5-2019

The Evaluation of Low-Use-Rate Zinc Fertilization Strategies on Seedling Canopy Coverage, Zn Concentration, Biomass, and Grain Yield

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Crop, Soil, and Environmental Sciences

by

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University of Arkansas
Bachelor of Science in Crop, Soil, and Environmental Sciences, 2016

May 2019
University of Arkansas

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Abstract

Zinc (Zn) is the most common micronutrient deficiency in flooded rice (*Oryza sativa* L.). Some new Zn fertilization methods have been advertised, but have limited research supporting their efficacy. This study mainly compared the effect of Zn-seed treatment rate in combination with other low-use-rate Zn-fertilization methods to the standard of 11 kg Zn ha^{-1} as $ZnSO_4$ on rice early-season canopy cover, tissue-Zn concentration, and grain yield. A secondary objective evaluated an alternative method (to seed treatment with ZnO) of enhancing seed-Zn concentration using post-heading foliar-Zn application on seedling tissue-Zn concentration and grain yield. For the main objective, rice seed was treated with 0 or 3.3 g Zn kg^{-1} using ZnO. The treated rice seed was planted and received the following Zn treatments in the field: i) no-Zn, ii) granular $ZnSO_4$ applied at 11 kg Zn ha^{-1} (GRAN), iii) 1.68 kg Zn ha^{-1} as MicroEssentials (MESZ), iv) 1.1 kg Zn ha^{-1} as foliar-applied Zn-EDTA (EDTA), and v/vi) 0.56 and 1.12 kg Zn ha^{-1} of WolfTrax Zn-DDP (DDP). For the second objective, in 2017, rice seed was biofortified by applying 0, 1, 2, or 3 applications of 1.75 kg Zn ha^{-1} as $ZnSO_4$ solution after 100% panicle emergence. In 2018, a greenhouse experiment evaluated non-fortified rice seed treated with ZnO compared to Zn-biofortified seed without a ZnO coating. In the field, each level of biofortified rice was planted with and without a ZnO-seed treatment. For the first objective, canopy coverage at two site-years was significantly affected by Zn-fertilization method or the significant Zn-seed treatment rate and Zn-fertilization method interaction. Rice fertilized with MESZ had the greatest canopy coverage at these sites. Rice receiving GRAN, increased seedling-Zn concentration by at least 4.3 mg Zn kg^{-1} above rice not receiving Zn. A ZnO-seed treatment increased seedling-Zn concentration above rice that did not receive a ZnO-seed treatment. In general, low-use-rate Zn fertilizers provide minimal Zn nutrition for rice seedlings, and should be avoided on fields where Zn deficiencies are probable. For the second objective investigating

biofortification of rice seed with Zn, the ZnO-seed treatment provided greater Zn nutrition for seedling rice compared to biofortified rice grains indicating that ZnO-seed treatments are more advantageous than Zn biofortification for early-season Zn nutrition of seedling rice.

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Chapter 1

Literature Review

Introduction

Zinc (Zn) is the most common micronutrient deficiency in flooded rice (*Oryza sativa* L.) production around the world. Cakmak (2008) estimated that 50% of the soils used to grow cereal crops have deficient Zn concentrations, contributing to reductions in crop yield and less nutritional quality of grain. Economically, rice is an important commodity grown in the United States with 1.27 million ha planted in 2016 (USDA-NASS, 2017). Arkansas has been the top rice producer in the United States since 1973, with 49% of the harvested area for the United States in 2016 (USDA-NASS, 2017). Harvested rice grain was valued at nearly $2.37 billion and $1.0 billion (USD) for the United States and Arkansas, respectively, in 2016 (USDA-NASS, 2016).

In the United States, Zn deficiency has been reported in all major rice-producing areas. Zinc deficiency can cause potential yield losses near 100%, if severe and left uncorrected, but typically results in 10-60% yield loss (Norman et al., 2003). Much of the rice in Arkansas is grown on alkaline soils due to long-term irrigation with ground water high in Ca and Mg carbonates. Slaton et al. (2002) reported that 79% of the soils used for rice production in Arkansas had a pH> 6.0 and may require Zn fertilization for normal rice growth and yield. In Arkansas, recommendations to apply Zn are given when three criteria are met including i) sandy or silt loam soil texture, ii) soil pH > 6.0, and iii) soil-test Zn concentrations below the critical value (Mehlich-3 Zn ≤ 4.0 mg Zn kg^{-1}) (Slaton et al., 2002; Norman et al., 2013). A wide variety of Zn fertilizer formulations (liquid, granular, and powder) varying in chemical and physical composition and application methods have been successfully utilized for correcting or preventing Zn deficiency of rice (Slaton et al., 2005). The standard method of Zn fertilization in Arkansas for the past 50 years has been the application of 11 kg Zn ha^{-1}; however, applying preplant or postemergence Zn solutions at 1 kg Zn ha^{-1} (Norman et al., 2003) or seed treated with 2.5 to 5.0

g Zn kg^{-1} (Slaton et al., 2001) have increased in popularity since the early 2000s. Fertilizer manufacturers have developed new Zn-containing fertilizers that are sold to producers with limited research verifying their efficacy. This literature review describes Zn deficiency in rice and, Zn fertilizer application recommendations and methods, and examines research results on new Zn fertilization methods, fertilizer properties, and Zn biofortification of rice grain. This literature review also examines data on how crop development stage influences crop canopy coverage and interception of applied solutions.

Soil Zn Availability

Zinc deficiency has been reported for plants grown on nearly all soil orders and textures in most countries around the world (Alloway, 2009). The plant-available fraction of soil Zn is controlled by several properties including soil pH, high soluble P content, agronomic practices, soil texture, soil Zn concentration, and their interactions.

Soil pH is regarded as the single most important soil factor influencing soil Zn availability to plants. Lindsay (1972) reported that Zn solubility declines 100-fold for every 1.0 unit increase of soil pH. In Arkansas, 61% of the soils used for rice production have pH ≥ 6.3 (DeLong et al., 2015). The neutral to alkaline soil pH in Arkansas' row-crop producing area is largely the result of long-term use of irrigation water with high levels of Ca and Mg bicarbonates that precipitate causing the soil pH to increase. Soil pH can also influence Zn availability through sorption onto soil colloids. The relationship of Zn sorption to soil colloids is also dependent upon the amount and type of clay present in the soil, organic matter content, and the presence of oxides (Singh et al., 2008). In general, increasing clay content and organic matter in the soil results in higher cation exchange capacity (CEC). Shuman (1975) found that soils with more clay content and organic matter had higher adsorptive capacity and bonding energy for Zn, versus sandy soils. However, in sandier soils, pH had more of an influence on adsorption than soils high in clay content and organic matter. The type of clay present in the soil can also influence adsorption, for example, 1:1 clays such as kaolinites tend to have a more rapid cation exchange than the 2:1 clays illite and montmorillonite (Barrow, 1993). When Zn is added to the soil it can bind to hydrated Al and Fe oxides becoming unavailable to plants (Stanton and Burger, 1967). Kalbasi et al. (1977) reported that Fe_2O_3 had a higher Zn adsorption capacity than Al_2O_3 at the same pH.

In soils where high amounts of soluble P are present, Zn deficiency can be induced in soils that are low in total Zn (Olsen, 1972). Many crops have experienced P-induced Zn deficiency including, okra (*Abelmoschus esculentus* L.; Loneragan et al., 1982) cotton (*Gossypium hirsutum* L.; Cakmak and Marschner, 1986); wheat (*Triticum aestivum*, Singh et al., 1986); and bean (*Phaseolus vulgaris* L., Singh et al., 1988). One mechanism of P-induced Zn deficiency might be caused by diluting Zn tissue concentration inducing Zn deficiency (Singh et al., 1988). A second mechanism of P-induced Zn deficiency could result from reduced translocation of Zn from roots to the tops of the plant (Singh et al., 1988).

Land leveling can remove topsoil and organic matter and increase the likelihood of Zn deficiency. Land leveling is a common agronomic practice in Arkansas as it facilitates uniform and rapid distribution of irrigation water across fields, conservation of soil and water, and more uniform crop growth and yield (Whitney et al., 1950). In the land leveling process, topsoil is removed, the field put to a uniform grade, and the topsoil is redistributed back onto the field. Despite topsoil replacement, infertile subsoils are often exposed or the topsoil depth is very shallow creating spatial variability in soil fertility and productivity. In Arkansas, many farmers report a decline in soil fertility and reduced crop productivity due to land leveling, and it is estimated between 28,000 and 33,000 ha are land leveled annually (Brye et al., 2006).

Water use efficiency has been of recent research interest due to decreased irrigation water availability. In areas where water is scarce, the lack of water has forced some rice producers to shift irrigation practices from continuous flooding to alternate wetting and drying (Gao et al., 2006). Gao et al. (2006) reported that rice grown in alternate wetting and drying had lower shoot Zn concentration at the tillering stage than flooded rice when no Zn fertilizer was applied. This suggests that upland production of rice may result in lower soil Zn availability as compared to continuous flooding. Giordano and Mortvedt (1972) also found that, in alkaline soils (7.5 pH), total Zn uptake for flooded rice was much greater than for rice grown under nonflooded conditions. However, in the Philippines, Zn uptake by rice grown on acidic soil was lower in flooded than non-flooded (Karim and Vlamis, 1962).

Soil analysis can be critical in predicting possible Zn deficiency. A variety of methods are used to determine soil Zn concentrations, and Diethylenetriamine pentaacetate (DTPA), HCl and Mehlich-3 extraction methods can differ in the amount of Zn extracted from the soil. Lindsay and Norvell (1978) developed a critical DTPA-extractable Zn concentration in soils that ranges from 0.6 to 0.8 mg Zn kg^{-1} and corresponds to 1.2 to 1.8 mg Zn kg^{-1} Mehlich-3 extractable Zn. California is the only rice-producing state to use a critical soil Zn level using the DTPA method to estimate soil Zn availability. The critical DTPA-Zn concentration for rice used to recommend Zn fertilization is $\leq$0.5 mg Zn kg^{-1} (Williams, 2010). The HCl extraction method is used in other rice-growing countries with a critical concentration set at 1.0 mg Zn kg^{-1} (Ponnamperuma et al., 1981). In Arkansas, soil-Zn availability is determined using the Mehlich-3 method. The Arkansas recommendations are based on soil pH and four levels of Mehlich-3 extractable Zn including very low (≤1.5 mg Zn kg^{-1}), low (1.6-2.5 mg Zn kg^{-1}), medium (2.6-4.0 mg Zn kg^{-1}) and optimum (≥4.0 mg Zn kg^{-1}; Slaton et al., 2002; Norman et al., 2013). Previous recommendations for applying Zn in Arkansas were based on soil pH and soil texture, and did not account for residual Zn from annual application of $ZnSO_4$ (Slaton et al., 2002).

Zn Deficiency Symptoms in Rice

Zinc deficiency is considered the most common micronutrient disorder for crop production in the world (Brown et al., 1993). Crops differ in their susceptibility to Zn deficiency with corn (*Zea mays*), onion (*Allium cepa*), and sorghum (*Sorghum bicolor*) considered highly susceptible to Zn deficiency (Martens and Westermann, 1991). In general, cereal crops are prone to Zn deficiency, which can cause reduced grain yields and grain with low Zn concentration. The first recorded Zn deficiency symptoms were identified in maize (Mazé, 1914). Sommer and Lipman (1926) determined that Zn was generally essential for plants. Zinc has a key role in plant metabolism including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) replication, cell division, and protein synthesis (Marschner, 1995). Zinc is also important in catalyzing enzymatic processes, and is a key component in alcohol dehydrogenase. In situations where Zn deficiency occurs, changes to the metabolism of carbohydrates, proteins, and auxins can lead to impaired cellular membrane integrity (Römheld and Marschner, 1991).

Zinc deficiencies were first reported in rice grown in the USA in the 1960s (Norman et al., 2003). Although Zn deficiencies were present prior to the 1960s, symptoms were often misdiagnosed as other nutrient deficiencies. Observable Zn deficiency symptoms in rice can manifest at any time during the season, but are typically and most prominently expressed within 1 to 2 wk after flooding. Zinc deficiency symptoms in rice may include basal chlorosis of the youngest leaves, the midrib of the oldest leaves turns yellow to white, loss of leaf turgidity, bronzing of older leaves, reduced tillering, stand loss (after flooding), stacking of leaf collars, and delayed maturity (Norman et al., 2003). During the reproductive growth phase, the Zn-deficient symptoms of rice include chlorotic leaves and glumes and brown flecking and spotting of leaves and panicles (Sedberry et al., 1978).

Zinc has intermediate mobility in plants, and has been shown to travel through the xylem and phloem (Longnecker and Robson, 1993; Marschner, 1995). When Zn is taken up by roots it is rapidly translocated to the shoots primarily through xylem transport (Longnecker and Robson, 1993). Zinc can be translocated to other plant organs when Zn availability is low (Longnecker and Robson, 1993; Haslett et al., 2001). Haslett et al. (2001) demonstrated that nearly one-half of the Zn applied to the leaves of 5-wk old wheat plants was exported from the leaves to stems (34%), young leaves (8%) and roots (6%) while the rest remained in the leaf to which the Zn was applied.

Zinc is also essential for normal plant metabolism and enzymatic function, and has been found in all six enzyme classes (Barak and Helmke, 1993). Enzymes containing Zn include alcohol dehydrogenase, Cu-Zn-superoxide dismutase, carbonic anhydrase, and RNA polymerase. (Römheld and Marschner, 1991). Alcohol dehydrogenase is the predominant enzyme involved in anaerobic metabolism in plant roots reducing acetaldehyde to ethanol (Pedrazzini and McKee, 1984). Zinc is a cofactor for alcohol dehydrogenase. Under Zn-deficient conditions, it has been reported that alcohol dehydrogenase activity is significantly reduced to a level that anaerobic root metabolism in rice is impaired (Moore and Patrick, 1988).

