight of 14 mg seed^{-1} (Table 1.6). This finding suggests that at the site with the highest soil organic matter and available N supply, in-season applications of *Source* in combination with the highest N fertilizer rate may be improving leaf photosynthetic activity later in the season, thereby increasing kernel dry matter accumulation. This phenomenon of maintaining green leaf tissues commonly occurs due to applications of fungicides that include strobilurin, leading to a longer period for dry matter transport and accumulation into the grain (Byamukama et al., 2018) and is caused by a reduction in the rate of chlorophyll degradation (Thomas and Howarth, 2000). This stay-green effect has also been observed when applying biostimulant products on corn and soybean (Ertani et al., 2011; Briglia et al., 2019).

When combined over all three locations, N rate influenced yield and both yield components, while *Source* treatment had an effect on yield and kernel number (Table 1.5). The main effects of in-furrow and V4 foliar *Source* applications were increases of 5 and 6 bushels acre^{-1}, respectively, compared to the untreated control; and these yield increases were associated with greater kernel production (181 or 130 kernels m^{-2} more for in-furrow and foliar *Source* applications, respectively) (Table 1.6). There was also a tendency of plots treated in-furrow with *Source* to produce a greater number of kernels compared to those treated with V4-foliar-applied *Source* and in contrast, foliar applications of *Source* tended to generate heavier kernels compared to in-furrow treatments (Table 1.6). This finding suggests that delaying *Source* applications later in the season has a lasting effect on the photosynthetic activity that persists into grain fill.

Nitrogen Accumulation

The total plant accumulation of N when no fertilizer N or *Source* treatment was applied was quantified as the amount of N supplied from the soil. Corresponding with each location's soil test values and grain yields, the soil N-supplying power was the highest at Yorkville with 106 lbs N $acre^{-1}$ and the lowest at Harrisburg with 39 lbs N $acre^{-1}$ (Table 1.7). This finding is in agreement with the high positive correlation between soil N analysis and the amount of corn N accumulation observed in other studies (Spencer, 1966).

Nitrogen fertilizer rate and *Source* treatment had an influence on grain, stover, and total N uptake at Harrisburg, while only N rate affected N accumulation values at Champaign and Yorkville (Table 1.8). At Harrisburg, applying either in-furrow or foliar applications of *Source* increased total plant N accumulation by 9 or 7 lbs N $acre^{-1}$, respectively, compared to the untreated control (Table 1.7). Increases of total plant N uptake were observed due to *Source* applications, regardless of N rate, suggesting that *Source* applications result in improvement of plant N accumulation, even when sufficient levels of fertilizer N were applied. Similar responses to *Source* treatment were observed in the accumulation of grain and stover N (Table 1.7). At Champaign, *Source*-induced increases in N uptake were not as apparent as in southern Illinois, although there were trends of increase from both in-furrow and foliar *Source* treatment in combination with the

60 lbs N acre^{-1} fertilizer rate (Table 1.7). At Yorkville, there was little to no effect of *Source* treatment on N accumulation except for an additional accumulation of 7 lbs N acre^{-1} from the V4 application of *Source* over the control at the fertilizer rate of 220 lbs N acre^{-1} (Table 1.7). We speculate that *Source* treatment only had a minor effect on the soil microbes that fix N_2 at Yorkville, most likely due to that soil's high fertility, organic matter level, and overall N-supplying power (Table 1.3). Additionally, both Champaign and Yorkville experienced weather conditions that typically promote soil N mineralization, but that were not conducive to N loss through leaching or denitrification, resulting in adequate amounts of available N (Table 1.4). Past research has shown that total N released from mineralization increases with warmer temperatures (Cassman and Munns, 1980). It is also well known that nitrate is susceptible to loss from the soil with excess water and that denitrification occurs in soils that have a lack of oxygen when waterlogged (Bernhard, 2010). In May, temperatures were warmer than average, which, in combination with average rainfall amounts promoted adequate N availability. These environmental conditions may have been the reason for the minimal effects of *Source* treatment on plant N accumulation at these higher-yielding locations.

Nitrogen Use Efficiency

At Harrisburg, N fertilizer rate and *Source* treatment affected both yield efficiency and recovery efficiency (Table 1.8). Compared to the control, the main effects of in-furrow and foliar applications of *Source* increased yield efficiency by 0.18 and 0.14 bushel lb N^{-1} applied, and these treatments led to the plants becoming 14.7 % and 10.5 % more efficient at recovering fertilizer N, respectively (Table 1.9). This finding shows that either application of *Source* can be implemented at locations with lower inherent fertility to improve nitrogen use efficiency by aiding the plant to recover more of the applied N; consequently increasing yields. Utilization of biostimulants to improve nutrient use efficiency has been studied in a vast array of crops (Vernieri et al., 2006; Berlyn and Sivaramakrishnan, 1996; Colla et al., 2015; Khaliq at al., 2006). However, in the current study at Champaign and Yorkville, where the grain yields were larger, responses of yield efficiency and recovery efficiency to *Source* applications were less apparent (Table 1.9). Additionally, both yield efficiency and recovery efficiency values were the least at Yorkville, where the check plot produced plants with the greatest yields and total N accumulation (Table 1.9).

2018 Conclusions

In the 2018 growing season, *Source* application, regardless of the timing, increased grain yield, N accumulation, and N use efficiency values compared to the control at Harrisburg. These results suggest that at lower-yielding environments, *Source* applications may have a lasting effect on the activity of N_2-fixing bacteria in the soil to improve plant N availability, resulting in an increase in season-long plant N accumulation and N use efficiency. These increases in N uptake and N use were associated with increases in grain yield. Similar trends were observed at the higher-yielding locations of Champaign and Yorkville. At environments with the least N limitation (220 lbs N acre^{-1} of fertilizer at Yorkville), foliar applications of *Source* at the V4 growth stage may improve leaf photosynthetic activity, resulting in an increase in kernel weight and a tendency for increased total N accumulation. These findings suggest that early applications of *Source* improved the yield potential of corn in all environments. However, when N was not limiting, delaying *Source* applications until later in the season might have had a lasting effect on the photosynthetic activity in the plant through grain fill. These findings were the groundwork for the trial that was designed and implemented in the 2019 growing season, based on the idea of simultaneously improving kernel number and average kernel weight from a combination of early- and late-season applications of *Source*.

Influence of Application Timing of Source on Nitrogen Uptake and Use, 2019

Trial Redesign

Due to the large interval between the nitrogen fertilizer rates of 60 and 220 lbs N acre^{-1} in the 2018 study, a rate of 120 lbs N acre^{-1} was added in 2019. Additionally, due to the initial finding that V4 foliar-applied *Source* increased kernel weight in non-limiting N environments, the trial was redesigned to compare single foliar applications of *Source* at the four-leaf plant growth stage (V4) or at the tassel stage (VT), as well as the synergy of applying *Source* twice in the growing season at both V4 and VT (Table 1.2). The hypothesis tested was to determine if corn kernel number and kernel weight could be increased with earlier and/or later season applications of *Source*.

Soil Characteristics

Before planting, composite soil samples were taken at each field site to measure organic matter, pH, CEC, nitrate, ammonium, phosphorus, potassium, calcium, magnesium, sulfur, zinc, manganese, and boron levels (Table 1.10). Similarly to 2018, the soil test values varied across the locations, and in general, native soil organic matter and CEC levels trended to increase moving from the southern to the northern location, with Yorkville soils containing the highest inherent soil fertility levels (Table 1.10).

Weather

The 2019 production year was characterized by less than ideal growing conditions across the state of Illinois. All three of the research sites received higher than average precipitation in April and May, delaying planting until June (Table 1.11). Following the above-average rainfall in the spring, all locations endured drier than normal conditions in June and July, and this deficiency was most apparent during pollination, which likely hindered growth and final grain yields (Table

1.11). Also, the Ewing site continued with precipitation deficits of 0.9 inches in August and 3.2 inches in September (Table 1.11). Grain yield can be markedly affected by a lack of moisture during flowering and pollination due to improper fertilization (Setter, 2001). However, the northern Illinois site received continued elevated rainfall of 8.9 inches more than the 30-year average in September (Table 1.11). The temperature at all locations in 2019 remained close to the 30-year average for the majority of the growing season (Table 1.11).

Grain Yield and Yield Components

At the southern Illinois site of Ewing in 2019, grain yield and kernel number were affected by N fertilizer rate and *Source* treatment, while there was no N rate by *Source* interaction (Table 1.12). Additionally, while N rate influenced average kernel weight, neither *Source* treatment nor the interaction of N rate and *Source* treatment affected average kernel weight at Ewing (Table 1.12). With the wet spring followed by dry June, July, August, and September months, the corn grain yields at Ewing were less than normal, with an average of 75.5 bushels acre^{-1} (Tables 1.11 and 1.13). Even though the yields were low, they were increased by 6 bushels acre^{-1} when *Source* was applied twice, at both the V4 and VT growth stages (Table 1.13). Notably, the largest yield response to *Source* applications of 14 bushels acre^{-1} occurred at the 120 lbs N acre^{-1} rate, equivalent to providing another 100 lbs N acre^{-1} without *Source* (Table 1.13). Plant growth-promoting rhizobacteria had been found to have a larger effect on grain yields when applied in soils with lower to intermediate inherent soil fertility, corresponding with the corn grain yield responses from applications of *Source* in the study presented here (Laudick, 2017). The single applications of *Source* at V4 or VT at Ewing tended to increase grain yield at most N rates; but especially at the higher rates of fertilizer applied N (120 and 220 lbs N acre^{-1}), regardless of plant growth stage at the time of application (Table 1.13). Yield responses from *Source* applications at Ewing were due to alterations in both kernel number and average kernel weight (Table 1.13). The V4 application of *Source* tended to foster the development of more kernels at the higher rates of applied N; however, adding the VT application along with the V4 treatment resulted in a greater increase in kernel number (Table 1.13). This finding suggests that there may be a synergistic response to applying *Source* twice in the growing season. Even though supplying *Source* at VT led to fewer kernels compared to the control, those kernels tended to be heavier, regardless of N rate (Table 1.13). Similarly, kernels tended to be heavier in response to the dual application of *Source* at Ewing (Table 1.13). Applications of biostimulants have been shown to help reduce environmental stresses, especially during the plant reproductive stages, to keep the plants healthier and greener longer, thereby resulting in more photosynthates and improved yields (Bulgari et al., 2019).

At Champaign, grain yield, kernel number, and kernel weight were affected by N fertilizer rate; however, there was no influence of *Source* application or the interaction of N rate and *Source* on these parameters (Table 1.12). The check plot yield at Champaign was 90 bushels acre^{-1} greater than the check plot yield at Ewing, indicating greater soil mineralization of N and the availability of other essential nutrients (Table 1.13). When *Source* was applied at VT with no additional N fertilizer, yield tended to increase by 14 bushel acre^{-1}, and when that application was combined with 220 lbs N acre^{-1}, there was a yield increase of 6 bushels acre^{-1} (Table 1.13). Interestingly, the dual application of *Source* in combination with the 120 lbs N acre^{-1} rate produced yields comparable to plots receiving 220 lbs of N acre^{-1} without *Source*, similar to the yield results at Ewing. Applying *Source* led to variable yield component changes at Champaign, affecting mostly average kernel weights (Table 1.13). *Source* applied at V4+VT with either 60 or 120 lbs N acre^{-1} tended to increase kernel weight by 10 mg kernel^{-1}, while application at VT tended to generate heavier kernels in combination with 60 lbs of applied N acre^{-1} (Table 1.13).

At Yorkville, N rate affected grain yield and both yield components; however, neither the *Source* applications nor the interaction of N rate and *Source* treatment led to any changes in these parameters (Table 1.12). Due to the above-average rainfall throughout the season, N in the soil had a higher susceptibility to loss, resulting in a lower check plot yield at Yorkville compared to Champaign (Tables 1.11 and 1.13). It has been shown that elevated rainfall can cause N loss through leaching due to the negative charge of nitrate (Nangia et al., 2010; Bernhard, 2010). Losses of N can have a substantial impact on grain yield due to the large quantities of this nutrient required by corn (Davis and Westfall, 2009). The dual application of *Source* at V4+VT tended to increase grain yield in combination with most N rates, with the greatest response of 6 bushels acre^{-1} at 120 lbs N acre^{-1} (Table 1.13). The yield response from the V4+VT application of *Source* was mainly due to heavier kernels, as average kernel weight tended to increase at every N rate (except 220 lbs N) when *Source* was applied twice (Table 1.13).

