VERSATILITY OF MYCORRHIZAL FUNGI APPLICATIONS TO INCREASE MAIZE PRODUCTIVITY

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ABSTRACT

With rising fertilizer prices and continual water contamination issues, it is vital to establish management factors that maximize productivity, while minimizing nutrient losses to the environment. One potential practice for improving nutrient use and grain yields includes utilizing mycorrhizal fungi. The objectives of this research were to determine the efficacy of various mycorrhiza applications on root colonization and the subsequent grain yield responses of maize (*Zea mays* L.). Three field trials were conducted at Champaign, IL in either 2018 or 2021. In all cases, soybean [*Glycine max* (L.) Merr.] was the previous crop and maize was grown at a stand density of 36,000 plants acre^{-1} with a sufficient amount of applied nitrogen fertilizer. A commercial mycorrhizal fungi product, *MycoApply EndoPrime SC* from Valent U.S.A. LLC (San Ramon, CA), was utilized in a variety of application methods. In 2018, mycorrhiza was applied in-furrow at planting with water as a carrier. In 2021, mycorrhiza was applied in-furrow at planting in combination with ammonium polyphosphate (APP; 10-34-0) starter fertilizer. Additionally, in 2021, mycorrhiza was impregnated with a slow-release polymer-coating [Pursell Agri-Tech (Sylacauga, AL)] on urea (46-0-0), which was applied in a pre-plant sub-surface band 6 inches directly below the crop row. In all three trials, mycorrhiza applications were compared to an untreated control. In the 2018 trial, the mycorrhiza treatment successfully generated mycorrhizal colonies on the treated maize roots compared to no fungal colonies on untreated plant roots and tended to increase yield by 2 bu acre^{-1}. Conversely, in 2021, supplying mycorrhiza in combination with slow-release urea or APP increased maize grain yields ($P \leq 0.10$) by 9 or 13 bu acre^{-1}, respectively, compared to the untreated controls. These findings demonstrate that supplying mycorrhizal fungi by multiple application methods can increase maize production, especially when paired with fertility.

INTRODUCTION

Phosphorus (P) is one of the most important mineral nutrients in maximizing maize production, with the second-greatest fertilizer demand in the world (FAO, 2019). Food production accounts for 90% of the global demand for P, totaling approximately 163 million tons of phosphate rock per year (Cordell et al., 2009). As the global population and food demand continue to increase, crop phosphorus (P) fertilizer requirements are predicted to increase by 50 to 100% by 2050 (Cordell et al., 2009). Inherent soil P levels are notably affected by increases in maize grain yield due to a large proportion of P removed with the grain (P harvest index). Of the essential mineral nutrients for maize, P has the highest harvest index of 79% (Bender et al., 2013).

Despite the importance of maintaining soil P levels, there are consequences of extensive fertilizer P applications, including increased eutrophication of water sources due to P loss from the soil. Phosphorus can be lost to the environment through soil erosion and runoff and 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. Although projections are variable, rock phosphate is a finite resource and will ultimately become depleted (Vaccari, 2009; Van Kauwenbergh, 2013). Therefore, further research is vital to discover new grower practices that improve fertilizer use efficiency in efforts to minimize P loss and extend the lifespan of mineral P reserves.

Phosphorus fertilizer use efficiency is low in cereal crops with estimations of world P use efficiency of between 10-16% (Roberts & Johnston, 2015; Dhillon et al, 2017). Dobermann (2007) claimed that at best most agriculture crops recovered only 20 to 30% of applied P under favorable conditions. Phosphorus use efficiency is low because 75 to 90% of applied P fertilizer becomes unavailable to plants through precipitation with soil cations (Sharma et al., 2013). Phosphorus is abundant in agricultural soils; however, P is still a limiting nutrient for maize growth as it is present mainly in unavailable forms. In soils similar to Champaign, IL (silty clay loam with 3% organic matter and 18 CEC), there was a total of 1,200 lbs P acre^{-1} in the top 6 inches of the soil profile. However, only a range of 0.01-0.1 lbs P acre^{-1} was in plant-available forms (Gardner et al., 1985). The rest of the total P in the soil is either contained in the organic pool or in mineral complexes that have a wide range in solubility.

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. Inorganic forms of P mainly exist as insoluble mineral complexes also called fixed P, and often occur following multiple fertilizer applications (Sharma et al., 2013). Soil P that is available for plant uptake is in the forms of $H_2PO_4^-$ and HPO_4^{2-} ions that are dissolved in the soil solution. Soil P cycling is a dynamic process where soluble P can move between organic and inorganic forms. Organic P is mineralized into readily available P for plant uptake and precipitated 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 plant-available P.