In conjunction with soil sampling, routine plant analysis can aid in diagnosing possible Zn deficiencies. Although plant sampling methods to determine Zn concentrations can vary among crops, the critical Zn concentrations in the mature leaves for many crops is around 15 mg

kg^{-1} (Jones, 1991). Ohki (1984) reported that the critical concentration for Zn in sorghum could be determined by sampling the youngest mature leaf during maximum vegetative growth with a critical concentration of 10 mg Zn kg^{-1}. In rice, Zn deficiency normally affects seedlings after flooding, so whole plant samples are much easier to collect than the youngest mature leaf (Norman et al., 2003). Yoshida et al. (1973) found that when Zn was deficient there was no difference in the Zn concentrations of leaf blades, leaf sheaths plus culms, and whole shoots of rice plants so they concluded that whole shoot could be used for Zn analysis. Yoshida et al. (1973) established the following criteria for diagnosing Zn deficiency based on whole-shoot analysis by collecting rice plants 3 wk after transplanting: < 10 mg Zn kg^{-1}, definite deficiency; 10 to 15 mg Zn kg^{-1}, very likely deficiency; 15 to 20 mg Zn kg^{-1}, likely deficiency; and > 20 mg Zn kg^{-1}, unlikely deficiency.

Zn Fertilizer Sources

China, Peru, Australia, the United States, and Mexico account for 68% of the global Zn production while another 45 countries mine the remaining 32% of the world's Zn (USGS, 2017). The majority of mined Zn is used for galvanizing steel to protect from corrosion (60%) and production of Zn alloys with various metals including copper and aluminum (17%) (IZA, 2016). More Zn fertilizers are being applied to crops and mining Zn for fertilizer production has increased (Montalvo et al., 2016). There has been a 2% increase from 2010 (6%) to 2015 (8%) of mined Zn that was used for making Zn oxide (ZnO) and Zn sulfate ($ZnSO_4$) compounds that are commonly used as fertilizers (IZA, 2016). Zinc fertilizer sources vary in water solubility, behavior in the soil, Zn concentration, chemical composition, and price. Three main classes of Zn fertilizers are inorganic, chelates and natural organic complexes (Mortvedt, 1991).

Water solubility is an important characteristic in predicting the availability of Zn fertilizers, and a virtual consensus in the literature suggests that granular Zn fertilizers should contain a minimum of 40-50% water solubility for immediate effectiveness for crops (Mortvedt, 1992; Amrani et al., 1999; Liscano et al., 2000). The most common Zn fertilizers used in agriculture are the inorganic sources ZnO (60-78% Zn; 1.6 mg L^{-1} water solubility) and $ZnSO_4$ (36% Zn; 960 g L^{-1} water solubility) that are typically sold as granules or powders (ChemicalBook, 2016). Partially acidulated ZnO with sulfuric acid (H_2SO_4) followed by dehydration can create oxysulfate fertilizers, and the fraction of water-soluble Zn in these fertilizers is related to the amount of $ZnSO_4$ formed (Mortvedt, 1992). Due to the additional processes required to make $ZnSO_4$ it is more expensive compared to ZnO. Ethylenediaminetetraacetic acid (EDTA) is an organic molecule commonly used in the chelating process, and when EDTA binds to a Zn^{2+} ion it forms Zn-EDTA (9% Zn; water soluble). The organic molecule EDTA protects the nutrient from reacting with the soil to become immobilized, increasing the bioavailability of the chelated nutrient to plants, and EDTA-containing fertilizers are typically more expensive than inorganic-Zn sources (Gangloff et al., 2006). Gangloff et al. (2006) compared the mobility of inorganic, chelated and natural organic complexed Zn sources, and reported that water-soluble Zn content was the predominate factor affecting the movement of Zn in the soil, regardless of total Zn content and complexation. Gangloff et al. (2006) reported that soil mobility of Zn-EDTA was greatest among the sources tested and only limited amounts of the Zn added as Zn-EDTA were recovered via extraction with DTPA in the deepest part of the column (8-11 cm) suggesting the added Zn had leached through the column. Zinc added as $ZnSO_4$ was found below the 2 cm depth, but did not reach the deepest part of the soil column. Zinc added as ZnO did not travel below the top 2 cm of the soil profile. Mortevedt and Gilkes

(1993) also reported that the downward movement of Zn in soil columns was greatest with Zn-EDTA fertilizer as compared to ZnO and $ZnSO_4$, and that Zn in chelated fertilizers can be prone to leaching because of the high water solubility and the ability to resist adsorption by the soil.

The effectiveness of EDTA is the chelating molecule's ability to keep nutrients in a soluble and mobile form in the soil (Norvell and Lindsay, 1969). However, when Zn-EDTA is added to the soil, the chelated Zn can be substituted with another cation taking the place of Zn. Norvell and Lindsay (1969) found that the rate of cation substitution was pH dependent, with the chelation of Zn being most stable at pH 6.7. At neutral soil pH, Norvell (1991) reported the stability constant for Zn-EDTA was 17.5 and higher than for Ca-EDTA (11.6). The higher stability constant for Zn-EDTA than Ca-EDTA results in little substitution of Ca for Zn making EDTA a reliable chelating agent in most soils with near neutral pH (Mortevedt and Gilkes, 1993). Zinc in EDTA is much less stable in strongly acidic (pH=5.7) and alkaline (pH=7.85) soils where the Zn is more likely to be substituted by Fe and Ca, respectively (Norvell and Lindsay, 1969).

Natural organic complexes also bond to Zn to protect Zn from being immobilized by the soil, but many of these bonds are not understood to the extent of chelating agents (Mortvedt, 1991). Lignosulfonates are the most common natural organic fertilizer used to complex Zn and are primarily produced by reacting Zn salts with lignin from wood pulp (Montalvo et al., 2016). Natural organic complexes have an array of complexing capacity depending on the lignin source and production method. For example, Martín-Ortiz et al. (2009) showed that softwood had a higher Zn-complexing capacity than hardwood.

Soil pH is known to influence leaching of fertilizers through the soil. Alvarez et al. (2001) reported that in a slightly acidic soil (pH=6.1) a Zn-lignosulfonate fertilizer did not leach through the soil while Zn-EDTA did. However, in a neutral soil (pH=7.07) leaching was not significant for either Zn-lignosulfonate or Zn-EDTA. Alvarez and Gonzalez (2006) compared the efficiency of several Zn chelating agents including ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid; EDDHA) and EDTA to natural organic complexed (Zn-phenolate, Zn-lignosulfonate, Zn-polyflavonoid and Zn-glucoheptonate) fertilizers and showed that Zn uptake by corn was greater for the chelated sources than the organic-complexed sources.

Methods of Zn Fertilization

When applications of Zn are recommended, three primary methods for correcting Zn deficiency in crops include applying Zn to the i) soil surface, ii) plant foliage during vegetative growth, or iii) seed before planting (Farooq et al., 2012). The application rate and time are determined by the Zn source and strategy. Thus, careful attention is needed to select the ideal Zn fertilizer for the particular cropping system.

For the last 50 years the most common method of Zn fertilization has been to apply 11 kg Zn ha^{-1} as granular $ZnSO_4$ to the soil surface before emergence (Norman et al., 2003). Carsky and Reid (1990) concluded that a single application of 11 kg Zn ha^{-1} was sufficient to prevent Zn deficiency for five years with corn response to a single application being similar to that of corn that received annual applications of 11 kg Zn ha^{-1}. Although broadcasting <11 kg Zn ha^{-1} as granular $ZnSO_4$ has been successful in correcting Zn deficiency in corn, application of relatively low Zn rates with sufficient spatial distribution has been noted as a problem, especially when Zn is applied alone (Mortvedt and Gilkes, 1993). To assist in uniform distribution of $ZnSO_4$, granular $ZnSO_4$ is often bulk blended with other N, P, and K granular fertilizers before application to the field (Mortvedt and Gilkes, 1993). Segregation of fertilizer granules having different sizes and densities during transportation and handling can occur, leading to uneven distribution of Zn during application. For example, Hoffmeister et al. (1964) demonstrated that the main cause of Zn segregation during handling and application of bulk-blended fertilizers was caused by the difference in particle size from mixing triple superphosphate with potassium chloride.

Fertilizer granules containing multiple nutrients have been developed by fertilizer manufacturers to assist in even distribution of nutrients across the field. Nutrient availability from these sources are being examined. Degryse et al. (2016), investigated the oxidation rates of elemental sulfur (S^0) from various multinutrient fertilizers (MES10; 5% S^0 and Tiger 90; 90% S^0) by collecting SO_4-S in leached through soil columns. At the conclusion of the study (392 d), MES10 was nearly fully oxidized (>80%) while only 20% of the S^0 in Tiger 90 was oxidized and its granules were still intact. Ruffo et al. (2016) evaluated MicroEssentials® MESZ (120 g N, 175 g P, 100 g S, and 10 g Zn kg^{-1}) as a Zn fertilizer compared to increasing rates of monoammonium phosphate (MAP), ammonium sulfate, and $ZnSO_4$ as a bulk-blended fertilizer. They found that corn fertilized with MESZ applied at 2.24 kg Zn ha^{-1} yielded 1004 kg ha^{-1} more than the corn fertilized with bulk-blended treatments at the same total Zn rate.

Zinc oxide nanoparticles (ZnO NPs; nominal diameter <20 nm, 99.9%) are commonly coated to macronutrient granular fertilizers, and have been successful in reducing nutrient segregation in bulk-blended fertilizers (Mortvedt and Gilkes, 1993). Increased surface area from using ZnO powders and NPs coated to macronutrient fertilizers should increase solubility, dissolution, and distribution of Zn in the soil, but depending on the macronutrient that NPs are coated onto can influence Zn availability in the soil (Milani et al., 2015). Mortvedt and Giordano (1969) reported that powdered ZnO coated onto urea resulted in less water-soluble Zn than compared with other macronutrient fertilizers. Milani et al. (2012) coated MAP and urea with

ZnO NPs so that the final analysis of each amended fertilizer contained about 1.5% Zn by weight. The Zn-amended fertilizers were applied to moistened sand columns to allow the fertilizers to dissolve and speciate the Zn compounds. Milani et al. (2012) concluded that Zn coated to MAP granules tended to form water-soluble Zn ammonium phosphate [$Zn(NH_4)PO_4$], but Zn-coated urea granules showed that Zn speciation was not affected, and water-insoluble ZnO was the main form of Zn detected.

Some Zn fertilizer manufacturers market their products as being more effective than $ZnSO_4$ and express the greater efficacy as an ‘efficiency factor’ (Shaver and Westfall, 2008). For example, an efficiency factor of 10:1 suggests that 10 kg Zn ha^{-1} applied as $ZnSO_4$ would be equivalent to 1 kg Zn ha^{-1} applied as another Zn-containing fertilizer claiming enhanced properties. Among the products currently being marketed, Wolftrax® DDP (dry dispersible powder; Compass Minerals, Overland Park, KS) and Zn lignosulfonate (granular fertilizer) products make efficiency factor claims. Wolftrax states that the Zn-DDP product has a 9:1 efficiency ratio because of micro-static adhesion that allows the Zn powder to adhere to each fertilizer granule enhancing the distribution of Zn across the field compared to application of granular $ZnSO_4$. The Wolftrax Zn-DDP label states that a maximum of 1 kg (0.62 kg Zn) of their product should be applied to 100 kg of granular fertilizer. Applying more than 1 kg of Wolftrax Zn-DDP will result in the product not adhering to the fertilizer granules. Recommended foliar application rates of Zn-DDP are 980 g Zn-DDP ha^{-1} (608 g Zn ha^{-1}) that can be applied until pollination.

Origin® (Winfield Solutions, LLC, Saint Paul, MN) is a granular fertilizer that contains 10% Zn complexed with lignosulfonate and claims a 7:1 efficiency ratio. The lignosulfonate is a natural organic material that protects Zn from being tied up by the soil and is marketed as being more efficient than granular Zn sulfate. Shaver and Westfall (2008) reported that when corn was planted into Zn-deficient soil (0.4 mg Zn kg^{-1} DTPA), seedling corn had higher Zn concentrations when fertilized with granular $ZnSO_4$ (7.6 mg Zn kg^{-1}) compared to corn fertilized with Wolftrax Zn-DDP (5.6 mg Zn kg^{-1}) at its efficiency ratio. Research that compares Zn fertilizer sources and supports these efficiency ratio claims is seldom published in peer-reviewed journals making it difficult for unbiased practitioners to refute or support the claims.

Foliar application of Zn has been used to prevent and correct crop Zn deficiency, and is commonly used in high value crops such as fruits and vegetables (Mortvedt and Gilkes, 1993). When $ZnSO_4$ was sprayed to mango (*Mangifera indica* L.) foliage, mango uptake of Zn was more rapid than soil-applied $ZnSO_4$ with the same application time (Bahadur et al., 1998). Hamza and Sadanandan (2005) found that the highest Zn concentration in the leaf and berry of black pepper (*Piper nigrum* L.) was when plants received a foliar application of 0.5% $ZnSO_4$ solution compared with the application of a 0.1% Zn-EDTA solution. In rice, Zn solutions are typically applied to seedling foliage at the 2-leaf stage with the majority of the solution contacting the soil surface rather than plant foliage. Karak et al. (2005) evaluated split applications of Zn-EDTA and $ZnSO_4$ solutions sprayed to the soil surface and found that Zn-EDTA was more effective at increasing soil Zn while increasing the yield of rice by 26.1% as compared with $ZnSO_4$ at the same application rate and timing. Rice plants sprayed with 1.1 kg EDTA-Zn or 2.2 kg $ZnSO_4$-Zn ha^{-1} produced similar yields compared with the traditional 11 kg Zn ha^{-1} broadcast preplant as granular $ZnSO_4$ (Slaton et al., 2005).