When averaged over the three locations, N fertilizer rate affected grain yield and both yield components, while *Source* treatment influenced grain yield and average kernel weight (Table 1.12). The dual application of *Source* increased yield when averaged across N rates and was most apparent at higher rates of N, with the largest response of 8 bushels acre^{-1} observed with 120 lbs N acre^{-1} (Table 1.13). This data shows that there may be a synergistic response of applying *Source* twice in the growing season and suggests a continued enhancement of N availability late in the season from two applications. Similar results were documented on applications of a foliar biostimulant at the V4 and V15 growth stages producing greater corn grain yields compared to a single application at V5, indicating synergy when applying at both early and later growth stages (Trivedi at al., 2017). Nitrogen availability during grain fill is critical for maximum yields. Any N stress during filling reduces the kernel sink capacity (Paponov et al., 2005; Melchiori and Caviglia, 2008). A large portion of N in the grain is due to remobilization from stover tissue; however, it has been observed that more than 25 % of N in the grain was accumulated into the plant during grain fill and not supplied by remobilization (Bender, 2012). Supplying *Source* at VT also tended to increase yield in combination with either 0 or 220 lbs N acre^{-1} (Table 1.13). Treatments that included the VT application of *Source* led to an increase of 2 (VT) or 3 (V4+VT) mg seed^{-1} in kernel weight when averaged over N rates (Table 1.13).

Nitrogen Accumulation

In 2019, N rate had an effect on grain, stover, and total N uptake at all three sites, while *Source* treatment affected stover N accumulation at Ewing and Champaign and total N accumulation when averaged across the three locations (Table 1.14).

At the site with the lowest inherent soil fertility (Ewing), plants with no N fertility or *Source* application accumulated 46.1 lbs N acre^{-1}, which was the least of all three sites (Table 1.15). Additionally, the ratio of grain to stover N uptake was much less than the other two sites, indicating a limitation in sink capacity, which also corresponded to fewer and lighter kernels on average at Ewing (Tables 1.13 and 1.15). Similarly, when sink capacity was reduced in wheat grain, N accumulation was reduced (Dordas, 2009). All applications of *Source* at Ewing tended to increase grain N accumulation, and these increases were most apparent in combination with greater rates of N fertilizer (120 or 220 lbs/acre) (Table 1.15). Additionally, applying *Source* at both V4 and VT led to the greatest grain N accumulation response (2.7 lbs N acre^{-1}) when averaged across N fertilizer rates (Table 1.15). Treatments that included an application of *Source* at tasseling (VT and V4+VT) increased both stover and total plant N accumulation (by 5.3 and 8.0 lbs N acre^{-1}, respectively) (Table 1.15). Soil permanganate oxidizable carbon (POXC), is a soil health parameter that is used to determine microbial biomass and can correlate these results to activity of soil microbes. Peak microbial biomass of POXC has been determined to occur under a corn-soy-wheat rotation and conventional tillage in mid-July in Michigan (Culman et al., 2013). This period corresponds to when corn plants are typically in the VT or R1 growth stages and also is the stage that corn root biomass is typically at its maximum (Anderson, 1987). These conditions of elevated soil microbial activity and maximum root biomass at VT coinciding with *Source* applications potentially led to greater N availability in the soil. Similar to grain N accumulation, *Source* applications tended to have a greater effect on stover and total plant N uptake in combination with the higher rates of fertilizer N (Table 1.15). This response to the double *Source* application was especially apparent at the N rate of 120 lbs acre^{-1}, which resulted in more stover and total N accumulation than those plants receiving 220 lbs N acre^{-1} with no *Source* treatment (Table 1.15). Additionally, plants treated with *Source* throughout the growing season (twice) tended to accumulate more plant N compared to either single application of *Source* (Table 1.15), further showing that *Source* can have a synergistic effect when applied at both the V4 and VT growth stages.

At Champaign, the soil was able to supply corn plants with more than double the amount of N compared to Ewing, resulting in check plot plants accumulating 116.8 lbs N acre^{-1} (Table 1.15). Therefore, the soil and environmental conditions at Champaign combined to mineralize more N than at Ewing. When *Source* was applied at V4 at Champaign in 2019, there were no changes in plant N accumulation (Table 1.15). However, plants that were supplied with *Source* at VT alone in combination with either no N fertilizer or 220 lbs N acre^{-1} tended to accumulate more N into grain tissues. But these grain N increases from VT applications of *Source* resulted in less stover N content when averaged over the N rates (Table 1.15). This data suggests that delaying *Source* applications to VT can help improve the uptake and remobilization of N later in the season into grain tissues; however, it may hinder the final N content in the stover. Applications of biostimulants have been found to have promoted glutamate production, and in turn increased the remobilization of N to storage forms in jute plants (Carillo et al., 2019). In a similar manner, *Source* applications at VT in Champaign remobilized N from the stover into the grain. On the other hand, applying *Source* at both V4 and VT resulted in the greatest average total N accumulation (180.0 lbs N acre^{-1}) compared to the other *Source* treatments, and these trends of increases in total N content were observed regardless of N fertilizer supply (Table 1.15). This same trend was found in stover N uptake when *Source* was applied at V4+VT (Table 1.15), indicating that dual applications of *Source* increased N uptake compared to none or a single treatment.

Even though the Yorkville soil analysis revealed greater organic matter, NO_3^-, and NH_4^+ levels compared to the soils at Champaign (Table 1.10), check plot plants at Yorkville accumulated 16.8 lbs N acre^{-1} less than at Champaign (Table 1.15). This discrepancy is most likely due to the 10.6 inches above average rainfall that Yorkville received in the growing season resulting in the potential for an extensive loss of N from leaching and denitrification. The lesser N uptake in Yorkville compared to Champaign also correlated with lower grain yields (Tables 1.13 and 1.15). Applications of *Source* at V4, either alone or also at VT, tended to increase total N accumulation by 2.6 and 2.8 lbs N acre^{-1}, respectively, when averaged across N fertilizer rates (Table 1.15). These responses in plant total N accumulation to V4 applications of *Source* were most notable in conditions when no N fertilizer was applied or at a rate of 220 lbs N acre^{-1} at Yorkville. Slight increases in both grain and stover N contents contributed to the tendency for increased total N accumulation observed (Table 1.15)

When averaged over all locations, total plant N accumulation increased by 4.9 lbs N acre^{-1} when *Source* was applied twice (Table 1.15). Additionally, at the highest fertilizer N rate of 220 lbs N acre^{-1}, all *Source* applications numerically increased total plant N uptake (Table 1.15). Therefore, across the state of Illinois, dual applications of *Source* at V4 and VT increased N accumulation at a typical fertilizer N rate that growers utilize.

Nitrogen Use Efficiency

In 2019, N fertilizer rate affected both yield efficiency and recovery efficiency at all three locations individually, and when averaged across locations (Table 1.14). The *Source* treatment also influenced both yield efficiency and recovery efficiency when averaged across locations, as well as recovery efficiency at the Champaign site (Table 1.14). Additionally, the two variables interacted with each other in terms of recovery efficiency at Champaign, and when the three locations were averaged (Table 1.14). Similarly to the 2018 growing season, both N efficiency values decreased as N fertilizer rates increased (Table 1.16).

At Ewing, *Source* treatment tended to increase yield and recovery efficiency at the higher N rates of 120 and 220 lbs N acre^{-1}, regardless of the timing of application (Table 1.16). The greatest increase over the control for yield efficiency was 0.11 bushel lb N^{-1} and 11.7% for recovery efficiency, both corresponding with the dual application of *Source* with 120 lbs N acre^{-1} of fertilizer (Table 1.16). Nitrogen use efficiency values in Ewing were less than the other sites due to the low levels of plant N accumulation (Table 1.13) caused by a low kernel sink capacity (Dordas, 2009). When calculating nutrient use efficiency parameters, the check plot accumulation of that nutrient is subtracted from the total amount of that nutrient in the plant and then divided by the applied amount of that nutrient. The amount of N accumulated was typically lower than the amount applied, resulting in inefficiency of N use.

At Champaign, corn plants treated with *Source* twice, at V4 and VT, were the most efficient in N use compared to the other treatments or the other locations (Table 1.16). Foliar applications of a biostimulant at both early and late vegetative stages reportedly have resulted in increased corn nutrient uptake as well as grain yields compared to a single foliar application, thereby improving nutrient use efficiency values (Trivedi et al., 2017). Yield efficiency values tended to be slightly increased by the dual *Source* treatment in combination with the 120 and 220 lb acre^{-1} rates of N at Champaign. Also, N recovery efficiency was increased over the control in response to the dual application of *Source*, increasing by 36.1% at the fertilizer N rate of 60 lbs acre^{-1} (Table 1.16). The single applications of *Source*, regardless of the timing, had no effect or tended to decrease plant N utilization compared to the control. In contrast, the recovery efficiency decreased when plants were grown at 120 lbs N acre^{-1} and Source applied at VT due to less N in the stover of these plants at Champaign (Tables 1.15 and 1.16).

At Yorkville, there were similar, but lesser, *Source* treatment responses for N use efficiency values than those recorded at Champaign. Single applications of *Source* led to no effect or lower yield and recovery efficiency values, but the dual application of *Source* tended to increase both efficiency values over the control (Table 1.16).

The consistent trend of increases in both yield and recovery efficiencies due to the dual applications of *Source* at V4 and VT at each site led to overall increases in these parameters when averaged across the three sites in Illinois (Table 1.16). This data further suggests that regardless of environment, applying *Source* at both an early vegetative stage and the start of grain fill can increase the uptake and use of N by corn plants to produce grain yield. These responses of both yield efficiency and recovery efficiency can aid farmers in the utilization of the fertilizer that they apply or even lessen fertilizer rates and obtain similar grain yields; furthermore reducing N loss to the environment (Zhou et al., 2019).

2019 Conclusions

In 2019, some foliar *Source* applications increased grain yield, N uptake, and N use efficiency values over the untreated control at all locations, but were most apparent at the lowest yielding environment (Ewing), similar to the 2018 growing season. Most notably, the *Source* treatment leading to the largest yield and N-based responses compared to the control consistently occurred when *Source* was applied foliarly at both the V4 and VT growth stages in combination with the higher N fertilizer rates of 120 and 220 lbs N acre^{-1}. This finding suggests an additive or synergistic effect of applying *Source* twice during the growing season. As expected, treatments that included a V4 application of *Source* fostered a greater number of kernels, while delaying the treatment to VT resulted in heavier average kernel weights.

The overall results of the *Source* microbial enhancer trials from the 2018 and 2019 growing seasons show the potential of applying *Source* in a corn management system to increase plant N uptake and grain yields, while reducing the effects of N loss in the environment and increasing farmer profits.

TABLES

Table 1.1 Nine treatments used in the evaluation of the influence of three *Source* applications combined with differing rates of nitrogen fertilizer on corn production at Harrisburg, Champaign, and Yorkville, IL in 2018.

Nitrogen Rate (lbs N/acre)†	***Source* Application‡**
0	None In-furrow (IF) Foliar
60	None In-furrow (IF) Foliar
220	None In-furrow (IF) Foliar

† Nitrogen rates applied as urea pre-plant broadcast and incorporated into the soil.

‡ *Source* applied either in-furrow (IF) at planting or foliarly at the V4 growth stage at rate of 17 oz acre^{-1}.

Table 1.2 Sub-treatments used to create sixteen final treatments for evaluating the influence of four *Source* application timings combined with differing rates of nitrogen fertilizer on corn production at Ewing, Champaign, and Yorkville, IL in 2019.

Nitrogen Rate (lbs N/acre)†	***Source* Timing‡**
0	None
60	V4
120	VT
220	V4 + VT

† Nitrogen rates applied as urea pre-plant broadcast and incorporated into the soil.

‡ *Source* applied foliarly at rate of 17 oz acre^{-1}.

Table 1.3 Pre-plant soil properties (0-12” depth) and Mehlich 3-extraction-based mineral test results for the *Source* experimental corn sites conducted at Harrisburg, Champaign, and Yorkville, IL in 2018.

OM†	CEC	pH	NO_3	NH_4	P	K	Ca	Mg	S	Zn	Mn	B
%	Meq/100g	units	ppm									
Harrisburg												
2.3	17.1	6.5	15.5	5.2	20	143	2602	299	9	2.5	42	0.3
Champaign												
3.5	18.4	6.4	7.5	4.1	27	132	2405	439	5	0.8	48	0.4
Yorkville												
7.2	30.6	5.8	73.7	22.1	263	350	3553	712	18	11.8	16	1

† OM, organic matter; CEC, cation exchange capacity.

Table 1.4 Monthly weather data between 1 April and 31 October at Harrisburg, Champaign, and Yorkville, IL in 2018. Values presented are the average daily air temperature and the average monthly accumulated rainfall, with deviations from the 30-year average in parentheses (Illinois State Water Survey, 2020).

Location	April	May	June	July	August	September
Temperature, ºF						
Harrisburg	50 (-6)	73 (7)	78 (3)	78 (0)	76 (-1)	72 (3)
Champaign	46 (-6)	72 (9)	75 (3)	75 (0)	75 (2)	71 (5)
Yorkville	40 (-10)	67 (6)	71 (1)	72 (-2)	71 (-1)	66 (1)
Precipitation, Inches						
Harrisburg	5.3 (0.8)	5.0 (-0.1)	6.1 (1.6)	3.1 (-0.7)	5.0 (2.0)	7.8 (4.7)
Champaign	2.5 (-1.1)	4.2 (-0.7)	7.3 (3.0)	3.2 (-1.5)	4.0 (0.1)	4.7 (1.6)
Yorkville	1.0 (-2.9)	6.5 (2.2)	7.1 (2.8)	1.9 (-2.8)	2.8 (-1.3)	2.4 (-0.7)

Table 1.5 Test of fixed effects for grain yield and yield components (kernel number and average kernel weight) as influenced by nitrogen fertilizer rate and *Source* treatment at Harrisburg, Champaign, Yorkville, and averaged over all locations in Illinois in 2018.