Challenges in P fertilizer management may be even greater in future years. Maize planting population in the United States has consistently increased each year since the 1960s (USDA-NASS, 2022). As planting densities increase, root biomass of each individual maize plant decreases, causing a smaller root surface area in contact with soil, and will ultimately cause issues with plant accumulation of immobile nutrients like P (Bernhard & Below, 2020).

The uncertainty of plant-available P, paired with the high P requirement for maize, indicates the need for improved management practices associated with P fertilization. Fortunately, there are a number of viable approaches to improve plant availability of P including placing fertilizer P near plant roots, increasing the surface area of roots, keeping fertilizer P from precipitating with soil cations, and promoting the cycling of P to plant-available forms either through mineralization (organic P forms) or solubilization (inorganic P forms).

One management strategy that can increase root surface area and enhance the uptake efficiency of fertilizer P is the utilization of arbuscular mycorrhizal fungi (AMF), which can form symbiotic associations with crop plants. These fungi colonize inside the root cortex, and the AMF grow hyphae outside the root. The plant provides carbohydrates as an energy source for the AMF, while the fungal hyphae act as an extension of the plant's root system, providing greater soil contact, and ultimately increasing plant accumulation of non-mobile nutrients such as P as well as water. Maize roots occupy only 1-3% of the soil volume in the top 0-8 inches, indicating a significant opportunity to improve nutrient use through greater root surface area (Barber, 1984). In addition, AMF can interact with unavailable soil P by releasing organic acids and phosphatase enzymes that solubilize inorganic forms or release organic forms of P in the soil. Organic acids can chelate cations that bind inorganic P complexes to promote the solubilization of plant-available P, while phosphatase enzymes are catalysts that enhance mineralization of organic P, by cleaving phosphate from the organic moiety. Thus, AMF inoculants can provide multiple modes of action to improve P fertilizer use efficiency through increasing root surface area and promoting levels of plant-available P in the rhizosphere.

MATERIALS AND METHODS

Field Characteristics and Cultural Practices

Three field trials were implemented at the Crop Sciences Research and Education Center at Champaign, IL in either 2018 or 2021. All fields were in a maize-soybean rotation with conventional tillage practices consisting of a deep ripping chisel plow in the fall followed by a field cultivator in the spring. All trials were planted with an ALMACO precision plot planter to achieve a density of 36,000 plants acre^{-1}. In 2018, the trial was planted on May 14, while in 2021 the two trials were both planted on May 1. All trials experienced average total rainfall and normal temperatures during the growing season.

Treatment Applications

To ensure adequate nitrogen (N) fertility, a base rate of 180 lbs N acre^{-1} as urea ammonium nitrate (UAN; 32-0-0) was pre-plant broadcast applied to all plots in 2018 and incorporated into the soil. Experimental treatments consisted of an uninoculated control or a commercial liquid mycorrhizal fungi inoculant, *MycoApply EndoPrime SC*, applied in-furrow at planting at a rate of 2 fluid ounces acre^{-1}. The inoculant was blended with water as a carrier for a total application volume of 12 gallons acre^{-1}.

In 2021, a base rate of 180 lbs N acre^{-1} as UAN was pre-plant broadcast applied at all plots to ensure adequate N availability and incorporated into the soil. Three treatments were implemented to test the compatibility of *MycoApply* with starter fertilizer. Ammonium polyphosphate (APP; 10-34-0) starter fertilizer was applied at 5 gallons acre^{-1}, supplying 20 lbs P_2O_5 acre^{-1}. Ammonium polyphosphate was applied with *MycoApply* at a product rate of 2 fluid ounces acre^{-1} or left uninoculated. These two treatments were compared to an untreated control with no in-furrow treatment. All in-furrow treatments were blended with water as a carrier for a total application volume of 12 gallons acre^{-1}. In a separate trial, *MycoApply* was impregnated with a slow-release polymer coating on urea (46-0-0), which was then applied in a pre-plant sub-surface band 6 inches directly below the crop row at a rate of 150 lbs N acre^{-1}. *MycoApply* was impregnated inside of the polymer coating on the urea prills at a rate of 2 fluid ounces acre^{-1} or left uninoculated. These two treatments were compared to an unfertilized control.

Measured Parameters

For the 2018 trial, root systems of four plants per plot were removed at the V8 growth stage from the outside rows using a shovel. Excess soil was gently removed by washing with a garden hose, leaving the rhizosheath soil that was directly in contact with the roots. The washed roots were sent to a third-party laboratory to be analyzed for abundance of mycorrhizal colonies.

Grain yield and harvest moisture were measured in all three trials by harvesting the center two rows of each plot with an ALMACO research plot combine and the subsequent grain yield values were standardized to 15.5% moisture. Subsamples of the harvested grain were evaluated for yield components of kernel number and average kernel weight. Kernel weights are presented at 0% moisture.