Zinc fertilization of crops by Zn-seed treatments has become more popular in the last two decades, because it can improve crop emergence, stand establishment, and yield (Farooq et al., 2012). The Zn source used to treat seeds can influence seed germination. Peanut (*Arachis* *hypogaea*) seeds treated with 1000 mg Zn L^{-1} as ZnO NP solution had 100% germination while seeds treated with 1000 mg Zn L^{-1} as $ZnSO_4$ chelated with EDTA had 90% germination (Prasad et al., 2012). The ZnO-treated seed had greater seedling vigor, which resulted in 34% higher pod yield per plant compared to application of chelated $ZnSO_4$ at the same Zn rate. However, germination declined when seeds were treated with 2000 mg Zn L^{-1}, regardless of Zn source (Prasad et al., 2012). Slaton et al. (2001) reported that rice seed treated with $ZnSO_4$, or without seed treatment, had significantly longer radicle and shoot lengths than seed treated with Zn-EDTA. The latter, inhibited germination when applied at the highest rates and encouraged fungal growth on seeds during germination tests. Slaton et al. (2001) also showed that seed-Zn treatments increased the Zn concentration of rice seedlings by 4.7 mg Zn kg^{-1} above the Zn concentration of seedling rice without seed treatment. For rice, seed treatments were deemed agronomically viable if seeds had at least 2.2 to 5.7 g Zn kg^{-1} (seed) (Slaton et al., 2001).

Biofortification of Zn

Zinc deficiency is an important human health issue affecting nearly one-third of the world's population, especially in areas where cereal grains are consumed as a staple food source (Phattarakul et al., 2012). One method for increasing grain Zn concentrations is through genetic breeding, but breeding for greater grain Zn may require years to develop an acceptable cultivar with enhanced Zn concentrations. Wild emmer wheat (*Triticum turgidum ssp. dicoccoides*) showed genetic potential for Zn uptake ranging in Zn concentrations from 14 to 190 mg Zn kg^{-1} (Cakmak et al., 2004). Brown rice Zn concentrations ranging from 13.5 to 58.4 mg Zn kg^{-1} were reported among varieties examined at the International Rice Research Institute (Welch and Graham, 2002). Agronomically, increased grain Zn concentrations can act as a starter fertilizer, and rice containing 67 mg Zn kg^{-1} had significantly longer coleoptiles than rice grain with 18 mg

Zn kg^{-1} (Boonchuay et al., 2013). An agronomic approach for enhancing Zn in the grain has been achieved through a combination of fertilization methods including soil and foliar applications (Phattarakul et al., 2012; Zou et al., 2012).

Cereal crops such as wheat and rice have shown the most promise for increasing grain Zn concentrations thorough fertilization. A wheat study that included 23 site-years in seven countries, concluded that a soil applications of 50 mg Zn ha^{-1} sprayed to the soil surface combined with two foliar applications (heading and milk stage) of 0.5% (w/v) $ZnSO_4$ solution at a rate of 600-800 L ha^{-1} increased grain-Zn concentration to 49 mg Zn kg^{-1} compared to wheat receiving no Zn (27 mg Zn kg^{-1}) (Zou et al., 2012). A similar study evaluating Zn fertilization methods for rice to increase grain Zn concentration across five countries showed that brown rice Zn concentrations were increased by 66% from two foliar applications made at panicle initiation and 1 wk after flowering compared to 50 kg Zn ha^{-1} applied to the soil preplant, which increased grain Zn concentrations by only 2.4% (Phattarakul et al., 2012). Greater Zn concentrations in polished rice were measured when rice was fertilized with 2.5 kg Zn ha^{-1} with either Zn amino acid or $ZnSO_4$ compared to Zn-EDTA or Zn-Citrate (Wei et al., 2012). The timing of foliar Zn application significantly affected the accumulation of Zn in the rice grain. When rice was fertilized with Zn after flowering there was a 56% percent increase in Zn concentration in brown rice compared to minimal increases from foliar Zn applied during panicle initiation and booting (Boonchuay et al., 2012).

Summary

Zinc has been the most problematic micronutrient deficiency for rice around the world. Many researchers have measured rice yield increases from Zn fertilization (Westfall et al., 1971; Sedberry et al., 1978; Slaton et al., 2005) and Zn fertilization options and guidelines have been developed for many crops. Numerous studies have been conducted evaluating the efficacy of Zn availably from various Zn fertilizer sources and nearly all published reports suggest that the fertilizer source should contain 40-50% water-soluble Zn to be immediately plant available (Mortvedt, 1992; Amrani et al., 1999; Liscano et al., 2000). To correct or prevent Zn deficiency, applications of $ZnSO_4$ at 11 kg Zn ha^{-1} to the soil preplant, foliar application early postemergence or applying Zn to seed have been the most common Zn fertilization methods for rice during the last 50 years (Norman et al., 2003). The need for farmers to reduce crop production costs that fit into established crop management practices and aggressive marketing and development of new fertilizers and fertilization methods by fertilizer suppliers have resulted in an influx of products and practices.

New low-use-rate Zn fertilization methods are being marketed to Arkansas rice growers with insufficient, unbiased research verifying their efficacy. Examples of these fertilizers include, but are not limited to, Wolftrax Zn-DDP and MESZ. Wolftrax Zn-DDP, according to its label, should be applied at rates up to 1 kg $ha^{-1}$of Zn-DDP product to 100 kg of granular fertilizer. The MESZ fertilizer is a granular P source that is mostly applied preplant and would supply 1.0 to 1.5 kg Zn ha^{-1} when applied to satisfy the typical P rate applied to rice. Additionally, it has been noted that the amount of Zn commercially applied to rice seed is often less than the minimum recommended rate, which probably diminishes the effectiveness of Zn-seed treatments to prevent Zn deficiency. Thus, there is the need to evaluate the effectiveness of established and new low-use-rate Zn fertilization methods to determine which ones used alone or in combination are viable methods for supplying Zn to seedling rice. The objectives of this research are to:

1. Evaluate the effect of Zn-seed treatment rate in combination with other Zn-fertilization methods including the standard of 11 kg Zn ha^{-1}, on rice early season canopy cover, seedling-Zn concentration, and grain yield.
2. Evaluate an alternative method (to seed treatment with ZnO) of enhancing seed-Zn concentration and seedling vigor by post-heading foliar-Zn application.

Based on previous research by Liscano et al. (2000) and Slaton et al. (2001), we hypothesized that seedling-Zn concentration and canopy coverage will be different among fertilizer sources and their seed treatment combinations. We additionally hypothesized, based on Boonchuay et al. (2013), that seedling-Zn concentration of Zn-biofortified rice grain will be similar to rice treated with ZnO as a seed treatment.

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Chapter 2

Effect of Low-Use-Rate Zinc Fertilization Strategies on Rice Seedling Zinc Concentration, Canopy Coverage, Biomass and Grain Yield

Abstract

Low-use-rate Zn fertilization methods have been developed and marketed for rice (*Oryza sativa* L.) fertilization with limited research validating their efficacy. Our research objectives were to evaluate the effect of Zn-seed treatment rate combined with six Zn-fertilization methods on early-season canopy coverage, tissue-Zn concentration at the midtillering stage, and rice grain yield. The field experiment was conducted on six silt loam soils and one clay soil. Rice seed was treated with 0 or 3.3 g Zn kg^{-1} as ZnO and combined with i) no Zn, ii) granular $ZnSO_4$ applied at 11 kg Zn ha^{-1} (GRAN), iii) 1.68 kg Zn ha^{-1} as MicroEssentials (MESZ), iv) 1.1 kg Zn ha^{-1} as foliar-applied Zn-EDTA (EDTA), and v/vi) 0.56 and 1.12 kg Zn ha^{-1} of WolfTrax Zn-DDP (DDP). Canopy coverage of seedling rice was measured at six sites and analyzed by site. Four sites were not affected by Zn-seed treatment rate or fertilization method. At two sites, canopy coverage was significantly affected by Zn-fertilization method or the significant Zn-seed treatment rate and Zn-fertilization method interaction. Rice receiving MESZ had the greatest canopy coverage at these sites. When averaged across sites and Zn fertilization methods, application of 3.3 g Zn kg^{-1} increased tissue-Zn concentration and biomass by 1.5 mg Zn kg^{-1}, 63 kg ha^{-1} respectively. Rice receiving GRAN, increased tissue-Zn concentration by 4.3 mg Zn kg^{-1} above rice not receiving Zn. Low-use-rate Zn fertilizers provide minimal Zn nutrition for rice seedlings, and should be avoided on fields where Zn deficiencies are probable.

Introduction

Rice is among the plants considered sensitive to zinc (Zn) deficiency (Takkar and Singh, 1978). Zinc deficiency of rice has been reported in nearly all rice-producing countries (Alloway, 2009), and all of the rice-producing states in the United States (Giordano, 1977). Zinc deficiency typically causes yield losses of 10 to 60%, but, in severe cases, plant death and stand loss can occur (Norman et al., 2003) making Zn deficiency a serious problem for rice production. Rice grown under flooded conditions is generally considered more susceptible to Zn deficiency than is rice managed as upland or alternate wetting and drying irrigation systems (Yoshida et al., 1973; Neue et al., 1998; Johnson-Beebout et al., 2009), although Gao et al. (2006) and Giordano and Mortvedt (1974) observed more Zn deficiency in non-flooded conditions compared with flooded conditions. Zinc deficiency is the most common micronutrient deficiency of rice in Arkansas, where, according to Delong et al. (2018), 58% of the soil-sampled acreage tests very low or low in Zn and is at risk to Zn deficiency.

One of the most common recommendations for prevention of Zn deficiency is to apply 11 kg Zn ha^{-1} as zinc sulfate ($ZnSO_4$) (Sharma and Katyal, 1986; Amrani et al., 1999; Norman et al., 2013). Recommendations for fertilization with relatively high granular Zn rates have existed since research was first initiated investigating how to prevent crop Zn deficiency (Sommer and Lipman, 1926). Bulk blending 11 kg Zn ha^{-1} as $ZnSO_4$ granules with other preplant-applied macronutrient fertilizers has been the standard recommendation for rice grown in Arkansas since the early 1970s (Wells et al., 1973). Applying Zn at 11 kg Zn ha^{-1} has consistently prevented Zn deficiency and builds soil-Zn levels to help reduce the likelihood of Zn deficiency for several years (Takkar et al., 1975; Carsky and Reid, 1990; Slaton et al., 2005). One disadvantage of bulk blending Zn granules is the potential for granule segregation, due to differences in granule size, leading to uneven application of nutrients (Mortvedt and Gilkes, 1993). Development of Zn fertilizers with Zn sources other than $ZnSO_4$ (e.g., Zn oxides, oxysulfates, lignosulfonates, and synthetic chelates) required granular Zn fertilizer recommendations be modified to account for differences in efficacy among fertilizers attributed to the variation in Zn bioavailability. For example, regardless of the Zn source, granular Zn fertilizers should contain 40 to 50% of the total Zn in the water-soluble form (Mortvedt, 1992; Amrani et al., 1999; Liscano et al., 2000; Gangloff et al., 2002). Application of 11 kg Zn ha^{-1} is not guaranteed to prevent Zn deficiency because the water-soluble Zn content of a granular fertilizer is not always required information for fertilizer labels. Using Zn fertilizers with a low water-soluble Zn content may not provide sufficient Zn nutrition, and require rescue Zn applications if Zn deficiency symptoms are observed.

The high costs of elemental Zn has increased the price of Zn fertilizers over the past 20 yr. The price of elemental Zn was \$1.12 kg^{-1} in 1996 (Plachy, 1998), gradually increased to \$1.48 kg^{-1} in 2005, peaked at \$3.50 kg^{-1} in 2006 (Tolcin, 2008) and has since declined and stabilized around \$2.10 kg^{-1} in 2015 (Tolcin, 2017; Fig. 1). The risks associated with using granular Zn fertilizers coupled with the high cost associated with this Zn-fertilization strategy have lead growers to seek effective but low cost alternative Zn-fertilization methods. Many of the alternative Zn-fertilization strategies lack unbiased research to validate their efficacy compared to the standard preplant application of 11 kg Zn ha^{-1} as granular $ZnSO_4$. Alternative, low-use-rate, Zn-fertilization methods include applications of Zn solutions at preplant or post-emergence, in-furrow Zn applications during planting, application of Zn directly to seed, surface application of Zn to macronutrient fertilizers, and inclusion of Zn as an element in multinutrient fertilizers.