Source of Variation	**Grain Yield**	**Yield Components**	
		Kernel Number	**Kernel Weight**
		P > F	
		Harrisburg	
Nitrogen (N)	<.0001	<.0001	<.0001
Source (S)	0.0440	0.0187	0.9652
N x S	0.6337	0.5995	0.9703
		Champaign	
Nitrogen (N)	<.0001	<.0001	<.0001
Source (S)	0.7797	0.4861	0.3426
N x S	0.8366	0.9507	0.5794
		Yorkville	
Nitrogen (N)	<.0001	<.0001	<.0001
Source (S)	0.6942	0.6111	0.3643
N x S	0.5436	0.9788	0.0241
		Averaged over Locations	
Nitrogen (N)	<.0001	<.0001	<.0001
Source (S)	0.0457	0.0044	0.2357
N x S	0.7761	0.9834	0.4153

Table 1.6 Grain yield and yield components (kernel number and average kernel weight) as influenced by nitrogen fertilizer rate and *Source* treatment at Harrisburg, Champaign, Yorkville, and averaged over all locations in Illinois in 2018. Corn grain yields and kernel weights are expressed at 15.5% and 0% moisture, respectively.

Nitrogen Rate	**Grain Yield**			**Kernel Number**			**Kernel Weight**		
	***Source* Treatment**								
	None	**IF**	**Foliar**	**None**	**IF**	**Foliar**	**None**	**IF**	**Foliar**
lbs N acre^{-1}	—— bushels acre^{-1} ——			—— number m^{-2} ——			—— mg seed^{-1} ——		
Harrisburg									
0	58	70	67	1526	1781	1729	203	207	204
60	115	135	130	2746	3253	3145	221	220	219
220	188	190	194	3830	3920	3980	260	258	259
Means	120	132*	130*	2701	2985*	2951*	228	228	227
Champaign									
0	95	92	98	2611	2767	2803	188	174	184
60	156	162	166	3964	4029	4007	208	213	219
220	237	238	234	4813	5029	4865	263	251	256
Means	163	164	166	3796	3942	3892	220	213	220
Yorkville									
0	171	176	175	3854	4026	3901	235	233	238
60	211	213	206	4718	4737	4755	237	239	229
220	250	253	266	5247	5394	5294	253	249	267*
Means	211	214	215	4606	4719	4650	242	240	245
Averaged over Locations									
0	108	113	113	2663	2858	2811	209	204	209
60	161	170	167	3809	4006	3969	222	224	223
220	225	227	231	4630	4781	4713	255	253	261
Means	165	170*	171*	3701	3882*	3831*	229	227	231

* Denotes a significant response ($P \leq .10$) from *Source* treatment compared to control within the same fertilizer rate.

Table 1.7 Plant nitrogen accumulation (grain, stover, and total) at physiological maturity as influenced by nitrogen fertilizer rate and *Source* treatment for corn grown at Harrisburg, Champaign, Yorkville, and averaged over all locations in Illinois in 2018.

Nitrogen Rate	Grain			Stover			Total		
	***Source* Treatment**								
	None	**IF**	**Foliar**	**None**	**IF**	**Foliar**	**None**	**IF**	**Foliar**
	lbs N acre^{-1}								
	Harrisburg								
0	27	32	31	12	14	13	39	46	44
60	52	63	60	22	27	26	74	90	86
220	99	102	101	43	44	44	142	146	145
Means	60	66*	64*	26	28*	28*	85	94*	92*
	Champaign								
0	36	35	39	15	15	16	51	50	55
60	61	63	66	26	27	28	87	90	94
220	109	109	104	47	47	45	156	156	149
Means	69	69	69	29	30	30	98	99	99
	Yorkville								
0	74	75	75	32	32	32	106	107	107
60	93	93	86	40	40	37	132	132	123
220	123	122	128	53	53	55	176	175	183
Means	97	97	96	41	41	41	138	138	137
	Averaged over Locations								
0	45	47	48	19	20	20	64	67	68
60	68	72	69	29	31	30	97	103	99
220	108	109	109	46	47	47	154	156	156
Means	74	76	75	31	33	32	105	109	107

* Denotes a significant response ($P \leq .10$) from *Source* treatment compared to control within the same fertilizer rate.

Table 1.8 Test of fixed effects for plant nitrogen accumulation (grain, stover, and total) at physiological maturity, yield efficiency, and nitrogen recovery efficiency as influenced by nitrogen fertilizer rate and *Source* treatment at Harrisburg, Champaign, Yorkville, and averaged over all locations in Illinois in 2018.

Source of Variation	**Nitrogen Accumulation**			**Yield Efficiency**	**Recovery Efficiency**
	Grain	**Stover**	**Total**		
	P > F				
			Harrisburg		
Nitrogen (N)	<.0001	<.0001	<.0001	<.0001	<.0001
Source (S)	0.0501	0.0501	0.0501	0.0858	0.0910
N x S	0.7888	0.7888	0.7888	0.1235	0.1533
			Champaign		
Nitrogen (N)	<.0001	<.0001	<.0001	<.0001	<.0001
Source (S)	0.9531	0.9531	0.9531	0.8347	0.8613
N x S	0.4596	0.4596	0.4596	0.6249	0.4015
			Yorkville		
Nitrogen (N)	<.0001	<.0001	<.0001	0.0057	0.0212
Source (S)	0.9965	0.9965	0.9965	0.8818	0.2095
N x S	0.4804	0.4804	0.4804	0.8623	0.2345
			Averaged over Locations		
Nitrogen (N)	<.0001	<.0001	<.0001	<.0001	0.0617
Source (S)	0.1929	0.1929	0.1929	0.2490	0.3267
N x S	0.8001	0.8001	0.8001	0.3338	0.4760

Table 1.9 Yield efficiency and nitrogen recovery efficiency as influenced by nitrogen fertilizer rate and *Source* treatment at Harrisburg, Champaign, Yorkville, and averaged over all locations in Illinois in 2018.

Nitrogen Rate	Yield Efficiency			Recovery Efficiency		
	Source Treatment					
	None	IF	Foliar	None	IF	Foliar
lbs N acre^{-1}	——— bushels lb N^{-1} ———			——— % ———		
			Harrisburg			
60	0.94	1.28	1.19	58.8	86.1	78.9
220	0.59	0.60	0.62	47.0	49.0	47.9
Means	0.76	0.94*	0.90*	52.8	67.5*	63.3*
			Champaign			
60	1.14	1.12	1.19	68.4	65.4	72.6
220	0.65	0.65	0.64	46.9	47.3	44.2
Means	0.89	0.89	0.91	57.7	56.4	58.4
			Yorkville			
60	0.86	0.90	0.77	43.3	58.0	36.8
220	0.42	0.50	0.49	31.4	34.6	34.9
Means	0.64	0.70	0.63	37.3	46.3	35.9
			Averaged over Locations			
60	0.94	1.10	1.05	47.9	58.5	51.5
220	0.56	0.56	0.58	40.1	41.3	40.7
Means	0.75	0.83	0.81	44.0	49.9	46.1

* Denotes a significant response ($P \leq .10$) from *Source* treatment compared to control within the same fertilizer rate.

Table 1.10 Pre-plant soil properties (0-12” depth) and Mehlich 3-extraction-based mineral test results for the *Source* experimental corn sites conducted at Ewing, Champaign, and Yorkville, IL in 2019.

OM†	CEC	pH	NO_3	NH_4	P	K	Ca	Mg	S	Zn	Mn	B
%	Meq 100g^{-1}	units	——— ppm ———									
						Ewing						
1.2	8.9	7.4	4.9	3.1	32	35	1658	67	10	0.7	135	0.1
						Champaign						
2.6	19.3	6.0	6.0	1.1	44	160	2349	429	10	1.5	31	0.5
						Yorkville						
5.5	32	6.1	13.2	9.3	45	152	4069	781	22	2.9	11	1

† OM, organic matter; CEC, cation exchange capacity.

Table 1.11 Monthly weather data between 1 April and 31 October at Ewing, Champaign, and Yorkville, IL in 2019. Values presented are the average daily air temperature and the average monthly accumulated rainfall, with deviations from the 30-year average in parentheses (Illinois State Water Survey, 2020).

Location	April	May	June	July	August	September
			Temperature, ºF			
Ewing	58 (0)	67 (0)	73 (-2)	79 (1)	76 (0)	75 (6)
Champaign	53 (1)	64 (1)	72 (0)	77 (2)	74 (1)	72 (6)
Yorkville	48 (-2)	58 (-3)	69 (-1)	75 (1)	69 (-3)	67 (2)
			Precipitation, Inches			
Ewing	7.1 (2.3)	7.0 (2.3)	3.5 (-0.5)	2.1 (-1.5)	2.2 (-0.9)	0.3 (-3.2)
Champaign	5.3 (1.7)	5.2 (0.3)	3.7 (-0.6)	2.3 (-2.4)	2.1 (-1.8)	3.3 (0.2)
Yorkville	4.8 (0.9)	8.4 (4.1)	2.6 (-1.7)	2.8 (-1.9)	4.4 (0.3)	12.0 (8.9)

Table 1.12 Test of fixed effects for grain yield and yield components (kernel number and average kernel weight) as influenced by nitrogen fertilizer rate and *Source* treatment at Ewing, Champaign, Yorkville, and averaged over all locations in Illinois in 2019.

Source of Variation	**Grain Yield**	**Yield Components** **Kernel Number**	 **Kernel Weight**
		P > F	
		Ewing	
Nitrogen (N)	<.0001	<.0001	<.0001
Source (S)	0.0749	0.0034	0.2045
N x S	0.5329	0.8976	0.2166
		Champaign	
Nitrogen (N)	<.0001	<.0001	<.0001
Source (S)	0.5873	0.8045	0.9574
N x S	0.1750	0.3414	0.2572
		Yorkville	
Nitrogen (N)	<.0001	<.0001	<.0001
Source (S)	0.3810	0.5456	0.6623
N x S	0.9941	0.8709	0.2498
		Averaged over Locations	
Nitrogen (N)	0.0011	<.0001	0.0178
Source (S)	0.0928	0.2776	0.0931
N x S	0.3255	0.5235	0.1509

Table 1.13 Grain yield and yield components (kernel number and average kernel weight) as influenced by nitrogen fertilizer rate and foliar *Source* treatment at Ewing, Champaign, and Yorkville, IL and averaged across the three locations in 2019. Corn grain yields and kernel weights are expressed at 15.5% and 0% moisture, respectively.

Nitrogen Rate	Grain Yield				Kernel Number				Kernel Weight			
	Source Treatment											
	None	V4	VT	V4+ VT	None	V4	VT	V4+ VT	None	V4	VT	V4+ VT
lbs N acre^{-1}	bushels acre^{-1}				number m^{-2}				mg seed^{-1}			
					Ewing							
0	51	52	49	55	1720	1718	1635	1818	157	162	159	161
60	72	74	71	70	2384	2396	2252	2373	160	163	167	159
120	78	81	83	92	2587	2692	2449	2801	161	160	163	173
220	92	95	95	98	2880	2933	2854	3039	170	173	176	171
Means	73	75	75	79*	2393	2435	2298	2508*	162	164	166	166
					Champaign							
0	141	139	155	139	3523	3578	3863	3567	213	208	213	207
60	193	192	192	196	4427	4444	4281	4356	230	230	238	240
120	213	211	206	218	4686	4580	4560	4593	242	245	241	252
220	220	221	226	222	4609	4532	4741	4777	254	260	252	247
Means	192	191	195	194	4311	4284	4361	4323	235	235	236	236
					Yorkville							
0	129	132	127	133	3655	3688	3590	3654	187	190	187	193
60	176	171	173	176	4650	4420	4548	4618	201	206	202	203
120	195	195	191	201	5028	5085	4818	5083	206	204	210	210
220	221	217	220	223	5292	5482	5388	5528	222	210	217	214
Means	180	179	178	183	4656	4668	4586	4721	204	202	204	205
					Averaged over Locations							
0	107	107	113	109	2969	2994	3043	3013	185	186	186	187
60	147	145	145	147	3819	3748	3691	3784	197	200	202	200
120	162	162	160	170	4100	4119	3944	4167	203	203	205	212
220	178	178	181	182	4256	4310	4331	4449	215	215	215	211
Means	149	148	150	152*	3786	3793	3752	3853	200	201	202*	203*

* Denotes a significant response ($P \leq .10$) from *Source* treatment compared to control within the same fertilizer rate.

Table 1.14 Test of fixed effects for plant nitrogen accumulation (grain, stover, and total) at physiological maturity, yield efficiency, and nitrogen recovery efficiency as influenced by nitrogen fertilizer rate and *Source* treatment at Ewing, Champaign, Yorkville, and averaged over all locations in Illinois in 2019.