Experimental Design and Analysis

All trials were planted in a randomized complete block design with six replications. Experimental units were plots four rows wide and 37.5 feet in length with 30-inch row spacing. Statistical analysis was conducted using a linear mixed model approach using PROC MIXED in SAS (version 9.4; SAS Institute, Cary, NC). Treatment was considered a fixed effect, with replication as a random factor in the model. Treatment means were separated using Fisher's protected LSD test with significance declared at $P \leq 0.10$. Normality of the errors was conducted with PROC UNIVARIATE and the homogeneity of variance on the errors was assessed with PROC GLM.

RESULTS AND DISCUSSION

Effects of *MycoApply* in a Standard Management System

In the 2018 study, fungal colonies did not form on the untreated plant roots, but did in all plots receiving *MycoApply* in-furrow (Table 1). Although *MycoApply* led to mycorrhizal growth on maize roots, there was minimal effect of this inoculant on grain yield or yield components (Table 1).

Table 1. Influence of *MycoApply* application on V8 mycorrhizal fungi colony formation, grain yield, and yield components of maize grown at Champaign, IL in 2018.

Treatment	Mycorrhizal Colonies	Grain Yield†	Kernel Number	Kernel Weight††
		bu acre^{-1}	kernels m^{-2}	mg kernel^{-1}
UTC	0	238	4689	268
MycoApply	15	240	4702	273
LSD (0.10)	2	NS	NS	NS

†Grain yields reported at 15.5% moisture. †† Kernel weights reported at 0% moisture.

Synergies of *MycoApply* with P Fertility

In 2021, APP starter fertilizer tended to increase grain yield by 7 bushels acre^{-1} compared to the untreated control (Table 2). There was a synergistic effect of combining *MycoApply* with APP, resulting in a yield increase of 13 bushels acre^{-1} compared to the UTC (Table 2). Both applications of APP alone and APP + *MycoApply* promoted greater kernel production compared to the UTC, indicating either improved early-season growth leading to a greater ovule development or less kernel abortion (Table 2). In addition, *MycoApply* with APP tended to increase kernel number compared to APP alone (Table 2). Due to yield component compensation, the application of APP alone led to a lower kernel weight compared to the UTC. However, maize plants treated with APP + *MycoApply* produced a greater number of kernels with the same average kernel weight as the UTC (Table 2). This finding infers that applications of *MycoApply* had a season-long effect on P availability, resulting in late-season plant health during grain fill.

Table 2. Influence of *MycoApply* and ammonium polyphosphate starter fertilizer applications on grain yield and yield components of maize grown at Champaign, IL in 2021.

Treatment	Grain Yield†	Kernel Number	Kernel Weight††
	bu acre^{-1}	kernels m^{-2}	mg kernel^{-1}
UTC	270	5112	281
APP	277	5362	274
APP + *MycoApply*	283	5456	281
LSD (0.10)	12	194	NS

†Grain yields reported at 15.5% moisture. †† Kernel weights reported at 0% moisture.

New Potential *MycoApply* Application Method

Banded applications of slow-release urea (SR urea) significantly increased grain yield compared to the UTC; however, the addition of *MycoApply* in the polymer coating further increased yields compared to the uninoculated SR urea (Table 3). Grain yield benefits due to *MycoApply* application were a function of greater kernel production, with a similar average kernel weight (Table 3).

Table 3. Influence of *MycoApply* and slow-release urea fertilizer applications on grain yield and yield components of maize grown at Champaign, IL in 2021.

Treatment	Grain Yield†	Kernel Number	Kernel Weight††
	bu acre^{-1}	kernels m^{-2}	mg kernel^{-1}
UTC	148	3339	237
SR Urea	253	5303	255
SR Urea + *MycoApply*	262	5522	253
LSD (0.10)	9	426	NS

†Grain yields reported at 15.5% moisture. †† Kernel weights reported at 0% moisture.

CONCLUSIONS

The application of *MycoApply* alone, resulted in successful mycorrhizal colonization on maize roots, demonstrating the potential for greater root surface area and improved yield potential. However, grain yield benefits were not realized unless *MycoApply* was applied with concentrated fertilizer applications. When *MycoApply* was either combined with APP in-furrow or coated within a SR urea source in a pre-plant band, consistent grain yield increases were observed. The observed yield benefits from various application methods show the versatility of this mycorrhizal fungi inoculant. We conclude that *MycoApply* positively affects plant availability of fertilizer applied nutrients, especially when the fertilizer is concentrated in close proximity to the mycorrhizal fungi inoculant.

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