Pre- (soil) and post-emergence (foliar) application of solutions containing soluble inorganic and chelated Zn have been extensively researched and successfully used for both prevention and amelioration of Zn deficiency (Mortvedt, 1991). Ethylenediaminetetraacetic acid (EDTA) is a chelating agent used to enhance Zn mobility in soil and maintain Zn bioavailability to the plant following soil application (Norvell and Lindsay, 1969; Mortvedt and Gilkes, 1993). A foliar application of Zn-EDTA or liquid $ZnSO_4$ to rice at the 2-3 leaf stage is a common practice in Arkansas. Slaton et al. (2002) reported that Zn-EDTA or $ZnSO_4$ sprayed at 1.1 and 2.2 kg Zn ha^{-1} to rice foliage was effective at preventing Zn deficiency symptoms and resulted in comparable yields to rice fertilized with 11.2 kg Zn ha^{-1} as granular $ZnSO_4$ before planting. Golden et al. (2016) reported corn (*Zea mays* L.) fertilized with 2.24 kg Zn ha^{-1} foliar-applied Zn at the V4 growth stage as Zn-citrate (152.4 mg Zn kg^{-1}) resulted in greater tissue-Zn concentration compared to $ZnSO_4$ (110.5 mg Zn kg^{-1}) and Zn-EDTA (104.1 mg Zn kg^{-1}). Many product labels suggest using rates lower than the 1.1 to 2.2 kg Zn ha^{-1} commonly recommended by land-grant institutions (Camberato and Maloney, 2012; Norman et al., 2013).

Zinc application to rice seed at low rates was investigated in the 1970s, but seldom utilized until use guidelines were developed in the late 1990s (Slaton et al., 2001). Although there is a lack of statistics on how widespread Zn-seed treatments are used, zinc oxide (ZnO) is the form of Zn usually applied to rice seed. Treated rice seed should contain between 2.2 and 5.7 g Zn kg^{-1} seed, which equates to 0.06 to 0.16 and 0.19 to 0.48 kg Zn ha^{-1} with typical seeding rates for hybrid and inbred rice varieties, respectively (Norman et al., 2013). Unfortunately, the ZnO products used as the Zn source for treating rice seed are often difficult to mix and apply uniformly, and commercially-treated seed often contains less Zn than recommended (Slaton, personal communication, 2018).

Macronutrient fertilizers can also be coated with ZnO powders, such as Wolftrax Zn-DDP (dry dispersible powder, 620 g Zn kg^{-1}, Compass Minerals, Overland Park, KS), which are marketed to producers claiming enhanced efficiency compared with granular $ZnSO_4$ due to more uniform Zn distribution because Zn adheres to each macronutrient fertilizer granule. Shaver and Westfall (2008) reported that WolfTrax Zn-DDP did not increase the shoot Zn concentration of greenhouse-grown corn above the tissue-Zn concentration of corn receiving no Zn, while $ZnSO_4$ increased tissue-Zn concentration relative to the check, but similar to Zn-DDP.

Fertilizers containing multiple nutrients in a single granule have been developed to address segregation of granules in bulk-blended fertilizers, and aid in uniform nutrient distribution. Ruffo et al. (2016) reported that corn fertilized with MicroEssentials SZ (MESZ; The Mosaic Company, Plymouth, MN) yielded 11,680 kg ha^{-1}, which was significantly greater than corn fertilized with a bulk blend of monoammonium phosphate, ammonium sulfate, and $ZnSO_4$ granules applied at 2.24, 4.48, and 6.72 kg Zn ha^{-1}. Only the application of 11.2 kg Zn ha^{-1} as the physical blend was able to match the yields of 2.24 kg Zn ha^{-1} from MESZ.

The peer-reviewed literature contains few examples of research verifying manufacturer claims that multinutrient fertilizers and fertilizer coatings are effective methods of Zn-fertilization. Our research objective was to evaluate the effectiveness of low-use-rate Zn-fertilization methods, used singularly and in combination with seed-applied Zn, to increase rice seedling growth and tissue-Zn concentration as compared to the standard Zn-fertilization method of preplant soil application of 11 kg Zn ha^{-1} as $ZnSO_4$ granules.

Materials and methods

Site Description

A total of seven field trials were established in 2017 and 2018. Selected soil properties are summarized in Table 2.1. Each location is identified by the soil series and year (e.g., Calhoun-17) the trial was conducted. All trials having Calloway (fine-silty, mixed, active, thermic Aquic Fraglossudalfs) or Calhoun (fine-silty, mixed, active, thermic Typic Glossaqualfs) soils were conducted at the Pine Tree Research Station (PTRS) near Colt, AR. The Sharkey-18 trial was conducted on a Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) at the Rohwer Research Station (RRS). Composite soil samples from the 0- to 10-cm depth were collected from each block of each trial prior to treatment application and planting. Each composite sample consisted of six, 2.5-cm o.d. soil cores from the plot designated as the no-Zn control treatment. The soil samples were oven-dried at 65ºC, crushed to pass through a sieve with a 2-mm diameter screen, and analyzed for soil pH (1:2 soil:water mixture; Sikora and Kissel, 2014), organic matter by weight loss on ignition (Schulte and Hopkins, 1996), and soil nutrient concentrations extracted with Mehlich-3 solution (Zhang et al., 2014). The Mehlich-3 extracts were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Arcos-160 SOP, Spectro, NJ).

Treatments

Each trial was a randomized complete block design with a two (Zn-seed treatment rate) by six (Zn-fertilization method) factorial treatment structure containing five blocks. Individual plots for the six trials on Calloway and Calhoun soils were 1.71-m wide and 5.21-m long, allowing for 9 rows spaced 19-cm apart. For the Sharkey-18 trial, plots were 4.9-m long with 15-

cm row spacing and 9 rows. Individual plots were separated from each other by a plant-free alley that was at least 0.4-m wide.

'Roy J', 'Diamond', or 'LaKast' rice seed was treated with Zinche ST (325 g Zn kg^{-1} and 488 g Zn L^{-1}, Drexel Chemical Company, Memphis, TN) at a rate of 5 g Zn kg^{-1} seed (10.4 mL Zinche ST kg^{-1} seed). Briefly, 11.34 kg of seed was placed in a cement mixer, sprayed with a Zn suspension using a CO_2-pressurized sprayer, and allowed to mix for 10 min to ensure that the Zn evenly coated the rice seed. Rice seed was also treated with AV-1011 (50% 9,10-Anthraquinone, ARKION® Life Sciences LLC, New Castle, DE) bird repellent at 11.65 mL kg^{-1} seed (5.8 g 9,10-Anthraquinone kg^{-1} seed). Rice was drill-seeded at a rate of 80 kg seed ha^{-1} on the dates listed in Table 2.2. Subsamples (n = 3) of treated and untreated seed were digested with concentrated HNO_3 and 30% H_2O_2 to determine the seed-Zn concentration (Jones and Case, 1990). The average seed-Zn content of the treated seed lots was 3.3 g Zn kg^{-1} seed (s = 0.23), which is within the recommended range (Slaton et al., 2001; Norman et al., 2013).

The six Zn-fertilization methods included: i) no Zn, ii) granular $ZnSO_4$ applied at 11 kg Zn ha^{-1} (GRAN; 355 g Zn kg^{-1}, Winfield Solutions, LLC, Shoreview, MN), iii) MESZ applied at 1.68 kg Zn ha^{-1} [MESZ; 28% water-soluble (WS) Zn, 120 g nitrogen (N), 175 g phosphorus (P), 100 g sulfur (S), and 10 g Zn kg^{-1}], iv) 1.12 kg Zn ha^{-1} as liquid Zn-EDTA (Ultra-Che Zinc 9% EDTA; 92.4 g N and 119 g Zn L^{-1}; Winfield Solutions, LLC, Shoreview, MN) applied at the 2-leaf stage (EDTA), and v and vi) 0.56 and 1.12 kg Zn ha^{-1}, respectively, as Zn-DDP coated onto triple superphosphate and muriate of potash (DDP0.5 and DDP1, respectively). The DDP0.5 (0.9 kg product) and DDP1 (1.8 kg product) treatments were applied to a total of 280 kg fertilizer ha^{-1}, which is below the maximum, labeled rate for adherence of the product to granular fertilizer (1 kg Zn-DDP 100 kg^{-1} fertilizer).

Water-soluble Zn (WSZn) and total Zn (TZn) contents of GRAN (358 g TZn kg^{-1} and 321 g WSZn kg^{-1}), MESZ (12.0 g TZn kg^{-1} and 3.4 g WSZn kg^{-1}), and Zn-DDP (657 g TZn kg^{-1} and 75 g WSZn kg^{-1}) were determined by an independent laboratory using Association of Official Analytical Chemists (AOAC) methods 965.09 and 957.02, respectively (AOAC, 1990). Results showed that 90, 28 and 11% of TZn was present as WSZn in GRAN, MESZ, and Zn-DDP, respectively. Granular triple superphosphate and muriate of potash were broadcast to the soil surface to provide equal P (28 kg P ha^{-1}) and K (67 kg K ha^{-1}) rates for all treatments. At each site, preplant treatments were applied to the surface of a tilled soil before planting (Table 2.2). Fertilizer treatments were incorporated by tillage only at Calhoun-17. At the 4-leaf stage, urea was applied at 168 kg N ha^{-1} at each site at PTRS and 200 kg N ha^{-1} for the clay soil at RRS, and a flood was established within 2 d after N application. Standard disease, insect, and weed management practices, based on University of Arkansas Cooperative Extension Service guidelines, were followed throughout the season to ensure pests did not limit yield (Hardke et al., 2013).

Measurements and Plant Analysis

Canopy coverage was measured three to five times during early vegetative growth using Canopeo (http://www.canopeoapp.com), an iPad application. Canopy coverage data was measured at six of the seven sites (Calhoun-18a excluded). Canopeo is an image analysis tool (Mathworks, INC., Natick, MA) that uses red-green-blue (RGB) color values (Patrignani and Ochsner, 2015). The program classifies all pixels in the image during processing and results in a black and white image. In the final image, the green pixels are classified as white pixels and all non-green pixels are classified as black. Canopy measurements started following the application of the EDTA treatment and continued until after the flood was established (Table 2.2).

An iPad (5th generation; 8-megapixel camera; Hon Hai Precision Industry Co., Ltd., Tucheng District, Taipei, Taiwan) was attached to a tripod with a bracket for stability and set to a consistent 0.9-m height above the soil surface. The tripod arm was extended so that a photograph of the middle five rows (1.23 m^2) in each plot was captured to determine canopy coverage. Only five of the Zn-fertilization methods were included in canopy coverage measurements (0.56 kg Zn ha^{-1} as Zn-DDP was excluded). The number of growing degree units (GDU) of each sample time were calculated using the DD10 (DD50 when calculated using degrees Fahrenheit) program. The DD10 program calculates cumulative GDU using the daily mean temperature minus 10°C, the low temperature threshold for rice growth, and has maximum daily high (34.4°C) and low temperatures (21.1°C) that limit daily GDU to 17.8 GDU d^{-1} (Hardke et al., 2013).

A 1.8-m section of seedlings from an inside row was cut 2.0-cm above the soil surface to measure aboveground dry matter and aboveground tissue Zn concentration at the midtillering growth stage. The samples were placed in paper bags, oven-dried at 55ºC to a constant weight, weighed, and ground to pass through a sieve with 1-mm openings. Subsamples were digested, as previously described for rice seed analysis, and elemental Zn concentration in the digest was determined by ICP-AES (Jones and Case, 1990).

At maturity, a 5-m^2 section of the middle five or six (Sharkey-18) rows of each plot was harvested for grain yield using a small-plot combine. Grain weight and moisture were measured immediately after harvest. Grain yields were calculated after grain moisture content was adjusted to 120 g H_2O kg^{-1} grain.

Statistical Analyses

Analysis of variance was performed using the GLIMMIX procedure in SAS (v.9.4, SAS Institute, Inc., Cary, N.C). For seedling aboveground dry matter, tissue-Zn concentration, and grain yield, Zn-seed treatment rate and Zn-fertilization method were treated as fixed effects. Block, field trial, and their interactions were treated as random effects using a gamma distribution. For canopy measurements, data were analyzed as a two-factor factorial, repeated-measure ANOVA model, with sample time (expressed as cumulative GDU) as the repeated measure. The ANOVA on canopy coverage was performed separately for each trial with measurement time, Zn-seed treatment rate, and fertilization method included as fixed effects, and block as a random effect using a beta distribution. When appropriate, means were separated using Fisher's protected least significant difference at a significance level of 0.10.

Results and Discussion

Canopy Coverage

Canopy coverage was affected by the main effects or their interaction at two of the six sites where canopy coverage was measured (Table 2.3). At four of the sites, canopy coverage was not affected by Zn-seed treatment rate or fertilization method, but all six sites were significantly affected by GDUs as canopy coverage increased with each successive sample time (Table 2.4). At Calloway-17, canopy coverage was significantly affected by the interaction between Zn-seed treatment and Zn-fertilization method (Table 2.3). Planting Zn-treated rice seed, and fertilizing with MESZ resulted in the numerically greatest canopy coverage, averaged across sample timings, but was only significantly greater than the rice that received no Zn, with or without Zn-seed treatment, and rice treated with GRAN plus the Zn-seed treatment.

Additionally, rice fertilized with GRAN and not planted with a seed treatment resulted in greater canopy coverage than the no-Zn control without Zn-seed treatment, while the remaining combinations of fertilizer Zn and Zn-seed treatments did not significantly influence canopy coverage relative to the no-Zn control with or without Zn-seed treatment. For any individual fertilizer-Zn source, the addition of a Zn-seed treatment did not significantly affect canopy coverage, relative to seed planted without a Zn-seed treatment.