Source of Variation	**Nitrogen Accumulation**			**Yield Efficiency**	**Recovery Efficiency**
	Grain	**Stover**	**Total**		
			P > *F*		
			Ewing		
Nitrogen (N)	<.0001	<.0001	<.0001	<.0001	0.0004
Source (S)	0.1497	0.0081	0.0103	0.3400	0.1394
N x S	0.4354	0.5701	0.3862	0.1964	0.2302
			Champaign		
Nitrogen (N)	<.0001	<.0001	<.0001	<.0001	<.0001
Source (S)	0.4400	0.0268	0.2540	0.3034	0.0004
N x S	0.3043	0.7719	0.2737	0.8475	0.0021
			Yorkville		
Nitrogen (N)	<.0001	<.0001	<.0001	<.0001	<.0001
Source (S)	0.5689	0.5791	0.5342	0.3916	0.2029
N x S	0.9926	0.2126	0.7067	0.9821	0.6158
			Averaged over Locations		
Nitrogen (N)	<.0001	0.0073	0.0075	0.0211	0.0241
Source (S)	0.5560	0.1471	0.0589	0.0255	0.0003
N x S	0.9973	0.1332	0.2021	0.7604	0.0998

Table 1.15 Plant nitrogen accumulation (grain, stover, and total) at physiological maturity as influenced by nitrogen fertilizer rate and foliar *Source* treatment for corn grown at Ewing, Champaign, and Yorkville, IL and averaged across the three locations in 2019.

Nitrogen Rate	Grain				Stover				Total			
	Source Treatment											
	None	V4	VT	V4+ VT	None	V4	VT	V4+ VT	None	V4	VT	V4+ VT
	lbs N acre^{-1}											
	Ewing											
0	25.7	25.6	24.9	27.5	20.3	19.0	24.1	23.4	46.1	43.4	48.9	50.9
60	34.5	34.4	33.4	33.8	24.8	24.4	23.8	26.9	59.3	58.2	57.2	60.6
120	37.2	38.5	40.1	44.1	23.7	26.6	26.2	34.0	60.7	65.1	66.3	78.0
220	44.9	46.5	49.2	47.7	27.0	28.8	33.1	33.0	72.1	75.5	82.3	80.8
Means	35.6	36.3	36.9	38.3	24.0	24.7	26.8*	29.3*	59.6	60.5	63.7*	67.6*
	Champaign											
0	78.4	76.6	87.9	76.1	38.4	39.5	35.8	37.4	116.8	116.1	123.7	113.6
60	116.3	113.6	111.9	120.6	50.9	49.6	48.3	59.6	167.2	163.4	165.4	176.3
120	140.9	138.1	134.9	144.3	57.1	55.6	47.5	58.4	198.0	190.3	176.2	201.8
220	156.0	153.2	159.6	157.3	67.1	67.6	65.0	71.1	223.1	220.6	224.3	228.4
Means	122.9	120.4	125.6	124.6	53.4	53.0	49.2*	56.6	176.3	172.6	172.4	180.0
	Yorkville											
0	64.2	69.2	64.2	69.6	36.6	40.8	41.9	40.9	100.0	110.5	106.4	110.5
60	94.8	91.5	92.3	94.6	53.0	49.5	50.7	53.4	147.7	141.0	143.0	148.0
120	109.4	109.1	109.4	113.5	65.1	57.0	49.4	55.9	174.5	166.1	158.7	168.7
220	128.0	131.0	130.1	131.4	60.2	72.4	62.4	65.6	188.4	203.4	188.2	194.2
Means	99.1	100.2	99.0	102.3	53.7	54.9	51.1	53.9	152.6	155.2	149.1	155.4
	Averaged over Locations											
0	56.2	56.9	59.4	57.7	31.9	32.9	33.5	33.9	87.8	89.9	93.1	91.7
60	81.8	80.1	82.0	83.0	42.9	41.1	40.9	45.1	124.8	120.9	121.8	128.1
120	95.8	95.3	95.0	100.6	49.1	46.4	41.3	49.7	145.0	140.5	134.9	149.7
220	109.7	110.2	112.9	112.2	49.8	55.9	52.0	56.9	160.1	166.3	164.9	168.0
Means	85.9	85.6	87.3	88.4	43.4	44.1	41.9	46.4	129.4	129.4	128.7	134.3*

* Denotes a significant response ($P \leq .10$) from *Source* treatment compared to control within the same fertilizer rate.

Table 1.16 Yield efficiency and nitrogen recovery efficiency as influenced by nitrogen fertilizer rate and foliar *Source* treatment at Ewing, Champaign, and Yorkville, IL and averaged across the three locations in 2019.

Nitrogen Rate	Yield Efficiency				Recovery Efficiency			
	Source Treatment							
	None	V4	VT	V4+VT	None	V4	VT	V4+VT
lbs N acre^{-1}	bushel lb N^{-1}				%			
				Ewing				
60	0.37	0.39	0.29	0.36	30.2	23.0	21.4	26.6
120	0.24	0.26	0.28	0.35	16.4	17.3	18.3	28.1
220	0.21	0.23	0.23	0.22	13.7	14.6	17.2	17.3
Means	0.28	0.29	0.27	0.31	20.1	18.3	19.0	24.0
				Champaign				
60	0.86	0.83	0.74	0.84	74.5	67.4	70.2	110.6*
120	0.60	0.58	0.52	0.64	67.6	67.8	49.7*	67.7
220	0.36	0.36	0.37	0.37	48.3	47.6	48.8	50.7
Means	0.61	0.59	0.55	0.62	63.5	60.9	56.2	76.4*
				Yorkville				
60	0.78	0.68	0.71	0.77	79.9	60.4	73.9	84.2
120	0.54	0.54	0.51	0.59	58.1	51.1	45.0	59.1
220	0.43	0.39	0.37	0.42	37.9	44.8	38.5	47.2
Means	0.58	0.54	0.53	0.59	58.6	52.1	52.5	63.5
				Averaged over Locations				
60	0.61	0.63	0.58	0.66	61.8	50.6*	55.0	70.3*
120	0.46	0.46	0.44	0.53	47.4	45.4	38.1*	51.6
220	0.33	0.33	0.32	0.34	33.5	35.5	34.6	38.4
Means	0.47	0.47	0.45	0.51*	47.6	43.8	42.6*	53.5*

* Denotes a significant response ($P \leq .10$) from *Source* treatment compared to control within the same fertilizer rate.

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CHAPTER 2. IMPROVING FERTILIZER USE AND CORN PRODUCTIVITY WITH A PHOSPHORUS SOLUBILIZING BACTERIA

ABSTRACT

Biological products are becoming more prevalent in the agricultural market as products to improve nutrient use, plant growth, leaf health, grain yield, and to improve soil health. Research on these technologies is important to discover their impacts in a corn management system. The objective of this research was to study the effects of applications of a phosphorus solubilizing bacteria (PSB) called *iNvigorate* (Agrinos, Davis, CA) on corn grain yield and the accumulation of important mineral nutrients. In 2018, corn was grown at one of five rates of pre-plant nitrogen (N) (broadcast, incorporated urea) and phosphorus (P) (monoammonium phosphate, also known as MAP) fertilizer at 0/0, 180/60, 180/20, 90/60, and 90/20 lbs N/P_2O_5 per acre and was either treated with or without *iNvigorate* applied in-furrow at planting. While in 2019, corn was grown at four rates of P fertilizer (pre-plant banded as triple superphosphate, also known as TSP) at 0, 30, 60, and 90 lbs P_2O_5 per acre and received one of three *iNvigorate* applications: either an untreated control, in-furrow at planting, or Y-drop at the V6 growth stage. These studies were implemented at Champaign, IL in both years. In 2018, supplying *iNvigorate* in-furrow had minimal effect on corn grain yield and yield components; however, *iNvigorate* led to a 6 pounds acre^{-1} increase in plant total P_2O_5 accumulation on average, across fertility rates. In 2019, when the phosphorus fertilizer was applied in a band, in-furrow applications of *iNvigorate* increased corn grain yield by 8 bushels acre^{-1}, when averaged across P fertilizer rates. These yield responses to *iNvigorate* were driven by an increase of 304 kernel m^{-2} compared to the control. Additionally, these yield responses from supplying *iNvigorate* correlated with an increase in plant P_2O_5 accumulation by 8 pounds acre^{-1} as well as an increase in accumulation of other important mineral

nutrients. The results of this research show that *iNvigorate* applications can increase soil P availability and corn uptake, as well as grain yield and yield potential.

INTRODUCTION

As the global population increases along with the demand for food, crop phosphorus (P) fertilizer requirements are predicted to increase by 50 to 100% by 2050 (Cordell et al., 2009). As crop yields increase to combat the rise in population, growers are depending more on commercial fertilizers due to the depletion of native soil fertility levels. Phosphorus within the plant is essential for building DNA, phosphoproteins, phospholipids, sugar phosphates, enzymes, and energy-rich phosphate compounds. These plant components are essential for photosynthesis, genes duplication in plant growth, energy storage and use, and nutrient transport, storage, and metabolism within the plant (Armstrong et al., 1999). Around 90% of the global demand for P is for food production, totaling approximately 148 million metric tons of phosphate rock per year (Cordell et al., 2009). This reliance on commercial fertilizers is especially apparent in areas like the U.S. Corn Belt, where manure sources of P are diminishing as a consequence of fewer livestock operations. Inherent soil P levels are notably affected by increases in corn grain yield due to the large proportion of P removed with the grain (P harvest index). Of the essential mineral nutrients for corn, P has the highest harvest index, approximately 79% (Bender, 2013).

Despite the importance of maintaining P soil levels, consequences of extensive applications of P arise, including increasing eutrophication in water sources due to P loss from the soil and diminishing the world's supply of phosphate rock. Phosphorus is bound tightly to soil particles that can be carried off into local water sources through erosion and runoff. Eutrophication is characterized by excessive plant and algal growth in bodies of water; consequently, decreasing the quality for human consumption as well as the ability to support wildlife (Chislock et al., 2013).

Phosphorus fertilizer runoff is the leading source of river, stream, and lake contamination (Daniel et al., 1998) which ultimately leads to the intensification of the hypoxic zone in the Gulf of Mexico. In 2008, the hypoxic zone in the Gulf of Mexico was reported to be one of the largest in the world (Rabotyagov, 2010). Methods of restoring this zone and other water sources are often expensive and difficult to perform, resulting in a long term remediation strategy. Additionally, over-fertilization of P may cause a long term issue of depleting phosphate rock mines. Although most projections are variable, phosphate rock is practically a finite resource and will ultimately be used up (Reijnders, 2014). Because phosphate fertilizer is not a renewable resource, there is a need for further research to understand and discover new grower practices to improve agricultural yields while minimizing P loss.

One management strategy showing the potential to solve this problem is fertilizer placement. Broadcast applications evenly spread the fertilizer across the soil surface area and are a typical farmer practice. Broadcast applications of P fertilizer provide the most uniform distribution in the rooting zone and the most root contact with P. However, broadcast applications also promote the most P fixation because of the higher soil-to-fertilizer contact. Broadcast applications work well in environments of warm soil, high native soil test values, and adequate moisture, allowing root proliferation near the surface. In contrast, banded applications below the soil surface place the fertilizer nearer to the crop roots in a concentrated, narrow zone. Banded applications of P are especially important due to the immobility of P in the soil. With recent advancements in GPS technology, fertilizers can be applied in a band at a certain depth with minimum disruption to soil structure regardless of tillage system (Vyn, 2008). This method of application is beneficial in soils with low fertility, when soils are cool or wet-which likely limits root growth, and for soils with a high probability of fixing P in unavailable forms.

Phosphorus is abundant in soils in both organic and inorganic forms; however, P is still a limiting nutrient for corn growth as it is present mainly in unavailable forms. One of these unavailable forms includes organic P. Organic P is a large constituent of the total P present in the soil and includes plant and animal residues, soil organic matter, and soil micro-organisms. Soil inorganic forms of P mainly exist as insoluble mineral complexes and often occur following multiple fertilizer applications (Sharma et al., 2013). Only a small amount of P occurs in soils as soluble and plant available forms because P is susceptible to fixation. P fixation is described as the removal of available phosphate from the soil solution into a soil solid phase (Barber, 1995). Two types of P fixation can occur (a) phosphate sorption on the surface of soil minerals and (b) phosphate precipitation by iron, aluminum, and zinc (Mortvedt, 1991). The small amount of P in soils that is available for plant uptake are in the forms of $H_2PO_4^-$ and HPO_4^{2-} ions that are dissolved in the soil solution. Soil P is a dynamic process where soluble P can move between organic and other inorganic forms. Organic P is mineralized into readily available P for plant uptake and insoluble P forms can be solubilized into $H_2PO_4^-$ and HPO_4^{2-} ions in the soil solution. These processes are largely performed by native soil microorganisms and are crucial for sufficient available P for plant growth.