At Calloway-18b, canopy coverage was not affected by Zn-seed treatment and the Zn-seed treatment and Zn-fertilization method interaction was not significant (Table 2.3). However, Zn-fertilization method, averaged across Zn-seed treatment rates and sample times, significantly affected canopy closure. Canopy coverage was greatest when MESZ was the Zn-fertilizer source, while other Zn sources did not differ from each other or from the no-Zn control. There are two reasons why rice grown in Calloway-17 and Calloway-18b fertilized with MESZ tended to have greater canopy coverage than other Zn-fertilizer treatments, while no response to Zn was detected in other site-years. The greater early-season growth of rice fertilized with MESZ was visibly noticeable in both of these trials. First, these two trials were located on opposite ends of the same field that had a pH below 7.0 and is irrigated with water from a reservoir that does not contain dissolved Ca bicarbonate (Table 2.1). Second, MESZ was the only Zn-fertilizer treatment that included preplant N (20 kg N ha^{-1}), which could have influenced canopy development. The nitrification rate in alkaline soils used for rice production is known to be very rapid (Fitts et al., 2014) and the nitrification rate in soil is known to decline as soil pH declines (Sahrawat, 2008). The soil pH values < 7.0 may have limited nitrification and allowed for greater uptake of the preplant-applied N from MESZ. Wells et al. (1973) showed that rice receiving ammonium sulfate between planting and flooding produced larger seedlings with greater tissue-Zn concentrations than rice that received no ‘starter’ N.

The canopy coverage measurements highlight that seedling rice has limited potential to intercept foliar-applied solutions. The foliar application of EDTA occurred at the 2-3 leaf stage when canopy coverage averaged 5.7% (ranging from 0.75 to 14.1% among site-years, Table 2.4). It is a common misconception among growers and consultants that the in-season application of a chelated Zn source is intended for foliar uptake; however, our canopy coverage data indicates that the majority of fertilizer solution comes in contact with the soil surface instead of aboveground plant tissue making below ground uptake of fertilizer Zn very important. Haslett et al. (2001) reported that the EDTA chelate offers no advantage or disadvantage for Zn uptake through the leaf compared to inorganic Zn. The organic molecule EDTA enhances Zn mobility in soil increasing the likelihood that the Zn will be taken up by small seedlings (Norvell and Lindsay, 1969; Mortvedt and Gilkes, 1993).

Seedling Aboveground Biomass

Similar to tissue-Zn concentration, the Zn-seed treatment by Zn-fertilization method interaction had no significant effect on aboveground biomass ($P = 0.8514$), but aboveground biomass was significantly affected by Zn-fertilization method (Table 2.5) and Zn-seed treatment ($P = 0.0101$). Slaton et al. (2001) reported that rice total dry matter would be maximized for rice receiving Zn-seed treatments applied at 2.2 to 5.8 g Zn kg^{-1} seed under Zn deficient conditions. Rice fertilized with MESZ resulted in greater seedling biomass than rice in the no-Zn control or other Zn-fertilizer methods when averaged across Zn-seed treatment rates (Table 2.5). Similar to the explanation for the canopy coverage results, the preplant N from the MESZ treatment may have been responsible for the increased seedling biomass compared to other Zn-fertilizer treatments. Rice receiving Zn-EDTA or GRAN produced greater aboveground biomass than rice in the no-Zn control, DDP0.5, and DDP1 treatments, which produced similar aboveground biomass. Moore and Patrick (1988) correlated dry matter production with tissue-Zn concentration and found that as Zn concentration increased so did dry matter, which would be expected for Zn-deficient plants.

Tissue-Zn Concentration and Aboveground Zn Content of Seedling Rice

The Zn-seed treatment rate by Zn-fertilization method interaction had no significant effect on tissue-Zn concentration ($P = 0.6895$) or content ($P = 0.8857$). However, tissue-Zn concentration and aboveground Zn content were affected by each of the main effects. When tissue-Zn concentrations and content were averaged across Zn-fertilization methods and site-years, application of 3.3 g Zn kg^{-1} as a Zn-seed treatment increased tissue-Zn concentration from 20.7 to 22.2 mg Zn kg^{-1} ($P < 0.0001$) and Zn content ($P < 0.0001$) from 19.1 to 21.8 g Zn ha^{-1}. Slaton et al. (2001) reported an increase in tissue-Zn concentration of 4.7 mg Zn kg^{-1} above rice that was planted without seed-applied Zn. Placement of the Zn-seed treatment could be the primary factor for increasing tissue-Zn concentration. Placing Zn directly on the seed positions it near the seedling roots for early season uptake when Zn deficiency typically occurs and is often difficult to recognize until after flooding (Norman et al., 2013).

Seedling rice was significantly affected by Zn fertilization method (Table 2.5). Averaged across site-years and Zn-seed treatment rates, rice had the greatest tissue-Zn concentration when fertilized with GRAN at 11 kg Zn ha^{-1}. Application of EDTA also increased tissue-Zn concentration relative to the no-Zn control, DDP0.5, and DDP1 treatments. Application of MESZ resulted in a greater tissue-Zn concentration than the no-Zn control, but did not increase tissue-Zn concentration above that of the DDP0.5 and DDP1 treatments. Although EDTA and

MESZ significantly increased tissue-Zn concentrations above the no-Zn control the increase in tissue-Zn concentration was nominal. For aboveground Zn content, rice fertilized with GRAN, MESZ, and EDTA had equal Zn contents that were greater than rice receiving no Zn, DDP1 and DDP0.5.

The average rice tissue-Zn concentrations for all treatments (Table 2.5) were above the 15 to 20 mg Zn kg^{-1} critical concentration range (Yoshida et al., 1973). The tissue-Zn concentration of rice receiving no seed-applied Zn and no other Zn fertilizer among the seven trials ranged from 9.8 to 31.6 mg Zn kg^{-1} and was above 15 mg Zn kg^{-1} in only three of the seven trails, indicating that soil-Zn concentrations from 2.1 to 3.1 mg Zn kg^{-1}, at these locations, were adequate for supplying Zn to seedling rice. The percent WSZn contained in a fertilizer is an important indicator of plant-available Zn (Mortvedt, 1992; Amrani et al., 1999; Liscano et al., 2000; Gangloff et al., 2002) and could explain why Zn-DDP (11% WSZn) did not increase tissue-Zn concentration, while GRAN (90% WSZn), EDTA (100% WSZn), and MESZ (28% WSZn) did affect tissue-Zn concentrations. Shaver and Westfall (2008) reported that the Zn concentration of corn plants fertilized with Zn-DDP was not different from that of corn that received no Zn.

New fertilizers containing Zn often claim to have efficiency ratios, but have insufficient research to validate these claims. Fertilizer efficiency ratios result from properties, claimed by the manufacturer, of the fertilizer that could allow for enhanced plant uptake or distribution compared to inorganic-Zn fertilizer sources. For example, a manufacturer claims that their fertilizer has an efficiency ratio of 10:1 meaning that 1 kg of Zn from a common source such as GRAN is equivalent to 0.1 kg of Zn from the manufacturer's source. The advertised efficiency ratio of DDP results from micro-static adhesion allowing the powder to adhere to each macronutrient granule allowing for uniform distribution of Zn compared to the use of granular Zn (e.g., GRAN), which has larger granules and results in a less dense distribution pattern. Our results showed that DDP applied at the label recommended rate did not increase tissue-Zn concentration above the no-Zn control. Several researchers have also claimed an efficiency ratio for Zn-EDTA as compared to Zn applied in the sulfate form (Boawn, 1973; Mortvedt, 1979). Comparably, in our study, rice fertilized with Zn-EDTA had greater aboveground tissue-Zn concentration than the no-Zn control, but less than seedlings receiving GRAN.

Grain Yield

Grain yield was not Zn-fertilization method (Table 2.5), or by their interaction (P = 0.8998). While Zn-seed treatment did not increase yield in this study (P = 0.1123), Slaton et al. (2001) and Rush (1972) reported that rice planted with a sufficient rate of seed-applied Zn produced grain yields comparable to rice fertilized with 11 kg Zn ha^{-1} as granular $ZnSO_4$, which were both greater than the yield of rice receiving no Zn.

Although Zn-fertilization method did not increase grain yield compared with the no-Zn control, several researchers have reported yield increases from Zn-fertilization. For example, Ruffo et al. (2016) reported that corn fertilized with MESZ or granular $ZnSO_4$ blended with granular fertilizers at a rate of 2.24 kg Zn ha^{-1} and 11.2 kg Zn ha^{-1}, respectively, produced similar yields that were greater than the yield of corn receiving no Zn fertilizer. Slaton et al. (2005) also reported yield increases from Zn fertilization of 12-180%. Although researchers have reported crop yield increases from Zn-fertilization, crop yield benefits from Zn fertilization are not universal. The literature also reports numerous instances of no crop yield response to Zn fertilization (Lindsay and Norvell, 1978; Slaton et al., 2002), especially on soils with medium soil-test Zn levels and slightly acidic pH. In Arkansas and probably many other places, Zn deficiency still occurs but has become less frequent over time due in part to the residual effect of fertilization with granular $ZnSO_4$ at 11 kg Zn ha^{-1} in prior years plus the inclusion of low-use-rate Zn fertilization methods as preventative insurance.

Summary

Our research investigating flood-irrigated rice response to two seed-applied Zn rates and six Zn-fertilization methods showed that some low-use-rate Zn fertilization methods can provide nominal Zn nutrition benefits as evidenced by small increases in tissue-Zn concentration from seed-applied Zn, MESZ, and EDTA-Zn. However, some low-use-rate Zn fertilization methods, like Zn-DDP applied to P and K fertilizers, did not increase tissue-Zn concentration above that of rice receiving no Zn. The advertised advantages of some low-use-rate Zn products are not defensible in regards to the product's (and its use rate) ability to provide sufficient Zn nutrition to seedling rice in a single year trial. The use of two low-use-rate Zn products may provide cumulative effects provided each of the selected strategies are singularly effective. The only treatment to provide consistent and substantial tissue-Zn nutrition increases was the application of 11 kg Zn ha^{-1} as granular $ZnSO_4$. This research is novel in that it is the first field research we are aware of in the published literature to compare multiple low-use-rate Zn fertilization strategies.

Our research on soils with low to medium soil-test Zn levels showed that significant grain yield increases from Zn-fertilization are difficult to accurately predict from soil tests and do not occur with high frequency. Zinc fertilization is often performed as insurance against Zn deficiency, especially for rice because it can cause substantial seedling injury, delayed maturity, or plant death and rescuing Zn-deficient plants substantially alters the crop management. The management of Zn-deficient rice requires flood removal for rice recovery, additional fertilizer-N application to account for N loss, may require additional herbicide for weed control, and extra energy to reestablish the flood making the rescue process very costly. Given the cost and potential environmental issues (e.g., greenhouse gas emissions and excess water use) associated with rescuing Zn-deficient rice, the use of low-use-rate Zn fertilization strategies as low-cost insurance policies is a reasonable practice provided the selected strategy is indeed beneficial. Growers should select and use only the low-use-rate Zn fertilization strategies that benefit seedling rice nutrition, which should translate into improved seedling nutrition and yield performance under Zn-deficient situations. Low-use-rate Zn products used alone or in combination should be done with caution as some are more effective than others. Based on this research measureable benefits of low-use-rate Zn strategies were measured only for Zn applied to rice seed at a recommended rate, preplant-applied MESZ, and a timely post-emergence application of a Zn-EDTA solution.

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Tables and Figures

Figure 1. Zinc prices ($ kg^{-1}) of the previous 20 years. Data collected from United States Geological Survey (USGS) mineral commodity summaries.

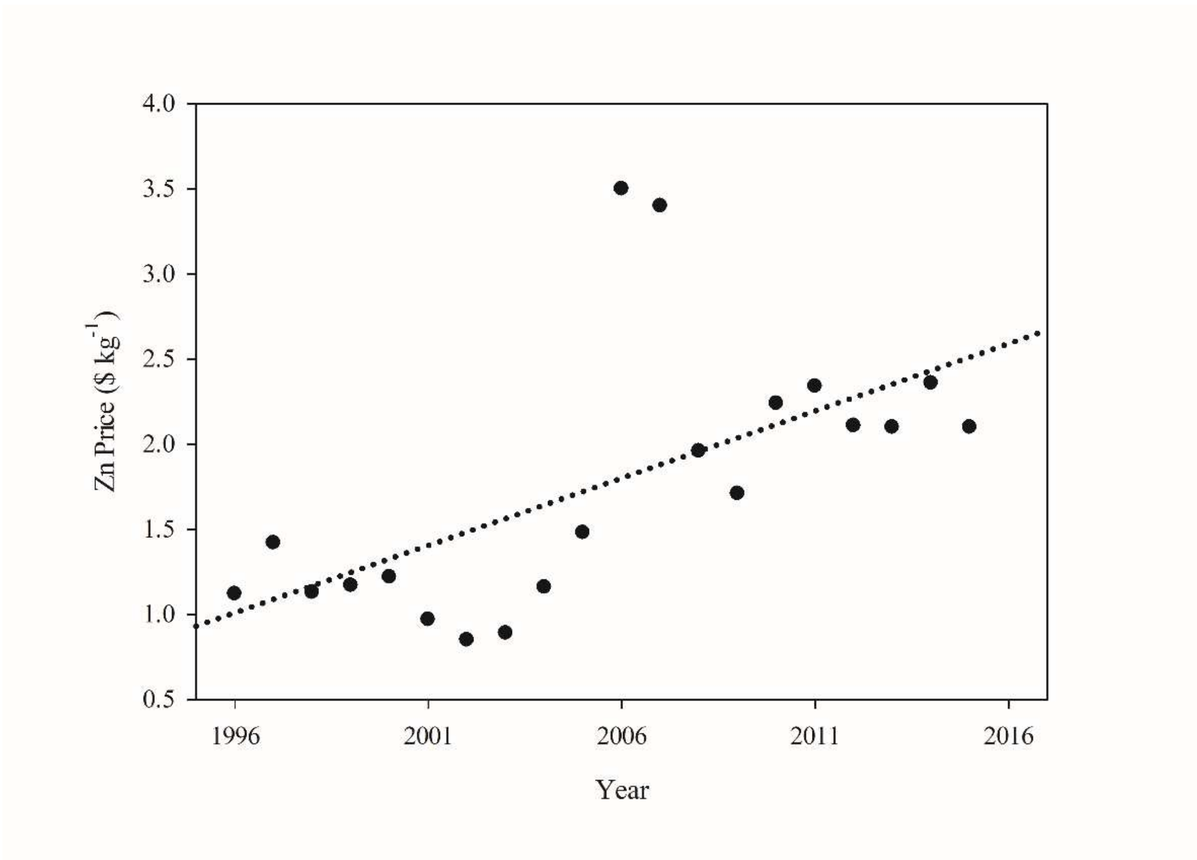

Table 2.1. Selected soil chemical property means (0-10 cm depth, *n* = 5) from sites used to evaluate rice response to different Zn fertilization methods at the University of Arkansas Pine Tree Research Station and Rohwer Research Station in 2017 and 2018.

			Mehlich-3 extractable soil nutrients§										
Soil-year	Soil pH†	Soil OM‡	P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	B
		$g\ kg^{-1}$	($mg\ kg^{-1}$)										
Calloway-17a	6.6	22	28	79	1335	204	14	48	345	444	3.1	0.8	0.5
Calhoun-17a	7.6	21	22	77	2002	311	7	33	314	303	2.5	1.0	0.6
Calhoun-18a	7.9	20	34	105	2948	406	25	113	437	203	2.0	1.4	0.4
Calloway-18a	7.9	21	16	47	1968	296	8	68	470	164	1.4	1.3	0.3
Calloway-18b	6.7	23	33	106	1278	243	12	64	352	263	2.1	1.4	0.2
Calhoun-18b	7.9	22	61	98	2529	379	14	61	411	281	2.2	2.1	0.3
Sharkey-18	7.9	2.6	62	268	5125	829	18	147	408	67	2.2	2.2	0.8

† Soil pH measured in a 1:2 soil: water mixture (Sikora and Kissel, 2014).
‡ OM, organic matter by weight loss on ignition (Schulte and Hopkins, 1996).
§ Extracted using Mehlich-3 method (Zhang et al., 2014).

Table 2.2. Dates of important agronomic management activities and treatment implementation for seven Zn fertilization trials conducted in 2017 and 2018 at the University of Arkansas Pine Tree Research Station and Rohwer Research Station. See Table 2.1 for selected site information.

Site-year	Planted	Foliar Zn application†	Flood established	Plant sample	Harvest	Canopeo measurement				
	Day – month									
Calloway-17	18 Apr	16 May	24 May	31 May	9 Sep	23 May	31 May	7 June	--	--
Calhoun-17	3 May	22 May	31 May	7 June	9 Sep	31 May	7 June	13 June	21 June	--
Calhoun-18a	10 Apr	15 May	31 May	12 June	10 Sep	--	--	--	--	--
Calloway-18a	19 Apr	15 May	30 May	5 June	27 Aug	15 May	24 May	31 May	5 June	12 June
Calloway-18b	20 Apr	15 May	31 May	12 June	10 Sep	15 May	24 May	31 May	5 June	12 June
Calhoun-18b	24 May	5 June	20 June	26 June	4 Oct	5 June	12 June	20 June	26 June	4 July
Sharkey-18	20 Apr	15 May	30 May	11 June	29 Aug	16 May	23 May	30 May	7 June	11 June

† Zinc-EDTA (Ultra-Che Zinc 9% EDTA, Winfield Solutions, LLC, Shoreview, MN) application at 1.12 kg Zn ha^{-1} at the 2-leaf stage.

Table 2.3. Rice canopy coverage as affected by the main effects of Zn-fertilization method and Zn-seed treatment, for each of the 6 locations averaged across sample times (*n*= 3-5).

Fertilizer†	Calloway-17	Calhoun-17	Calloway-18a	Calloway-18b	Calhoun-18b	Sharkey- 18a
	--- (Canopy Coverage %) ---					
No Zn	39.3 b§	38.4	27.6	19.7	19.8	10.4
EDTA	47.1 ab	37.3	29.5	19.2	24.2	11.4
DDP1	48.4 ab	40.5	26.1	19.5	15.6	12.3
GRAN	48.8 ab	39.9	27.0	19.1	19.3	11.3
MESZ	57.7 a	41.0	32.3	26.7	20.7	12.8
P- value	0.0044	0.8876	0.2665	0.0020	0.2283	0.8730
Seed Treatment‡						
0.0 g Zn kg^{-1}	48.9	38.0	28.5	20.6	19.4	11.7
3.3 g Zn kg^{-1}	47.6	40.8	28.4	20.9	20.2	11.6
P- value	0.6373	0.2828	0.9815	0.8105	0.7119	0.9704
Interaction *P*-value	0.0578	0.9042	0.7089	0.5119	0.6459	0.9529

† EDTA Ultra-Che Zinc 9% EDTA, Winfield Solutions, LLC, Shoreview, MN; DDP1, WolfTrax, Compass Minerals, Overland Park, KS; GRAN, granular zinc sulfate, Winfield Solutions, LLC, Shoreview, MN; MESZ, MicroEssentials The Mosaic Company, Plymouth, MN.
‡ Zinche ST, Drexel Chemical Company, Memphis, TN.
§ Means within a column followed by different lowercase letters are statistically different at the 0.10 level.

Table 2.4. Percent rice canopy coverage as affected by growing degree units (GDU) at each sample date for six Zn-fertilization trials conducted in 2017 and 2018.

Location	Sample Date	GDU	Canopy Coverage
		------- (DD10) -------	------- (%)-------
Calloway-17	23 May	275	14.1 c‡
	31 May	371	36.7 b
	7 June	472	89.5 a
P- value			<0.0001
Calhoun-17	31 May	221	4.7 d
	7 June	322	20.3 c
	13 June	404	62.5 b
	21 June	533	89.6 a
P- value			<0.0001
Calloway-18a	15 May	210	6.9 e
	24 May	391	15.2 d
	31 May	536	31.7 c
	5 June	635	40.2 b
	12 June	772	70.5 a
P- value			<0.0001
Calloway-18b	15 May	199	4.9 e
	24 May	351	8.4 d
	31 May	465	15.6 c
	5 June	544	23.3 b
	12 June	658	82.0 a
P- value			<0.0001
Calhoun-18b	5 June	161	3.0 e
	12 June	276	4.8 d
	20 June	415	16.6 c
	26 June	512	35.2 b
	4 July	652	84.6 a
P- value			<0.0001
Sharkey-18	16 May	151	0.75 e
	23 May	254	7.0 d
	30 May	359	12.3 c
	7 June	487	27.6 b
	11 June	555	56.5 a
P- value			<0.0001

† Canopy coverage sampling started at the 2-3 leaf stage once per week for 3-5 wk.
‡ Within the same column and location, means followed by different lowercase letters are statistically different at the 0.10 level.

Table 2.5. Rice aboveground tissue-Zn concentration, Zn content and biomass at the midtillering growth stage and grain yield as affected by Zn-fertilization method, averaged across Zn-seed treatment rates trials (*n*=7) conducted in 2017 and 2018.

Fertilizer†	Biomass	Tissue-Zn	Zn-Content	Grain Yield
	(kg ha^{-1})	(mg kg^{-1})	(g ha^{-1})	(kg ha^{-1})
no-Zn	951 d	19.4 d‡	17.4 b	9801
EDTA	1153 bc	23.0 b	22.7 a	9941
DDP0.5	924 d	20.1 cd	17.4 b	9685
DDP1	965 cd	20.5 cd	18.3 b	9723
GRAN	1049 b	25.4 a	24.9 a	9874
MESZ	1169 a	20.8 c	23.0 a	9802
P-value	<0.0001	<0.0001	<0.0001	0.5173

† EDTA Ultra-Che Zinc 9% EDTA, Winfield Solutions, LLC, Shoreview, MN; DDP0.5 and DDP1, WolfTrax, Compass Minerals, Overland Park, KS; GRAN, granular zinc sulfate, Winfield Solutions, LLC, Shoreview, MN; MESZ, MicroEssentials The Mosaic Company, Plymouth, MN.

‡ Within each column, means followed by different lowercase letters are statistically different at the 0.10 level.

Chapter 3

Determination of Agronomic Benefits from Zinc Biofortified Rice Compared to Zinc Seed Treatments

Abstract

Zinc (Zn) biofortification has been investigated to address human Zn deficiencies, but limited research has evaluated the effect of Zn-biofortified rice (*Oryza sativa* L.) on seedling vigor and early-season plant nutrition. In 2017, rice received 0, 1, 2, or 3 applications of 1.75 kg Zn ha^{-1} as $ZnSO_4$ solution after 100% panicle emergence. Our objectives evaluated four levels of biofortified rough rice compared to Zn applied as a seed treatment on seedling height, biomass, tissue-Zn concentration, and grain yield. In 2018, one greenhouse experiment and two field experiments were conducted on silt loam soils evaluating biofortified rice grain. For the greenhouse experiment, only non-fortified rice seed was treated with ZnO at 5 g Zn kg^{-1}. In the field, each level of fortified rice was treated with ZnO at the same rate. Tissue-Zn content at 26 d after emergence was numerically but not statistically increased by 1.1 (LaKast) and 1.4 (Diamond) mg Zn kg^{-1} for the ZnO-seed treatment compared to the highest tissue-Zn concentration of biofortified rice. In the field, Zn biofortification rate did not affect tissue-Zn concentration, but when ZnO-seed treatment was applied, tissue-Zn concentration increased by 1.6 mg Zn kg^{-1} for Diamond and 1.0 mg Zn kg^{-1} for LaKast. Grain yield was not affected by Zn biofortification, ZnO-seed treatment or their interaction. The ZnO-seed treatment provided greater Zn bioavailability for seedling rice crops compared to biofortified rice grains with high Zn concentrations indicating that ZnO-seed treatments may be more advantageous than biofortification for early-season Zn nutrition of seedling rice.

Introduction

Zinc (Zn) is one of the most common micronutrient deficiencies in humans. It is estimated that nearly one-third of the world's population suffers from Zn deficiency (Alloway, 2009), but the Zn-deficient population ranges between 4 and 73% in different countries (Hotz and Brown, 2004). Improving the nutritional value of edible parts of crops are defined as biofortification strategies (Cakmak and Kutman, 2018). Biofortification of cereal grains with Zn has been of recent interest as a means of increasing the amount of Zn in the human diet, especially in areas where cereal grains are the staple food source. The HarvestPlus (https://www.harvestplus.org) program researches strategies to enhance cereal crop nutrient concentrations in edible plant parts.

Enhancing nutrient concentrations for cereal grains has been through classical plant breeding strategies and fertilizer applications (Cakmak, 2008; Cakmak and Kutman, 2018). There exists considerable variation in grain-Zn concentrations for cereal crops. For example, Graham et al. (1999) found Zn concentrations range from 13.5 to 58.4 mg Zn kg^{-1} of rice varieties at the International Rice Research Institute (Los Baños, Laguna, Philippines), and Zn concentrations of 132 wheat (*Triticum aestivum* L.) varieties ranged from 28.8 to 56.5 mg Zn kg^{-1} (Monasterio and Graham, 2000), indicating the potential to develop cultivars with increased nutrient uptake efficiency. Although several researchers have developed transgenic plants with enhanced uptake and transportation of nutrients (Vasconcelos et al., 2003; Suzuki et al., 2008; Lee et al., 2009), much of these are greenhouse experiments where the environments do not mimic natural conditions. Thus, it would be difficult to predict how these transgenic varieties will respond when grown in a field environment.

Fortification of cereal grains with nutrients has been successful with timely fertilizer applications (Cakmak et al., 2010; Phattarakul et al., 2012; Boonchuay et al., 2013). Increasing Zn concentrations in cereal grains for the purpose of addressing Zn deficiencies in humans has also generated interest on the impact of biofortification on crop performance (Yilmaz et al., 1998; Boonchuay et al., 2013; Candan et al., 2018). Boonchuay et al. (2013) sprayed 0.5% Zn sulfate ($ZnSO_4$) solution at different growth stages applying at a rate of 900-1000 L ha^{-1}. Only applications after flowering increased Zn concentration in rough rice, and harvested rough rice was classified into low seed-Zn (18 mg Zn kg^{-1}), intermediate seed-Zn (42 mg Zn kg^{-1}), and high seed-Zn (67 mg Zn kg^{-1}). Rough rice grains were germinated and analyzed for the combined dry weight of roots and coleoptile, and dry weight was reported to increase as rough rice Zn concentration increased. Candan et al. (2018) also reported increased germination rates and taller seedling height 7 d after germination in a greenhouse study for wheat seeds with higher Zn concentrations. Yilmaz et al. (1998) reported wheat yield increases of 116% from high seed-Zn compared to low seed-Zn seeds when no Zn fertilizer was added, however, when low seed-Zn received soil applications of Zn as $ZnSO_4$, yield increased by 466%. They concluded that biofortified wheat grains partially alleviate Zn deficiency, but could not fully overcome the deficiency.

Several peer-reviewed studies have shown increased germination, seedling vigor, and grain yield from biofortified grains. However, there is no published research comparing biofortified rice seed to ZnO-seed treatments. Thus, our objectives were to evaluate Zn-biofortified rough rice compared to Zn applied as a seed treatment for seedling height, biomass, Zn concentration, and grain yield.