There is a large diversity of phosphorus solubilizing compounds or microorganisms in the soil that can create plant-available P forms. Mineralization of organic P is primarily carried out by enzymes (Sharma et al., 2013). These enzymes include phosphatases, phytases, phosphonatases, and carbon-phosphorus lyases. (Sharma et al., 2013). Solubilization of inorganic P forms is performed by bacteria and fungi. There exists a vast array of bacteria with P solubilization activity, including *Pseudomonas*, *Bacillu*s, *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium*, *Delftia* sp. (Wani et al. 2005; Chen et al. 2006), *Azotobacter* (Kumar et al. 2001), *Xanthomonas* (De Freitas et al. 1997), *Enterobacter*, *Pantoea*, and *Klebsiella* (Chung et al. 2005) *Vibrio proteolyticus*, *Xanthobacter agilis* (Vazquez et al. 2000). The particular product utilized for the research presented here has bacterial active ingredients of *Azotobacter* and *Clostridium* species. The main bacteria mechanism of P solubilization is the release of organic acid ions that chelate cations that are bound to P including calcium, aluminum, iron, and zinc. Following this process, the P is released and able to dissolve in the soil solution, thus becoming able for plant accumulation (Sharma et al., 2013). Recent technology has made it possible to culture these bacteria and apply them to the soil to restore this P solubilization activity.

Placement of phosphorus solubilizing bacteria (PSB) in-furrow would be advantageous for increased levels of plant-available P in the rooting zone for plants to accumulate. Recently, 360 Yield Center (Morton, IL) developed a technology with the ability to apply a liquid solution in a band on the soil surface directly next to the crop row, called Y-drop. With rain or heavy dew, the architecture of the corn plant leaves creates a water funneling system that flows down to the base of the plant and assists in incorporating the liquid solution into the ground. Little research on Y-drop applications of biological products has been conducted; however, with proper water incorporation, this PSB will be present in the rooting zone and create more available P for roots to intercept and acquire.

The objective of this research was to evaluate a PSB, called *iNvigorate*, regarding its optimal use to provide increased soil P availability and enhanced uptake of essential mineral nutrients in corn production. With improved nutrient accumulation, the corn plant is expected to produce greater grain yield. Determining the effects of supplementing corn growth with this bacterial product will help growers increase profits, while also reducing the environmental impact of P loss.

MATERIALS AND METHODS

Field Characteristics

The phosphorus-solubilizing bacteria (PSB) study was implemented in the 2018 and 2019 growing seasons at the Crop Science Research and Education Center (CSREC) located at the University of Illinois Urbana-Champaign. In both years, the trial site consisted of a Flanagan silt loam soil type.

Pesticide Applications

In both years, the location was maintained weed-free with a pre-emergence application of bicyclopryrone (bicyclo[3.2.1]oct-3-en-2-one, 4-hydroxy-3-[[2-[(2-methoxyethoxy)methyl]-6-[trifluoromethyl]-3-pyridinyl]carbonyl]) + mesotrione + S-metolachlor + atrazine known as Acuron (Syngenta, Basel, Switzerland) at a rate of 3 qt acre^{-1}. Additionally, in both years, all plots received an in-furrow soil insecticide application of tefluthrin ([2,3,5,6-tetrafluoro-4-methylphenyl]methyl-[1α,3α]-[Z]-[±]-3-[2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethylcyclopropanecarboxylate), known as Force 3G (Syngenta, Basel, Switzerland) at a rate of 4 oz acre^{-1}. In-season weed control in both growing seasons was applied at the V5 to V6 growth stages with atrazine, known as AAtrex 4L (Syngenta, Basel, Switzerland) at a rate of 1 qt acre^{-1}, topramezone (3-[4,5-dihydro-isoxazolyl]-2-methyl-4-[methylsulfonyl]phenyl [5-hydroxy-1-methyl-1H-pyrazol-4-yl]methanone), known as Armezon (BASF Corporation Ludwigshafen, Germany) at a rate of 0.75 oz acre^{-1}, glyphosate (N-phosphonomethyl glycine, in the form of a potassium salt), known as RoundUp PowerMax (Bayer, St. Louis, MO) at a rate of 32 oz acre^{-1}, and ammonium sulfate (AMS; 21-0-0-24S) at a rate of 0.2 gal acre^{-1}.

Agronomic Management

Soybean was the previous crop and conventional tillage was used in both seasons. A hybrid responsive to fertility, Golden Harvest G10T63-3122 with 110-day relative maturity (Syngenta, Basel, Switzerland), was planted to achieve a population of 36,000 plants acre^{-1}. Plots were planted with a Seed Pro 360 planter (ALMACO, Nevada, IA) on 7 May 2018 and 1 June 2019.

Treatment Applications

Applications were designed to evaluate a PSB, known as *iNvigorate* (Agrinos, Davis, CA), for its role in nutrient use and productivity of corn. In 2018, this product was supplied to corn plants in-furrow at planting and combined with differing rates of N and P from zero, limiting, to typically sufficient (Table 2.1). Fertility was broadcast-applied at rates of 0/0, 180/60, 180/20, 90/60, and 90/20 lbs N/P_2O_5 acre^{-1} pre-plant and incorporated into soil. The nitrogen fertilizer source was urea (46-0-0) and the P fertilizer used was monoammonium phosphate, i.e., MAP (11-52-0). In 2019, *iNvigorate* was applied in-furrow at planting and also by Y-drop (near the crop row) at the V6 (six fully collared leaves) growth stage and combined with differing rates of applied P (Table 2.2). Phosphorus was applied at 0, 30, 60, and 90 lbs P_2O_5 acre^{-1} as triple superphosphate i.e., TSP (0-45-0-15 Ca) at pre-plant and banded 4 to 6 inches below the soil surface directly under the crop row. For N fertility, 180 lbs N acre^{-1} was applied pre-plant by broadcasting and incorporated into the soil. All treatments of this *iNvigorate* were applied at a rate of 1 L acre^{-1}. The in-furrow treatments of *iNvigorate* were applied at planting to all plot rows with a planter-attached liquid starter applicator system (Surefire Ag Systems, Atwood, KS) and at a rate of 8 gal acre^{-1} with water as a carrier. Y-drop treatments of this bacteria were applied at the V6 growth stage by dribbling along the crop row using 8 oz bottles filled with *iNvigorate* and water at a rate of 15 gal acre^{-1}. In 2019, the y-drop applications of *iNvigorate* occurred on 27 June.

Experimental Design and Statistical Analysis

Treatments were arranged in a randomized complete block design with six replications and ten treatments for a total of 60 plots in 2018 and 12 treatments for a total of 72 plots in 2019. Each plot was four rows wide and 37.5 ft in length with 30 in row spacing. Statistical analysis was conducted using PROC MIXED in SAS (version 9.4; SAS Institute, Cary, NC). Phosphorus-solubilizing bacteria treatment and fertility rate were considered fixed effects in the model. Significance was declared at $P \leq 0.10$ due to the fastidious nature of biological products to be certain we would not miss differences that may exist. PROC GLM of SAS was utilized to conduct the Brown-Forsythe test of the Levene test for homogeneity of variance on the errors and significance was declared at $P \leq 0.05$. PROC UNIVARIATE of SAS was used to determine possible outliers and assess the normality of the errors, with significance declared at $P \leq 0.01$. In addition to the Shaprio-Wilk test, QQ plots and histograms were examined to determine normality of the errors, when the Shapiro-Wilk tests were significant. With homogeneity of variance and normality assumptions met, the data were analyzed separately by year due to differing treatments.

Measured Parameters

Pre-plant soil samples (0-12 in deep) were obtained from plot areas prior to planting and analyzed (A & L Great Lakes Laboratories, Fort Wayne, IN) to confirm soil fertility levels.

Following physiological maturity, the middle two rows of each plot were harvested with a SPC40 combine (ALMACO, Nevada, IA) to determine grain yield, with values adjusted to 15.5% moisture on 20 September 2018 and 4 November 2019. The ALMACO combine also collected subsamples of the harvested grain that were evaluated for grain quality (protein, oil, and starch concentrations at 0% grain moisture) by utilizing near-infrared transmittance spectroscopy (NIT) with an Infratec 1241 Grain Analyzer (Foss, Eden Prairie, MN). Average kernel weights were evaluated based on a representative subsample of 300 kernels and adjusted to 0% moisture. Kernel number on a per-acre basis was obtained from dividing total grain weight by the average kernel weight.

Total above-ground plant biomass sampling was performed at the R6 growth stage (physiological maturity) and conducted by manually selecting two random plants at the soil surface from each of the center two rows on 30 August 2018 and 2 October 2019. The plants were partitioned into grain and stover (including husk) components. Stover biomass accumulation was obtained by weighing the fresh plant stover and then passed through a BC600XL chipper (Vermeer Corporation, Pella, IA) to attain representative subsamples. The stover subsamples were immediately weighed to measure the fresh weight, and later weighed again when dried to 0% moisture in a forced air oven at 167 ºF to determine dry weight. The stover dry weight was then calculated by multiplying the total fresh weight by the quotient of the dry and fresh weight of the subsample. The corn ears that were partitioned were also dried, the grain was removed from the ear by a corn sheller (AEC Group, St. Charles, IA), and analyzed for moisture content using a Dickey John moisture reader (GSF, Ankeny, IA). Cob weight was calculated by difference, and dry stover and cob weights were added to obtain total R6 stover biomass. Dried stover subsamples were ground using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) to pass through a 2 mm mesh screen. Dried grain samples were ground into a powder using a Stein Mill (The Steinlite Corporation, Atchison, KS). Representative stover and grain subsamples were evaluated for nitrogen (N), phosphorus (P_2O_5), potassium (K_2O), sulfur (S), magnesium (Mg), and zinc (Zn) concentrations (A & L Great Lakes Laboratories, Fort Wayne, IN). These nutrient concentrations were multiplied by their corresponding plant fraction total biomass weights on a per acre basis to get total nutrient accumulation acre^{-1}.

RESULTS AND DISCUSSION

Influence of iNvigorate applied In-furrow on N and P Uptake and Utilization, 2018

Soil Characteristics

Prior to planting, a representative soil sample was taken at the field site to measure the qualities and inherent fertility levels in the soil. Soil samples were analyzed for organic matter, CEC, pH, nitrogen as nitrate, nitrogen as ammonium, phosphorus, potassium, calcium, magnesium, sulfur, zinc, manganese, and boron (Table 2.3). The phosphorus levels in the soil were lower than what we would typically expect at Champaign. These lower phosphorus soil test levels would suggest a high potential for plant responses to phosphorus fertilizer in uptake of this essential nutrient and grain yield.

Weather

In 2018, temperatures were 6 ºF cooler compared to the 30 year average in April, followed by a 9 ºF warmer month of May and the temperatures for the rest of the growing season were similar to the 30-year average (Table 2.4). The rainfall amounts in 2018 were fairly consistent with past rainfall, and timely rain events fostered a growing season with little weather-induced heat or moisture stress.

Grain Yield, Yield Components, and Grain Quality

Nitrogen and P_2O_5 fertility rates had significant effects on grain yield, yield components, protein, and starch concentrations in the grain, while the effects of *iNvigorate* treatment and the interaction of fertility and *iNvigorate* were nonsignificant (Table 2.5). As levels of nitrogen fertilizer increased, grain yield also increased as a result of a larger production of kernels as well as heavier average kernel weight. Even though the soil tests indicated low phosphorus levels, increases of P_2O_5 fertility within each N rate did not affect grain yield (Table 2.6). The effects of *iNvigorate* on grain yield were inconsistent; with the largest response of 4 bushels acre^{-1} when there was no fertility applied, originating from small increases in the constituent yield components in response to *iNvigorate*. As N rates increased, protein concentrations significantly increased in the grain, while starch concentrations were the highest when no fertilizer was applied (Table 2.7). These results were consistent with a study that evaluated the effects of varying rates of N fertilizer and N fertilizer sources on corn grain quality (Singh et al., 2005). Similar to grain yield, grain quality parameters were not affected when corn plants were treated with *iNvigorate* (Table 2.7).

Nutrient Accumulation

Although the in-furrow *iNvigorate* application did not affect grain yield in 2018, it affected the accumulation of important plant nutrients by the R6 growth stage (physiological maturity). Fertility rates affected the accumulation of all nutrients in the grain tissues, while *iNvigorate* affected grain P_2O_5, K_2O, and Mg accumulation (Table 2.8). For nutrients in the stover, fertility rates affected the accumulation of all nutrients measured except Zn, but *iNvigorate* treatment had no effect on these measurements. Fertility rates also affected the whole plant total uptake of N, K_2O, and Mg, while *iNvigorate* treatment influenced total P_2O_5 accumulation (Table 2.8). However, there was no interaction between the fertility and *iNvigorate* treatments on any nutrient accumulation parameters (Table 2.8).