Materials and Methods

Zn Biofortification of Rice Seed

Foliar Zn applications were made to biofortify rice grain Zn concentration in two field trials conducted at the Pine Tree Research Station in 2017. Selected soil chemical properties of the two fields are summarized in Table 3.1. 'Diamond' and 'LaKast' rice were drill seeded at 80 kg ha^{-1} in fields with soil mapped as a Calhoun (fine-silty, mixed, active, thermic Typic Glossaqualfs) and Calloway (fine-silty, mixed, active, thermic Aquic Fraglossudalfs) silt loam, respectively. Each location is identified by the soil series and year (e.g., Calhoun-17). Selected crop management dates of agronomic importance are summarized in Table 3.2. Pest management, fertilization, and irrigation of rice in both trials were managed with practices recommended for the direct-seeded, delayed-flood rice production system (Hardke, 2013). In each field, three 6.1-m long strips of rice were sprayed with 0, 1, 2, or 3 applications of Zn on the dates listed in Table 3.2. The foliar applications started when rice reached 100% panicle emergence and was repeated weekly until three applications were made. Foliar applications were made at 1108, 1217, 1306, and 1412 cumulative GDD10 units for Diamond, and 1066, 1175, 1265, and 1371 for LaKast. According to Castaneda-Gonzalez et al. (2016), Diamond and LaKast rice require 1190 (s = 25) and 1150 (s = 32) GDD10 units, respectively, between rice emergence and 50% panicle emergence in the direct-seeded, delayed-flood production system. The number of GDD required for key rice development stages may vary based upon management practices such as the N application rate and flood establishment time. The Zn applications were made after panicle emergence based on the results of Cakmak et al. (2010) and Boonchuay et al. (2013), indicating that Zn applied to rice foliage following panicle emergence was preferentially translocated to the developing rice grain. Each Zn application consisted of applying a $ZnSO_4$ (90% WS Zn, 355 g Zn and 175 g S kg^{-1}, Super Tel Zn Powder, Nutrien Ltd., Calgary, Alberta, Canada) solution using a CO_2 backpack sprayer calibrated to deliver 1.75 kg Zn ha^{-1} in a 164 L ha^{-1} spray volume. The intent of the three foliar applications was to produce rough rice seed with four different Zn concentrations. At maturity, rice was harvested when the grain moisture content averaged 176 g H_2O kg^{-1} for Diamond and 210 g H_2O kg^{-1} for LaKast. The grain was air dried to an equilibrium moisture of 76 g H_2O kg^{-1} and cleaned to remove foreign material.

Three 100 g subsamples of the harvested grain were collected from each cultivar and application. A portion of the grain sample was dehulled with Satake Rice Machine (Satake Engineering Co., LTD., Hoshidakita, Katano-shi, Osaka, Japan). Subsamples (n = 3) of rough rice, brown rice and rice hulls of each cultivar were digested with concentrated HNO_3 and 30% H_2O_2 to determine the Zn concentration, and whether the biofortified Zn was in the hull or in the grain (Jones and Case, 1990). The digests were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Arcos-160 SOP, Spectro, NJ). The Zn concentration means for each grain part are listed in Table 3.3. Two-way ANOVA was performed using the GLIMMIX procedure in SAS (v.9.4, Institute, Inc., Cary, N.C.) by cultivar to determine whether the seed parts (hull, brown rice, and rough rice) contained different Zn concentrations among seed receiving the three foliar Zn applications using subsample (replicate) as a random effect and Zn application number as a fixed effect. When appropriate, Fisher's protected LSD was used to separate means at the 0.10 significance level.

Greenhouse Trial

A greenhouse experiment was designed with grain from each cultivar harvested in 2017, Diamond and LaKast. The experiment was a randomized complete block design with five treatments and four blocks for each of the two rice cultivars. The treatments included the four seed-Zn concentration levels of rough rice seed plus seed that received no foliar-applied Zn that was hand-treated with Zinche ST (325 g Zn kg^{-1} and 482 g Zn L^{-1} Zn, Drexel Chemical Company, Memphis, TN) at a rate of 5 g Zn kg^{-1} (10.4 mL Zinche ST kg^{-1} seed). Subsamples (n = 3) of the ZnO-treated seed were digested as previously described to determine total Zn concentration by ICP-AES (Jones and Case, 1990). Seed treated with Zinche ST contained 3.4 g Zn kg^{-1} (s = 0.20) for Diamond and 4.3 g Zn kg^{-1} (s = 0.28) for LaKast.

Topsoil (0-10 cm) from a field at the Lon Mann Cotton Research Station mapped as a Calloway silt loam was weighed (3.2 kg pot^{-1}) into rectangular plastic containers (30 x 16 cm) having a surface area of 480 cm^2. After settling from surface irrigation, the average soil bulk density was 1.22 g cm^{-3}. Twelve seed were planted 0.7 cm deep in a single row in the middle of each container and thinned to 9 plants $container^{-1}$ 9 d after emergence (DAE) for a plant stand density equal to 193 plants m^{-2}. The experiment was performed during January and February 2018 in the University of Arkansas Altheimer greenhouse facility in Fayetteville, AR. The temperature ranged from 25°C during the night to 35°C during the day with a 14 h photoperiod. Twelve DAE the equivalent of 150 kg N ha^{-1} was applied by dissolving 1.6 g urea in 200 mL of deionized water. Irrigation was scheduled every 3 d with water added to bring soil to 30% volumetric water content which was estimated using the soil texture and organic matter in the SPAW software system (USDA-ARS, 2016) bringing a combined weight of soil and water to 4.2 kg pot^{-1}.

Each rice seedling was measured for height (cm) as an estimate of seedling vigor once per week beginning 6 DAE. Rice was allowed to grow until the three-leaf stage (26 DAE) at which time the nine seedlings from each pot were cut at the soil surface, rinsed in deionized water, placed in a paper bag, oven-dried at 55ºC to a constant weight, weighed, and ground to pass through a sieve with 1-mm openings. A subsample was digested for nutrient analysis as previously described. Tissue-Zn content was determined by multiplying biomass by tissue-Zn concentration and expressed as (mg Zn pot^{-1}).

Statistical Analysis

The greenhouse trial was a single factor (Zn treatment) randomized complete block design with four replications. The mean seedling height at each measurement sample time and seedling biomass and Zn content data at 26 DAE were analyzed by cultivar using ANOVA with Zn treatment as the fixed effect and block as a random effect fit to a gamma distribution. The ANOVA was performed using the GLIMMIX procedure in SAS (v.9.4, SAS Institute, Inc., Cary, N.C). When appropriate, means were separated with Fisher's least significant difference at a 0.10 significance level different.

Field Trial

In the summer of 2018, the Diamond and LaKast seed produced in the two 2017 field trials were planted in two field trials on soil mapped as Calhoun silt loam at the Pine Tree Research Station, near Colt, AR. Each biofortification trial in 2018 is indicated by soil series and year (e.g., Calhoun-18a and Calhoun-18b). The two cultivars were planted in adjacent areas on two different planting dates in a single field. The field areas for each planting date were about 300 m apart and managed independently. Each experiment was a randomized complete block design with a 2 by 4 factorial treatment arrangement and four blocks with the exception of location Calhoun-18b, which contained only three blocks due to limited space. Individual plots were 6.1-m long allowing for 9 rows spaced 19-cm apart. Planting dates and other management dates for each trial are summarized in Table 3.2.

Prior to planting, composite soil samples were collected from the plot representing the no Zn biofortification and 0 g Zinche ST kg^{-1} as a seed treatment in each block. Selected soil properties are summarized in Table 3.1. Each composite sample consisted of six, 2.5 cm o.d. soil cores. Soil samples were oven dried at 65°C, ground to pass through a 2-mm diameter sieve and tested for pH (1:2 soil:water mixture; Sikora and Kissel, 2014), soil organic matter by weight loss on ignition (Schulte and Hopkins, 1996), and Mehlich-3 extractable nutrients (Zhang et al., 2014). To ensure adequate P and K availability the research areas each received 28 kg P and 67 kg K ha^{-1} as triple superphosphate and muriate of potash applied to the soil surface.

LaKast and Diamond rice were seeded at the optimum density of 322 seeds m^{-2} resulting in 80 and 73 kg ha^{-1}, respectively, on the dates listed in Table 3.2. Trials planted on April 10 had a poor stand due to abnormally cool temperatures and excessive moisture, so yield was not taken from these trials, but seedling rice was sampled to examine treatment effects on tissue-Zn concentration. For each cultivar, the treatments included two rates of Zinche ST (0 and 5 g Zn kg^{-1}) and the four Zn-biofortified grain levels from the 2017 field trials (Table 3.4). The rice seed treated with Zinche ST was digested (*n* = 3) with concentrated HNO_3 and 30% H_2O_2 to determine the Zn concentration (Jones and Case, 1990) and analyzed by ICP-AES. Urea was applied at the four-leaf stage to supply 150 kg N ha^{-1} and the plots were flooded 1 d later.

At the midtillering growth stage (Table 3.2), 6 to 12 d after preflood-N application and flooding, a 1.8-m section from an inside row of rice was cut 2.0-cm above the soil surface to determine tissue-Zn concentration for each trial. Samples were bagged, oven-dried at 55ºC to a constant weight, and ground to pass through a sieve with 1-mm openings. A subsample of ground plant matter was digested and analyzed as previously mentioned. At maturity, a 5.24-m^2 section of the middle five rows of each plot was harvested for grain yield using a small-plot combine. Grain weight and moisture were measured immediately after harvest. Grain yields were calculated after grain moisture content was adjusted to 120 g H_2O kg^{-1}.

Statistical Analysis

The field experiments were randomized complete block designs. Two-way ANOVA was performed using the GLIMMIX procedure in SAS (v.9.4, SAS Institute, Inc., Cary, N.C) and analyzed by cultivar. The ANOVA model included the two levels of ZnO-seed treatment rate four levels of biofortified-Zn concentration and their interaction as fixed effects while block and site-year (or planting date) were treated as random effects. The model was fit to a gamma distribution, and site-year was only in the ANOVA model for comparing tissue-Zn concentration since grain yield was not measured on the April 10 planting date. When appropriate, means were separated with Fisher's least significant difference at a 0.10 significance level.

Results and Discussion

Zn Biofortification of Rice Seed

Zinc concentrations of rough rice, hulls, and brown rice were significantly increased compared to rice not receiving late-season foliar applications of $ZnSO_4$ solution for each cultivar (Table 3.3), which is consistent with previous research (Cakmak et al., 2010; Wu et al., 2010; Wei et al., 2012). The Zn concentration of Diamond rough rice was significantly increased only by two or three foliar-Zn applications, while the Zn-concentration of LaKast rough rice was increased incrementally with each application (Table 3.3). Zinc applied to rice foliage translocated primarily to the rice hull, resulting in the largest increase in Zn-concentration among the individual rice grain components. Boonchuay et al. (2012) also reported that rice hulls are the primary sink of the translocated Zn from late-season, foliar-Zn applications. Brown rice Zn concentrations was increased by 67.8% for Diamond and 48.8% for LaKast. Rengel and Grahamn (1995) suggested that high seed-Zn concentrations, especially in brown rice, could act as a “starter fertilizer” for rice seedlings.

Greenhouse Trial

Diamond rice seedlings had no difference in height among treatments at either of the three sample times (Table 3.5). Non-fortified LaKast seedlings with no ZnO-seed treatment were tallest only at 13 DAE measuring at least 1.2-cm taller than the next tallest treatment (Table 3.6). Boonchuay et al. (2013) showed that the rice coleoptile length was significantly longer in medium (42 mg Zn kg^{-1}) and high (67 mg Zn kg^{-1}) Zn rice grains for the first 5 d after germination compared to low Zn rice grains (18 mg Zn kg^{-1}), but after 5 d there was no significant difference in coleoptile length compared to low Zn rice grains. Increased seedling height occurred when wheat was planted with high Zn concentration seed compared to medium and low Zn concentration wheat grains (Candan et al., 2018).

Seedling biomass was not significantly affected by treatment for Diamond (Table 3.5) or LaKast (Table 3.6). However, Yilmaz et al. (1998) measured increased wheat biomass from seed fortified with Zn compared to non-Zn fortified seed. Similar results were published by Boonchuay et al. (2013) showing high Zn concentration rice seed produced greater combined root and coleoptile biomass 7 d after germination compared with rice seed having intermediate or low seed-Zn concentrations.

The tissue-Zn content of Diamond and LaKast rice seed treated with ZnO was numerically higher but not statistically greater than the tissue-Zn concentration from the other treatments (Tables 3.5; 3.6). All treatments produced seedlings that were below the critical range of 15 to 20 mg Zn kg^{-1} for Zn deficiency (Yoshida et al., 1973). Candan et al. (2018) reported that high seed-Zn concentrations significantly increased tissue-Zn concentration for wheat plants grown for 60 d in drought stress conditions in a greenhouse experiment regardless if Zn was applied to the soil. Yilmaz et al. (1998) reported similar results as presented in our research that there was no difference in Zn-concentration of field-grown wheat plants from low seed-Zn concentration (355 ng Zn $seed^{-1}$) compared to medium (800 ng Zn $seed^{-1}$) and high (1465 ng Zn $seed^{-1}$). In our trial, the seed-Zn concentrations for ZnO-seed treatments compared with biofortified rice were drastically different, which could explain the consistent numerical differences in tissue-Zn concentrations to be higher for ZnO-treated seed compared to Zn-biofortified rice. LaKast and Diamond rough rice seed treated with ZnO contained on average 4269 mg Zn kg^{-1} and 3351 mg Zn kg^{-1}, respectively. The highest rough rice seed-Zn level obtained from the biofortification process was 89.9 mg Zn kg^{-1} for Diamond and 95.9 mg Zn kg^{-1} for LaKast. ZnO fertilizer placed on the outside of the rice hull provided more Zn to seedlings than the biofortification process. Our greenhouse experiment did not corroborate results of other studies that observed a trend for increased seedling vigor from grain biofortified with Zn, however, when rice was treated with ZnO it tended to increased tissue-Zn concentration above biofortified rice.