On average, in this study, total plant P_2O_5 accumulation was 71 lbs acre^{-1} (Table 2.9), which was less than past research. A 230 bushel acre^{-1}-yielding corn crop can accumulate 101 lbs P_2O_5 acre^{-1} (Bender, 2012), while the trial presented here averaged 220.5 bushels acre^{-1} when averaged across all fertility rates (Table 2.6). Also, the typical removal of P_2O_5 has been documented to range from 0.37 to 0.43 lbs bushel^{-1} (Silva, 2017; Fernandez and Hoeft, 2009), which was a higher ratio than the removal of phosphorus in this trial. As P_2O_5 fertilizer rate increased, the total plant

P_2O_5 accumulation tended to increase (Table 2.9). But when *iNvigorate* was applied in-furrow, total phosphorus accumulation increased by 6 lbs acre^{-1} when averaged over the fertility rates (Table 2.9). These *iNvigorate*-driven responses were largely due to more phosphorus accumulated in grain tissues, suggesting that *iNvigorate* aids the plant in accumulating phosphorus while also reducing the probability of soil-P loss due to the removal with the grain (Table 2.9). Decreasing P loss from agricultural land is an important goal because water quality has been decreasing through eutrophication, lowering the ability to support wildlife, human consumption, and other recreational uses of rivers, streams, and lakes (Chislock et al., 2013; Daniel at al., 1998). This continued P loss from soils is ultimately leading towards further hypoxia of the Gulf of Mexico, which is noted to be one of the worst hypoxic zones in the world (Rabotyagov, 2010). When averaged across the *iNvigorate* treatment, total plant K_2O uptake increased with each elevation of N and P_2O_5 rate (Table 2.9). Nitrogen fertilizer rates have been previously found to affect the accumulation of essential nutrients, with higher N rates leading to an increase in K_2O uptake (Pasley et al., 2019). Nitrogen fertilizer affects yield potential, and when yield potential is elevated, the plants are in need for more nutrients, causing the response in plant K_2O content (Pasley at al., 2019). Additionally, *iNvigorate* application tended to increase total K_2O accumulation at most rates of fertilizer (Table 2.9). Similar to plant P_2O_5 uptake, the increase in K_2O accumulation was driven by more K_2O accumulated in the grain (Table 2.9). There were minimal changes in N uptake in response to *iNvigorate*, which corresponds with the lack of response in grain protein levels (Tables 2.7 and 2.9). Grain protein is known to function as nitrogen reserves in seeds (Tsai, 1983), so grain protein concentration and grain N accumulation are highly correlated. In addition, *iNvigorate* tended to increase total plant Mg and Zn accumulation at certain levels of fertility (Table 2.10).

These responses for Mg and Zn were mainly due to more accumulated in the grain, where *iNvigorate* increased grain Mg content when averaged across all fertility levels (Table 2.10).

Phosphorus Recovery Efficiency

With rising concerns of phosphorus loss to the environment, the recovery efficiency of this nutrient is important in the utilization of applied fertilizer. In 2018, both fertility rates and *iNvigorate* application affected phosphorus recovery efficiency separately, with no interaction between the two studied factors (Table 2.11). As expected, when P_2O_5 fertilizer rates increased, recovery efficiency decreased (Table 2.12). This response is because the applied P_2O_5 rate is the denominator of the equation and fertilizer applications follow the Law of Diminishing Returns (McNall, 1933). Additionally, *iNvigorate* treatment resulted in an increase of 20.1 % in phosphorus recovery efficiency when averaged across fertility rates (Table 2.12). This increase of phosphorus recovery efficiency was driven by the 58.1 % increase when *iNvigorate* was applied in-furrow to corn grown at 90/20 lbs N/P_2O_5 $acre^{-1}$ fertility level. This was the treatment with the largest response in total plant P_2O_5 uptake of 12 lbs $acre^{-1}$ (Table 2.9). These large increases in phosphorus recovery efficiency were due to the low check plot P_2O_5 content where plants receiving no fertility or *iNvigorate* application accumulated 62 lbs P_2O_5 $acre^{-1}$ (Table 2.9), which is less than expected. Also, the low phosphorus levels from the soil test could have contributed to the increase in phosphorus uptake and recovery efficiency values.

2018 Conclusions

In 2018, in-furrow applications of *iNvigorate* increased total plant P_2O_5 uptake and as well as elevated P_2O_5, K_2O, and Mg content in grain tissues showing the potential to increase grain quality and yield. Unfortunately, these applications of *iNvigorate* did not affect corn grain yields in 2018. Through greater phosphorus recovery efficiency, applications of *iNvigorate* can be utilized to assist in reduced phosphate loss to the environment.

Utilization of Differing iNvigorate Applications on Grain Yield and Nutrient Accumulation, 2019

Trial Redesign

Based on the data from 2018, the trial was redesigned to focus on the effects of *iNvigorate* in a more progressive corn management system with a base rate of N and various rates of phosphorus fertilizer. The differing N rates were omitted due to the minimal effects of *iNvigorate* on N accumulation and allowed for a larger range in P_2O_5 rates. Additionally, the phosphorus fertilizer was applied in a band below the crop row, concentrated in closer proximity to where *iNvigorate* was applied. A Y-drop application of *iNvigorate* was added to test for the product efficacy when side-dressed mid-season at the V6 plant growth stage. The sub-treatments are listed in Table 2.2. The objective of this study was to determine the effects of differing *iNvigorate* applications on the accumulation of essential nutrients and corn grain yield when phosphorus fertilizer was banded beneath the crop row.

Soil Characteristics

Prior to planting, a representative soil sample was taken at the field site and analyzed for organic matter, CEC, pH, phosphorus, potassium, calcium, magnesium, sulfur, zinc, manganese, and boron levels (Table 2.13). Soil phosphorus, organic matter, and CEC levels tended to be higher in 2019 when compared to the prior year (Tables 2.3 and 2.13). Plant accumulation of phosphorus is largely affected by the amount of available phosphorus in the soil (Fernandes and Soratto, 2016; Kavka and Polle, 2016).

Weather

In the 2019 growing season, the trial received 2.1 inches above-average rainfall in April and May, causing a later planting date compared to 2018, followed by a dry summer with 3.2 inches less precipitation compared to the 30-year average in July and August (Table 2.4). The temperature values were fairly consistent with the past 30 years (Table 2.4).

Grain Yield, Yield Components, and Grain Quality

The rate of P_2O_5 fertilizer influenced kernel number, kernel weight, grain oil concentration, and grain protein concentration, while *iNvigorate* treatments influenced grain yield, kernel number, and grain protein concentration (Table 2.15). Also, there were interactions between P_2O_5 fertilizer rate and *iNvigorate* treatment that influenced the grain yield and both yield component parameters (Table 2.15). Grain yield tended to increase as the P_2O_5 rates increased up to 60 lbs P_2O_5 acre^{-1}; however, these responses were minimal compared to yield responses gained from applying *iNvigorate* (Table 2.16). Applying *iNvigorate* in-furrow to plants grown at the 60 lbs P_2O_5 acre^{-1} rate increased grain yield by 16 bushels acre^{-1} as well as a generating an overall increase of 8 bushels acre^{-1} when averaged across all phosphorus fertilizer rates (Table 2.16). Many past studies have shown the ability of several phosphorus-solubilizing bacteria to increase corn grain yield (Hussain et al, 2013; Viruel et al., 2014; Amanullah and Khan, 2015). Applying *iNvigorate* via the Y-drop method also increased grain yield over the control, but at a lower magnitude of 3 bushels acre^{-1} (Table 2.16). These grain yield responses contrasted with data collected the year prior when fertility was broadcast applied (Table 2.6). The yield responses to both application methods of *iNvigorate* were driven by greater production of kernels, with the largest response in combination with the highest rate of 90 lbs P_2O_5 acre^{-1} (Table 2.16). The greater production of kernels consequently decreased the average kernel weight due to yield component compensation

(Table 2.16). This increase in kernel number due to *iNvigorate* application is consistent with previous reported findings on the effects of phosphorus-solubilizing bacteria (PSB) in corn production (Amanullah and Khan, 2015). In addition to improved grain yield, in-furrow applications of *iNvigorate* led to an increase in grain protein concentration and a tendency to increase oil concentration in the grain tissues at most P_2O_5 rates, while the Y-drop application tended to increase grain protein levels (Table 2.17). Similar results have been reported of an increase in corn grain protein levels when a PSB was applied in combination with phosphate fertilizer compared to no PSB application (Galavi et al., 2011). This data shows the potential to simultaneously improve grain yield and grain quality by applications of *iNvigorate* when phosphorus fertilizer is placed in a band directly below the crop row.

Nutrient Accumulation

In 2019, banding phosphorus fertility as TSP beneath the crop row did not affect nutrient accumulation in any plant part; however, the *iNvigorate* treatment affected grain and total P_2O_5, Mg, and Zn accumulations (Table 2.18). Increasing P_2O_5 fertilizer rates alone did not affect the accumulation of P_2O_5; however, when *iNvigorate* was applied, plant total P_2O_5 levels were increased by 8 lbs acre^{-1} over the control, regardless of application method (Table 2.19). This data implies that *iNvigorate* did solubilize soil phosphorus, ultimately for the plants to uptake and utilize. These *iNvigorate*-driven responses in total P_2O_5 accumulation were largely due to an increase of partitioning into grain tissues (Table 2.19), consistent with the trial conducted in 2018, further showing that *iNvigorate* is facilitating the plants' removal of more phosphorus from the soil, in turn reducing the likelihood of phosphorus loss to water sources. In addition to phosphorus, *iNvigorate*-treated plants tended to accumulate more N and K_2O compared to untreated plants in combination with higher P_2O_5 fertilizer rates (60 and 90 lbs P_2O_5 acre^{-1}) (Table 2.19). Furthermore, in-furrow applications of *iNvigorate* increased whole plant accumulation of Mg by 4 lbs acre^{-1} and Zn by 0.53 oz acre^{-1} (Table 2.20). Grain accumulated more Mg and Zn when *iNvigorate* was applied in-furrow, while Y-drop applications increased grain Zn content (Table 2.20), indicating that the changes in total accumulation of these elements were based on the changes in the grain. *iNvigorate* may be causing Mg and Zn to be more readily available for plant uptake, due to the product's mechanism of solubilizing phosphorus. Phosphorus-solubilizing bacteria release weak organic acids that chelate cations that are bound to phosphorus in the soil, in turn causing phosphorus to dissolve in the soil solution and become available for plant uptake (Sharma et al. 2013). The cations that were chelated are protected from binding with phosphorus or other anions in the soil and after the chelation is dissociated, these cations can become available for plant uptake later in the season (Krishnaraj and Dahale, 2014). Consequently, there was more Mg and Zn accumulated in the grain in the study presented here because these nutrients are somewhat immobile in plants, with only 15% of grain Mg, while 25% of grain Zn due to remobilization (Bender, 2012). Greater amounts of a substance in grain tissues suggests that they were accumulated by the plant later in the growing season during grain fill when the chelated Mg and Zn became available for uptake.

Phosphorus Recovery Efficiency

In the 2019 growing season, the applied treatments did not lead to any changes in phosphorus recovery efficiency (Table 2.21). However, in-furrow applications of *iNvigorate* tended to increase the recovery efficiency of phosphorus at all P_2O_5 rates and with an average response that was 12.2 % greater than the untreated control (Table 2.22). The phosphorus recovery efficiency values in 2019 were smaller in magnitude compared to the values in 2018 (Tables 2.12 and 2.22), which can be attributed to the greater accumulation of phosphorus by the unfertilized plants in 2019 (Tables 2.9 and 2.19). Corn plants that were not treated with fertilizer or *iNvigorate* accumulated 62 lbs P_2O_5 acre^{-1} in 2018 compared to 95 lbs P_2O_5 acre^{-1} in 2019 (Tables 2.9 and 2.19) and this difference of 33 lbs acre^{-1} had a large effect on the calculation of the efficiency of applied fertilizer. The reason for this large difference of P_2O_5 uptake can be attributed to the differences in soil test values, where the soils possessed 15 ppm higher phosphorus levels, 0.3% more organic matter, and 4.0 Meq 100 g^{-1} higher CEC values in 2019 vs. 2018 (Tables 2.3 and 2.13). Additionally, the 2019 trial was planted later in the season, resulting in higher soil temperatures when the phosphorus fertilizer was applied. These elevated soil temperatures can cause the fertilizer to be more mobile and accumulated more readily into the plant as well as influence the rate of mineralization of organic phosphorus to inorganic forms (Beegle, 2002).

2019 Conclusions

The results of this study in 2019 show the effect of phosphorus-solubilizing bacteria applications in a corn production system. Applications of *iNvigorate*, either in-furrow at planting, or as Y-drop at V6 increased grain yields, through increased availability of P_2O_5, Mg, and Zn and the accumulation of these minerals in the grain. Plant responses to *iNvigorate* applications were more apparent when phosphorus fertilizer was banded beneath the crop row, indicating the importance of fertilizer placement. Overall, there was a potential reduction in the environmental effects of nutrient runoff and an improvement in the efficiency of phosphorus fertilizer and yield from *iNvigorate* applications in corn in 2019

TABLES

Table 2.1 Sub-treatments used for the evaluation of an in-furrow *iNvigorate* application combined with five rates of nitrogen plus phosphorus fertilizer on corn production at Champaign, IL in 2018.

Fertilizer Rates†		***iNvigorate* Treatment‡**
N	**P_2O_5**	
lbs acre^{-1}		
0	0	None In-furrow
90	20	
180	20	
90	60	
180	60	

† Fertility rates expressed in lbs N or lbs P_2O_5 per acre and applied as urea and MAP pre-plant broadcast and incorporated in the soil. All fertility levels except the unfertilized control also received 3 gal/acre of 10-34-0 starter.
‡ *iNvigorate* applied in-furrow at planting at a rate of 1 L acre^{-1}.

Table 2.2 Sub-treatments used for the evaluation of the influence of *iNvigorate* application timing combined with differing rates of phosphorus fertilizer on corn production at Champaign, IL in 2019.

P_2O_5 Rate†	***iNvigorate* Applications‡**
lbs acre^{-1}	
0	None In-furrow Y-drop (V6)
30	
60	
90	

† P_2O_5 rates in lbs of P_2O_5 per acre and applied as TSP pre-plant banded four to six inches below the soil surface.
‡ *iNvigorate* applied either in-furrow at planting or Y-drop at the V6 growth stage at a rate of 1 L acre^{-1}.

Table 2.3 Pre-plant soil properties (0-12” depth) and Mehlich 3-extraction-based mineral test results for the *iNvigorate* experimental corn site conducted at Champaign, IL in 2018.

OM†	**CEC**	**pH**	**NO_3**	**NH_4**	**P**	**K**	**Ca**	**Mg**	**S**	**Zn**	**Mn**	**B**
%	Meq 100g^{-1}	units	ppm									
3.5	18.6	6.3	8.5	3.8	27	129	2472	420	7	0.8	41	0.3

† OM, organic matter; CEC, cation exchange capacity.

Table 2.4 Monthly weather data between 1 April and 31 October at Champaign, IL in 2018. Values presented are the average daily air temperature and the average monthly accumulated rainfall, with deviations from the 30-year average in parentheses (Illinois State Water Survey, 2020).

April	May	June	July	August	September	October
			Temperature, °F			
46 (-6)	72 (9)	75 (3)	75 (0)	75 (2)	71 (5)	54 (-1)
			Precipitation, Inches			
2.5 (-1.1)	4.2 (-0.7)	7.3 (3.0)	3.2 (-1.5)	4.0 (0.1)	4.7 (1.6)	2.2 (-1.0)

Table 2.5 Test of fixed effects for grain yield, yield components (kernel number and average kernel weight), and grain quality (oil, protein, and starch concentrations) as influenced by nitrogen and phosphorus fertility rates and *iNvigorate* treatment at Champaign, IL in 2018.

		Yield Component		Grain Quality		
Source of Variation	Yield	Kernel Number	Kernel Weight	Oil	Protein	Starch
			$P > F$			
Fertility (F)	<.0001	<.0001	<.0001	0.6028	<.0001	<.0001
iNvigorate (I)	0.7713	0.7426	0.8779	0.6692	0.7570	0.5970
F x I	0.9267	0.7449	0.9645	0.7454	0.9048	0.7368

Table 2.6 Grain yield and yield components (kernel number and average kernel weight) as influenced by nitrogen and phosphorus fertility rates and *iNvigorate* treatment at Champaign, IL in 2018. Corn grain yields and kernel weights are expressed at 15.5% and 0% moisture, respectively.

	Grain Yield		Kernel Number		Kernel Weight	
N/P_2O_5	*iNvigorate* Treatment					
Fertility Level†	None	In-furrow	None	In-furrow	None	In-furrow
lbs acre^{-1}	— bu acre^{-1} —		— number m^{-2} —		— mg seed^{-1} —	
0/0	148	152	3461	3476	223	227
90/20	232	227	4696	4629	260	258
180/20	249	250	4859	4821	270	273
90/60	227	230	4622	4715	258	257
180/60	246	248	4803	4872	270	268
Means	220	221	4488	4503	256	257

† Fertility levels in lbs N or lbs P_2O_5 per acre. All fertility levels also received 3 gal/acre of 10-34-0 starter except the unfertilized control.

Table 2.7 Grain quality (oil, protein, and starch concentrations) as influenced by nitrogen and phosphorus fertility rates and *iNvigorate* treatment at Champaign, IL in 2018.

N/P_2O_5 Fertility Level†	**Oil**		**Protein**		**Starch**	
	***iNvigorate* Treatment**					
	None	**In-furrow**	**None**	**In-furrow**	**None**	**In-furrow**
lbs acre^{-1}	%					
0/0	3.4	3.4	5.9	5.9	74.5	74.5
90/20	3.4	3.5	6.9	6.9	73.7	73.8
180/20	3.4	3.5	7.7	7.8	73.4	73.0
90/60	3.5	3.5	6.9	7.0	73.5	73.6
180/60	3.4	3.4	7.7	7.6	73.4	73.3
Means	3.4	3.5	7.0	7.0	73.7	73.6

† Fertility levels in lbs N or lbs P_2O_5 per acre. All fertility levels also received 3 gal/acre of 10-34-0 starter except the unfertilized control.

Table 2.8 Test of fixed effects for plant phosphorus, nitrogen, potassium, magnesium, and zinc accumulations in the grain, stover, and whole plant at physiological maturity as influenced by nitrogen and phosphorus fertility rates and *iNvigorate* treatment at Champaign, IL in 2018.

Source of Variation	**P_2O_5**	**N**	**K_2O**	**Mg**	**Zn**
	$P > F$				
			Grain		
Fertility (F)	0.0048	<.0001	<.0001	<.0001	0.0328
iNvigorate (I)	0.0452	0.4942	0.0154	0.0041	0.5901
F x I	0.6710	0.8279	0.7810	0.5301	0.8522
			Stover		
Fertility (F)	0.0118	<.0001	0.0006	0.0763	0.1245
iNvigorate (I)	0.5208	0.5857	0.9538	0.9189	0.7691
F x I	0.5889	0.0959	0.2781	0.7334	0.9277
			Whole Plant		
Fertility (F)	0.2342	<.0001	<.0001	0.0010	0.4861
iNvigorate (I)	0.0192	0.8528	0.4536	0.2465	0.5288
F x I	0.3451	0.8757	0.2766	0.6167	0.8191

Table 2.9 Plant phosphorus, nitrogen, and potassium accumulations in the grain, stover, and whole plant at physiological maturity as influenced by nitrogen and phosphorus fertility rates and *iNvigorate* treatment at Champaign, IL in 2018.

	P_2O_5		**N**		**K_2O**	
N/P_2O_5	***iNvigorate* Treatment**					
Fertility Level†	**None**	**In-furrow**	**None**	**In-furrow**	**None**	**In-furrow**
	lbs acre^{-1}					
			Grain			
0/0	43	50	65	69	36	41
90/20	51	62	116	111	45	52
180/20	56	57	130	126	47	49
90/60	57	58	109	110	49	50
180/60	59	66	133	127	49	55
Means	53	59*	111	109	45	49*
			Stover			
0/0	19	19	41	36	122	128
90/20	14	16	54	56	137	143
180/20	14	14	59	64	152	167
90/60	15	13	57	50	158	135
180/60	11	15	53	66	166	162
Means	15	15	53	54	147	147
			Whole Plant			
0/0	62	69	109	107	157	169
90/20	66	78	171	169	182	195
180/20	70	71	190	192	199	217
90/60	72	71	168	161	206	185
180/60	70	80	188	194	215	217
Means	68	74*	165	165	192	197

† Fertility levels in lbs N or lbs P_2O_5 per acre. All fertility levels also received 3 gal/acre of 10-34-0 starter except the unfertilized control.

* Denotes a significant response ($P \leq .10$) from *iNvigorate* treatment compared to control.

Table 2.10 Plant magnesium and zinc accumulations in the grain, stover, and whole plant at physiological maturity as influenced by nitrogen and phosphorus fertility rates and *iNvigorate* treatment at Champaign, IL in 2018.

N/P_2O_5 Fertility Level†	Mg		Zn	
	iNvigorate Treatment			
	None	In-furrow	None	In-furrow
	lbs acre^{-1}		oz acre^{-1}	
		Grain		
0/0	8.3	9.8	2.43	2.59
90/20	10.8	13.0	2.85	2.86
180/20	11.9	11.8	2.71	2.64
90/60	10.5	12.3	2.84	2.80
180/60	12.5	13.7	2.92	3.16
Means	10.8	12.1*	2.75	2.81
		Stover		
0/0	17.7	15.5	1.31	1.33
90/20	20.6	21.0	1.17	1.20
180/20	20.3	19.4	1.13	1.21
90/60	19.5	19.7	1.14	1.06
180/60	16.8	18.8	1.05	1.09
Means	19.0	18.9	1.16	1.18
		Whole Plant		
0/0	26.4	25.8	3.79	3.97
90/20	31.9	34.5	4.06	4.10
180/20	32.7	31.7	3.89	3.90
90/60	30.5	32.4	4.03	3.90
180/60	29.8	33.0	4.02	4.29
Means	30.3	31.5	3.96	4.03

† Fertility levels in lbs N or lbs P_2O_5 per acre. All fertility levels also received 3 gal/acre of 10-34-0 starter except the unfertilized control.

* Denotes a significant response ($P \leq .10$) from *iNvigorate* treatment compared to control.

Table 2.11 Test of fixed effects for phosphorus recovery efficiency as influenced by nitrogen and phosphorus fertility rates and *iNvigorate* treatment at Champaign, IL in 2018.

Source of Variation	Phosphorus Recovery Efficiency
	$P > F$
Fertility (F)	0.0055
iNvigorate (I)	0.0043
F x I	0.1713

Table 2.12 Phosphorus recovery efficiency as influenced by phosphorus fertilizer rate and *iNvigorate* treatment at Champaign, IL in 2019.

N/P_2O_5 Fertility Level†	Phosphorus Recovery Efficiency *iNvigorate* Treatment None	 In-furrow
lbs acre^{-1}	%	
90/20	23.4	81.5
180/20	41.0	47.1
90/60	17.4	15.8
180/60	12.7	30.3
Means	23.6	43.7*

† Fertility levels in lbs N or lbs P_2O_5 per acre. All fertility levels also received 3 gal/acre of 10-34-0 starter except the unfertilized control.

* Denotes a significant response ($P \leq .10$) from *iNvigorate* treatment compared to control.

Table 2.13 Pre-plant soil properties (0-6” depth) and Mehlich 3-extraction-based mineral test results for the *iNvigorate* experimental corn site conducted at Champaign, IL in 2019.

OM†	CEC	pH	P	K	Ca	Mg	S	Zn	Mn	B
%	Meq 100g^{-1}	units	ppm							
3.8	22.6	6.2	42	149	2839	533	18	1.9	53	0.6

† OM, organic matter; CEC, cation exchange capacity.

Table 2.14 Monthly weather data between 1 April and 31 October at Champaign, IL in 2019. Values presented are the average daily air temperature and the average monthly accumulated rainfall, with deviations from the 30-year average in parentheses (Illinois State Water Survey, 2020).

April	May	June	July	August	September	October
Temperature, °F						
53 (0)	64 (1)	72 (-1)	77 (2)	74 (0)	72 (5)	54 (-1)
Precipitation, Inches						
5.3 (1.6)	5.2 (0.5)	3.7 (-0.7)	2.3 (-1.9)	2.1 (-1.3)	3.3 (0.2)	5.0 (1.8)

Table 2.15 Test of fixed effects for grain yield, yield components (kernel number and average kernel weight), and grain quality (oil, protein, and starch concentrations) as influenced by phosphorus fertilizer rate and *iNvigorate* treatment at Champaign, IL in 2019.

Source of Variation	**Yield**	**Yield Components**		**Grain Quality**		
		Kernel Number	**Kernel Weight**	**Oil**	**Protein**	**Starch**
		$P > F$				
P_2O_5 Rate (P)	0.1963	0.0034	0.0082	0.0486	0.0047	0.3753
iNvigorate (I)	0.0585	0.0018	0.5022	0.1667	0.0926	0.9182
P x I	0.0705	0.0037	0.0457	0.3844	0.3629	0.1025

Table 2.16 Grain yield and yield components (kernel number and average kernel weight) as influenced by phosphorus fertilizer rate and *iNvigorate* treatment [none, in-furrow at planting (IF), or via Y-drop at V6] at Champaign, IL in 2019. Corn grain yields and kernel weights are expressed at 15.5% and 0% moisture, respectively.

	Grain Yield			**Kernel Number**			**Kernel Weight**		
P_2O_5 Rate	***iNvigorate* Treatment**								
	None	**IF**	**Y-Drop**	**None**	**IF**	**Y-Drop**	**None**	**IF**	**Y-Drop**
lbs acre^{-1}	bu acre^{-1}			number m^{-2}			mg seed^{-1}		
0	219	225	222	4665	4810	4664	250	249	253
30	224	225	234	4850	5027	5109	246	238	251
60	227	243^{*}	219	5074	5260	4811	238	246	242
90	219	228	224	4414	5121^{*}	5149^{*}	249	237^{*}	232^{*}
Means	222	230^{*}	225^{*}	4751	5055^{*}	4934^{*}	246	242	244

* Denotes a significant response ($P \leq .10$) from *iNvigorate* treatment compared to the control compared to the control within the same P_2O_5 rate.

Table 2.17 Grain quality (oil, protein, and starch concentrations) as influenced by phosphorus fertilizer rate and *iNvigorate* treatment [none, in-furrow at planting (IF), or via Y-drop at V6] at Champaign, IL in 2019.