Field Trial

The Zn-biofortified rice seed by ZnO-seed treatment interaction had no significant effect on tissue-Zn concentration for Diamond (*P*-value = 0.5692) or LaKast (*P*-value = 0.8936).

Tissue-Zn concentration was also not affected by Zn biofortification rate for either cultivar (Table 3.7), but was significantly affected by the application of ZnO-seed treatment rate averaged across biofortification levels for Diamond (*P*-value = 0.0029) and LaKast (*P*-value = 0.0219) increasing tissue-Zn concentration by 1.6 and 1.0 mg Zn kg^{-1}, respectively. The mean Zn concentration from each treatment ranged from 10.9 to 14.4 mg Zn kg^{-1} which is below the 15 to 20 mg Zn kg^{-1} critical range outlined by Yoshida et al. (1973). An increase in tissue-Zn concentration from ZnO-seed treatment was also reported by Slaton et al. (2001). Our trials clearly showed that placement of ZnO fertilizer on the outside of the rice hull was more advantageous for increasing tissue-Zn concentration compared to Zn-biofortified rice grains. The rice hull Zn-concentration was affected to the greatest extent by the foliar-applied, Zn biofortification process (Table 3.3). Brown rice Zn-concentrations increased as the number of foliar-Zn applications increased, but not to the same magnitude as rice hulls which suggest the limited bioavailability of Zn in rice hulls to rice seedlings. The much greater concentration of ZnO applied to the exterior of the rice hull may allow for some Zn movement through the rice hull to the seed or uptake by seedling roots. However, these theories have not been investigated.

Grain yield was not affected by the Zn-biofortified rice seed by ZnO-seed treatment interaction for Diamond (*P*-value = 0.5626) or LaKast (*P*-value = 0.9033), or by the main effect of Zn biofortification level (Tables 3.7). Additionally, the main effect of ZnO-seed treatment had no effect on grain yield of Diamond (*P*-value = 0.9566) or LaKast (*P*-value = 0.2536). Yilmaz et al. (1998) reported a yield increase from wheat grains that were fortified with Zn, containing 800 ng Zn $seed^{-1}$ and 1465 ng Zn $seed^{-1}$, of 92% and 116%, respectively, compared to low Zn content grain containing 355 ng Zn $seed^{-1}$. Although Yilmaz et al. (1998) measured a yield increase for high seed-Zn, the yield increase from soil-applied $ZnSO_4$ was greater than the yield increase from biofortified wheat grains. They concluded that Zn-fertilization may be more advantageous for increasing yield compared to biofortified grains.

Summary

Our field research evaluating flood-irrigated rice response to two levels of seed-applied ZnO rates and four levels of biofortification showed that rice grains biofortified with Zn did not supply more Zn to developing rice seedlings when compared to non-fortified rice. Seed-applied ZnO resulted in greater tissue-Zn concentrations compared to biofortified rice grains receiving no ZnO-seed treatment. Planting the highest level of biofortified rice seed added the equivalent of 6.0 g Zn ha^{-1} (LaKast) and 5.2 g Zn ha^{-1} (Diamond) more than rice that was not biofortified. In comparison, the ZnO-seed treatment added at least 214 g Zn ha^{-1} for LaKast and 166 g Zn ha^{-1} for Diamond more than biofortified rice not treated with ZnO. Fertilizer applied to the outside of the hull as ZnO supplied far more Zn than the biofortified seed resulting from three Zn-fertilizer applications.

The research described in this paper is novel in that it is the first field research we are aware of in the published literature to compare ZnO-treated rice seed and Zn-biofortified rice seed on seedling height, Zn concentration, biomass, and grain yield. Based on this research the only measureable Zn nutrition benefits were for rice receiving a ZnO-seed treatment at the recommended rate. The Zn-biofortification process resulted in nominal Zn concentrations in the brown rice grain, which may explain the lack of benefit to seedlings at the midtillering stage.

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Tables

Table 3.1. Selected soil chemical property means (0-10 cm depth, *n*= 5) from field trials at the Pine Tree Research Station (PTRS) and greenhouse (GH) trials used to evaluate rice response to Zn biofortification in 2017 and 2018.

Soil-year†	Soil pH‡	Soil OM§	**Mehlich-3 extractable soil nutrients¶**										
			P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	B
		g kg^{-1}	(mg kg^{-1})										
Calhoun-17	7.6	21	22	77	2002	311	7	33	314	303	2.5	1.0	0.6
Calloway-17	6.4	20	21	82	1180	226	8	69	385	444	2.0	1.0	0.5
Calloway-GH	7.1	17	49	145	1720	394	11	25	220	96	2.1	1.2	0.7
Calhoun-18a	7.9	20	34	105	2948	406	25	113	437	203	2.0	1.4	0.4
Calhoun-18b	8.0	25	33	107	2918	399	24	127	439	190	2.0	1.4	0.4

† Diamond rice was grown on the Calhoun-17 soil and LaKast rice was grown on the Calloway-17 soil.
‡ Soil pH measured in a 1:2 soil: water mixture (Sikora and Kissel, 2014).
§ OM, organic matter by weight loss on ignition (Schulte and Hopkins, 1996).
¶ Extracted using Mehlich-3 method (Zhang et al., 2014).

Table 3.2. Dates of important agronomic management events in two field trials used to biofortify rice grain via foliar Zn applications made after 100% panicle emergence in 2017, and four field experiments evaluating biofortified rice grains. See Table 3.1 for selected soil information.

				Zn application number‡				
Site-year†	Planted	Flooded	Sampled	0	1	2	3	Harvested
	------------------------------------ Day – month ------------------------------------							
Calhoun-17	3 May	31 May	--	26 July	2 Aug	8 Aug	15 Aug	9 Sept
Calloway-17	10 May	15 June	--	26 July	2 Aug	8 Aug	15 Aug	9 Sept
Calhoun-18a	10 Apr	31 May	12 June	--	--	--	--	--
Calhoun-18b	5 June	5 July	11 July	--	--	--	--	4 Oct

† For initial biofortification, Diamond rice was grown on the Calhoun-17 soil and LaKast rice was grown on the Calloway-17 soil. The biofortified rice seed was planted in trials named Calhoun-18a and Calhoun-18b.
‡ Super-Tel Zn (355 g Zn kg^{-1}, Nutrien Ltd., Calgary, Alberta, Canada) at a rate of 1.75 kg Zn ha^{-1} at each application timing.

Table 3.3. Seed-Zn concentrations as affected by the interaction between seed part and the number of biofortifying, foliar-Zn applications made after 100% panicle emergence for two trails seeded with either Diamond or LaKast rice cultivars in 2017.

Zn applications†	Diamond			LaKast		
	Brown rice	Hulls	Rough	Brown rice	Hulls	Rough
	---------------------------------- mg Zn kg^{-1} ----------------------------------					
0	21.4 gh‡	18.2 j	19.1 ij	24.8 h	15.6 j	20.3 i
1	22.7 g	30.1 e	20.5 hi	26.0 gh	50.0 d	29.3 fg
2	26.3 f	110.1 b	35.5 d	30.2 f	136.4 b	47.2 d
3	35.9 d	300.0 a	89.9 c	36.9 e	301.3 a	95.9 c
P- value		<0.0001			<0.0001	

† Super-Tel Zinc (355 g kg^{-1}, Nutrien Ltd., Calgary, Alberta, Canada) at a rate of 1.75 kg Zn ha^{-1} at each application timing.
‡ Within each cultivar for each plant part and Zn applications combination, means followed by different lowercase letters are statistically different at the 0.10 level.

Table 3.4. LaKast and Diamond rough rice seed-Zn concentrations of rice receiving 0 to 3 biofortifying, foliar-Zn applications and a post-harvest ZnO-seed treatment (*n*=3).

Cultivar	Zn application number†				SD‡
	0	1	2	3	
	-------------------------------- mg Zn kg^{-1}§ --------------------------------				
Diamond	2250	3577	3515	3338	626
LaKast	2667	2704	3298	3183	381

† Super-Tel Zinc (355 g kg^{-1}, Nutrien Ltd., Calgary, Alberta, Canada at a rate of 1.75 kg Zn ha^{-1} at each application timing.

‡ Standard Deviation.

§ Zinche ST, (Drexel Chemical Company, Memphis, TN) treated at a rate of 5 g Zn kg^{-1}.

Table 3.5. Diamond rice biomass and tissue-Zn concentration at 26 d after emergence (DAE) and seedling height as affected by the main effect of Zn biofortification rate or by ZnO-seed treatment from one greenhouse experiment conducted in 2018.

Zn applications†	Height			Biomass	Tissue Zn
	6 DAE	13 DAE	20 DAE		
	-------------------- cm --------------------			g pot^{-1}	mg pot^{-1}
0	7.1	9.3	22.8	0.63	8.4
1	7.5	9.7	21.7	0.61	8.0
2	7.7	19.9	22.9	0.64	8.6
3	7.5	20.3	22.8	0.60	7.4
3.4 g Zn kg^{-1}	7.4	19.1	21.7	0.60	9.7
P- value	0.4675	0.3123	0.5670	0.8729	0.1595

† Super-Tel Zinc (355 g kg^{-1}, Nutrien Ltd., Calgary, Alberta, Canada) at a rate of 1.75 kg Zn ha^{-1} at each application timing; Zinche ST, (Drexel Chemical Company, Memphis, TN) treated at a rate of 5 g Zn kg^{-1}.

Table 3.6. LaKast rice biomass and tissue-Zn concentration at 26 d after emergence (DAE) and seedling height as affected by the main effect of Zn biofortification rate or by ZnO-seed treatment from one greenhouse experiment conducted in 2018.

Zn applications†	Height 6 DAE	13 DAE	20 DAE	Biomass	Tissue Zn
	-------------------- cm --------------------			g pot^{-1}	mg pot^{-1}
0	9.4	23.5 a‡	24.0	0.70	7.1
1	8.8	22.3 ab	23.6	0.72	7.5
2	8.3	21.5 b	23.3	0.69	6.6
3	8.6	21.8 b	23.1	0.67	7.3
4.3 g Zn kg^{-1}	9.1	22.0 b	23.7	0.69	8.9
P- value	0.3056	0.0380	0.8214	0.9508	0.3757

† Super-Tel Zinc (355 g kg^{-1}, Nutrien Ltd., Calgary, Alberta, Canada) at a rate of 1.75 kg Zn ha^{-1} at each application timing; Zinche ST, (Drexel Chemical Company, Memphis, TN) treated at a rate of 5 g Zn kg^{-1}.

‡ Means within a column followed by different lowercase letters are statistically different at the 0.10 level.

Table 3.7. Tissue-Zn concentration, averaged across two planting dates, at the midtillering growth stage and grain yield (average from one trial) as affected by Zn applications made after 100% panicle emergence, averaged across ZnO-seed treatment rates for trials conducted in 2018.

	Diamond		LaKast	
Zn applications†	Tissue Zn	Grain Yield	Tissue Zn	Grain Yield
	mg Zn kg^{-1}	kg ha^{-1}	mg Zn kg^{-1}	kg ha^{-1}
0	13.0	7348	11.7	6884
1	13.7	7551	11.0	7328
2	13.8	7384	11.2	7025
3	13.7	7641	10.9	6793
P- value	0.7132	0.9374	0.5639	0.2285

† Super-Tel Zinc (355 g kg^{-1}, Nutrien Ltd., Calgary, Alberta, Canada) at a rate of 1.75 kg Zn ha^{-1} at each application timing.

Conclusion

Several low-use-rate Zn-fertilization strategies claiming to be more efficient than the current recommendation of applying 11 kg Zn ha^{-1} as granular zinc sulfate have not been thoroughly investigated. The overall objective of this study was to compare the effect of Zn-seed treatment rate in combination with other Zn-fertilization methods to the standard of 11 kg Zn ha^{-1}, on rice early season canopy cover, seedling Zn concentration, and grain yield. A secondary objective was to evaluate an alternative method (to seed treatment with ZnO) of enhancing seed-Zn concentration and seedling vigor by post-heading foliar Zn application on tissue-Zn concentration and rice grain yield.

Results showed that low-use-rate Zn-fertilization methods can provide nominal Zn nutrition benefits to flood-irrigated rice as evidenced by small increases in tissue-Zn concentration from seed-applied Zn, MESZ, and EDTA-Zn. The Zn-DDP treatment did not increase tissue-Zn concentration above that of rice receiving no Zn. The advertised advantages of fertilizer efficiency ratios of some low-use-rate fertilizers were not able to provide sufficient Zn nutrition to seedling rice. Application of 11 kg Zn ha^{-1} as granular $ZnSO_4$ was the only treatment to produce a consistent response increasing tissue-Zn concentration of rice above any low-use-rate strategy. However, the use of two low-use-rate Zn products may provide cumulative effects provided the fertilizer products of the selected strategies are effective.

Flood-irrigated rice response to two levels of seed-applied ZnO rates and four levels of biofortification showed that rice grains biofortified with Zn did not supply more Zn to developing rice seedlings when compared to non-fortified rice. Seed-applied ZnO resulted in greater tissue-Zn concentrations compared to biofortified rice grains receiving no ZnO-seed treatment due to the placement and the concentration of the ZnO on the outside of the hull.

Fertilizer applied to the outside of the hull as ZnO was more advantageous for Zn nutrition supplying more Zn to seedling rice than the biofortified seed resulting from three Zn-fertilizer applications after 100% panicle emergence.