	Oil			**Protein**			**Starch**		
P_2O_5 Rate	***iNvigorate* Treatment**								
	None	**IF**	**Y-Drop**	**None**	**IF**	**Y-Drop**	**None**	**IF**	**Y-Drop**
lbs acre^{-1}	%								
0	4.16	4.22	4.04	8.78	8.87	8.98	72.4	72.9	72.4
30	4.33	4.53	4.15	8.68	8.78	8.71	72.6	72.4	72.4
60	4.19	4.09	3.94	8.69	8.75	8.58	72.7	72.4	72.8
90	4.10	4.25	4.33	8.57	8.80	8.67	72.6	72.6	72.9
Means	4.19	4.27	4.11	8.68	8.80^{*}	8.73	72.6	72.6	72.6

* Denotes a significant response ($P \leq .10$) from *iNvigorate* treatment compared to the control within the same P_2O_5 rate.

Table 2.18 Test of fixed effects for plant phosphorus, nitrogen, potassium, magnesium, and zinc accumulations in the grain, stover, and whole plant at physiological maturity as influenced by phosphorus fertilizer rate and *iNvigorate* treatment at Champaign, IL in 2019.

Source of Variation	**P_2O_5**	**N**	**K_2O**	**Mg**	**Zn**
			P > F		
			Grain		
P_2O_5 Rate (P)	0.1223	0.1678	0.1121	0.1961	0.1154
iNvigorate (I)	0.0502	0.1669	0.2339	0.0728	0.0064
P x I	0.6055	0.2311	0.2932	0.6555	0.2385
			Stover		
P_2O_5 Rate (P)	0.5559	0.2448	0.1913	0.4922	0.0234
iNvigorate (I)	0.5937	0.1891	0.7507	0.1331	0.4125
P x I	0.7708	0.1567	0.1998	0.3388	0.1702
			Whole Plant		
P_2O_5 Rate (P)	0.3736	0.2069	0.5554	0.5816	0.5128
iNvigorate (I)	0.0199	0.1188	0.5499	0.0587	0.0192
P x I	0.4206	0.2817	0.2300	0.2346	0.1809

Table 2.19 Plant phosphorus, nitrogen, and potassium accumulations in the grain, stover, and whole plant at physiological maturity as influenced by phosphorus fertilizer rate and *iNvigorate* treatment [none, in-furrow at planting, or via Y-drop at V6] at Champaign, IL in 2019.

P_2O_5 Rate	P_2O_5			N			K_2O		
	iNvigorate Treatment								
	None	IF	Y-Drop	None	IF	Y-Drop	None	IF	Y-Drop
	lbs acre^{-1}								
	Grain								
0	70	75	77	171	172	174	51	53	54
30	75	78	81	166	166	176	53	54	57
60	76	86	78	169	185	169	54	60	54
90	77	80	80	161	171	164	56	57	56
Means	75	80[*]	79[*]	167	174	171	54	56	55
	Stover								
0	25	26	24	99	92	93	161	149	156
30	26	25	25	91	92	87	154	143	149
60	20	26	26	87	105	99	136	149	146
90	22	23	22	90	98	87	132	159	134
Means	23	25	24	92	97	92	146	150	146
	Whole Plant								
0	95	101	101	271	265	267	212	202	210
30	101	103	104	257	258	257	207	196	204
60	92	112	111	252	291	268	188	210	200
90	99	103	102	251	269	253	188	216	192
Means	97	105[*]	105[*]	258	271	261	199	206	201

[*] Denotes a significant response ($P \leq .10$) from *iNvigorate* treatment compared to the control within the same P_2O_5 rate.

Table 2.20 Plant magnesium and zinc accumulations in the grain, stover, and whole plant at physiological maturity as influenced by phosphorus fertilizer rate and *iNvigorate* treatment [none, in-furrow at planting, or via Y-drop at V6] at Champaign, IL in 2019.

P_2O_5 Rate	Mg			Zn		
	iNvigorate Treatment					
	None	**In-furrow**	**Y-Drop**	**None**	**In-furrow**	**Y-Drop**
	lbs acre^{-1}			oz acre^{-1}		
				Grain		
0	13.0	13.6	14.4	3.21	3.44	3.62
30	13.5	14.0	14.2	3.41	3.56	3.67
60	13.8	16.0	14.5	3.44	4.07	3.55
90	13.8	14.7	14.1	3.49	3.76	3.68
Means	13.5	14.6*	14.3	3.39	3.71*	3.63*
				Stover		
0	39.5	38.7	38.6	2.00	1.82	1.91
30	37.7	40.5	40.0	1.67	1.72	1.44
60	34.8	43.7	37.3	1.45	2.01	1.84
90	37.4	38.0	35.3	1.56	1.62	1.53
Means	37.3	40.2	37.8	1.67	1.79	1.68
				Whole Plant		
0	52.5	52.3	53.0	5.17	5.22	5.53
30	51.2	54.5	53.9	5.08	5.51	4.89
60	48.2	59.7	51.9	4.78	6.08	5.38
90	51.1	52.7	48.7	5.05	5.38	4.99
Means	50.8	54.8*	51.9	5.02	5.55*	5.20

* Denotes a significant response ($P \leq .10$) from *iNvigorate* treatment compared to the control within the same P_2O_5 rate.

Table 2.21 Test of fixed effects for phosphorus recovery efficiency as influenced by phosphorus fertilizer rate and *iNvigorate* treatment at Champaign, IL in 2019.

Source of Variation	Phosphorus Recovery Efficiency
	—— $P > F$ ——
P_2O_5 Rate (P)	0.2998
iNvigorate (I)	0.2958
P x I	0.7764

Table 2.22 Phosphorus recovery efficiency as influenced by phosphorus fertilizer rate and *iNvigorate* treatment [none, in-furrow at planting, or via Y-drop at V6] at Champaign, IL in 2019.

P_2O_5 Rate	Phosphorus Recovery Efficiency		
	***iNvigorate* Treatment**		
	None	**In-furrow**	**Y-Drop**
lbs acre^{-1}		%	
30	19.3	25.4	10.0
60	2.5	28.9	15.7
90	4.6	8.8	2.2
Means	8.8	21.0	9.3

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APPENDIX A: SUPPLEMENTAL TABLES

Table A.1 Test of fixed effects for grain quality (oil, protein, and starch concentrations) as influenced by nitrogen fertilizer rate and *Source* treatment at Harrisburg, Champaign, Yorkville, and averaged over all locations in Illinois in 2018.

Source of Variation	Oil	Protein	Starch
		P > F	
		Harrisburg	
Nitrogen (N)	0.6000	<.0001	0.0040
Source (S)	0.4762	0.8150	0.9677
N x S	0.6277	0.8541	0.5436
		Champaign	
Nitrogen (N)	0.0006	<.0001	0.6542
Source (S)	0.8746	0.9842	0.4704
N x S	0.7533	0.4152	0.9199
		Yorkville	
Nitrogen (N)	0.0014	<.0001	0.0993
Source (S)	0.1759	0.0769	0.2147
N x S	0.4372	0.0457	0.5359
		Averaged over Locations	
Nitrogen (N)	0.0072	<.0001	0.4705
Source (S)	0.9386	0.3849	0.8455
N x S	0.5374	0.0760	0.7450

Table A.2 Grain quality (oil, protein, and starch concentrations) as influenced by nitrogen fertilizer rate and *Source* treatment at Harrisburg, Champaign, Yorkville, and averaged over all locations in Illinois in 2018.

Nitrogen Rate	Oil			Protein			Starch		
	Source Treatment								
	None	IF	Foliar	None	IF	Foliar	None	IF	Foliar
lbs N acre^{-1}					%				
					Harrisburg				
0	4.26	4.16	4.30	6.10	6.08	6.15	73.3	73.5	73.3
60	4.32	4.22	4.26	6.16	6.17	6.10	73.0	73.2	73.3
220	4.24	4.30	4.34	6.98	7.13	6.98	73.0	72.6	72.7
Means	4.27	4.23	4.30	6.41	6.46	6.41	73.1	73.1	73.1
					Champaign				
0	4.40	4.47	4.49	5.05	4.95	5.17	73.4	73.5	73.4
60	4.33	4.26	4.34	5.12	5.17	5.16	73.5	73.8	73.5
220	4.24	4.15	4.13	6.03	6.03	5.85	73.3	73.6	73.5
Means	4.32	4.29	4.32	5.40	5.38	5.39	73.4	73.6	73.5
					Yorkville				
0	4.39	4.56	4.41	5.97	5.58	5.70	73.4	72.9	73.3
60	4.49	4.44	4.33	5.80	5.72	5.52	73.3	73.3	73.6
220	4.25	4.28	4.23	6.37	6.61	6.37	73.5	73.5	73.5
Means	4.38	4.43	4.32	6.05	5.97	5.86	73.4	73.2	73.5
					Averaged over Locations				
0	4.35	4.40	4.40	5.63	5.54	5.67	73.4	73.3	73.3
60	4.38	4.31	4.31	5.69	5.68	5.58	73.3	73.4	73.5
220	4.24	4.25	4.23	6.51	6.59	6.39	73.3	73.2	73.3
Means	4.32	4.32	4.31	5.94	5.94	5.88[*]	73.3	73.3	73.3

[*] Denotes a significant response ($P \le .10$) from *Source* treatment compared to control within the same fertilizer rate.

Table A.3 Test of fixed effects for grain quality (oil, protein, and starch concentrations) as influenced by nitrogen fertilizer rate and *Source* treatment at Ewing, Champaign, Yorkville, and averaged over all locations in Illinois in 2019.

Source of Variation	**Oil**	**Protein**	**Starch**
		P > F	
		Ewing	
Nitrogen (N)	0.0288	<.0001	0.0422
Source (S)	0.2715	0.3182	0.5003
N x S	0.2694	0.9227	0.0881
		Champaign	
Nitrogen (N)	0.0787	<.0001	<.0001
Source (S)	0.8235	0.3774	0.3472
N x S	0.9758	0.9296	0.6454
		Yorkville	
Nitrogen (N)	0.0304	<.0001	0.0065
Source (S)	0.7105	0.6615	0.2116
N x S	0.0956	0.8343	0.7725
		Averaged over Locations	
Nitrogen (N)	0.0762	<.0001	<.0001
Source (S)	0.5645	0.8394	0.405
N x S	0.559	0.9998	0.2995

Table A.4 Grain quality (oil, protein, and starch concentrations) as influenced by *Source* treatment and nitrogen fertilizer rate for corn grown at Ewing, Champaign, and Yorkville, IL and averaged over all locations in Illinois in 2019.

Nitrogen Rate	Oil				Protein				Starch			
	Source Treatment											
	None	V4	VT	V4+VT	None	V4	VT	V4+VT	None	V4	VT	V4+VT
lbs N/acre	%											
					Ewing							
0	3.42	3.33	3.44	3.50	6.69	6.47	6.69	6.58	74.4	74.8	74.8	74.2
60	3.26	3.41	3.54	3.60	6.35	6.20	6.23	6.35	74.7	74.5	74.2	74.8
120	3.56	3.53	3.58	3.62	6.28	6.30	6.37	6.37	74.5	74.7	74.4	74.1
220	3.58	3.58	3.63	3.42	6.53	6.43	6.53	6.41	74.5	74.0	73.9*	74.4
Means	3.46	3.47	3.55	3.54	6.47	6.35	6.46	6.43	74.5	74.5	74.3	74.4
					Champaign							
0	3.91	3.97	4.08	3.91	7.27	7.23	7.48	7.23	73.4	73.4	73.2	73.7
60	4.08	4.08	4.09	4.06	7.93	7.80	8.00	8.12	73.1	73	72.4	72.9
120	4.25	4.13	4.22	4.09	8.73	8.63	8.62	8.73	72.2	72.6	72.3	72.3
220	4.09	4.04	4.08	4.15	9.35	9.14	9.33	9.33	71.8	71.9	72.1	72.0
Means	4.08	4.05	4.12	4.05	8.32	8.20	8.36	8.35	72.6	72.7	72.5	72.7
					Yorkville							
0	4.70	4.98	4.66	4.66	6.74	6.98	6.73	6.87	71.9	71.7	72.2	71.8
60	4.73	4.58	4.74	4.80	7.13	7.05	7.07	7.08	72.1	71.8	71.9	71.6
120	4.81	4.58	4.47*	4.42*	7.40	7.38	7.55	7.43	71.9	72.2	71.8	71.8
220	4.18	4.65*	4.51*	4.63*	7.63	7.98	7.83	7.78	71.6	71.4	71.6	71.1
Means	4.60	4.70	4.59	4.62	7.23	7.35	7.30	7.29	71.9	71.8	71.9	71.6
					Average of Locations							
0	3.97	4.01	4.06	4.02	6.93	6.83	6.95	6.89	73.2	73.4	73.4	73.4
60	4.03	4.02	4.12	4.16	7.13	7.03	7.10	7.18	73.3	73.1	72.8	73.1
120	4.21	4.08	4.12	4.09	7.47	7.44	7.51	7.50	72.9	73.2	72.8	72.7
220	3.94	4.07	4.05	4.07	7.85	7.87	7.91	7.88	72.5	72.4	72.4	72.5
Means	4.04	4.04	4.09	4.08	7.35	7.29	7.37	7.36	73.0	73.0	72.9	72.9

* Denotes a significant response from *Source* compared to the control