Effect of low-use-rate zinc fertilization on rice growth and grain yield

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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 mid-tillering stage, and rice grain yield. The field experiment was conducted on six silt loams and one clay. Rice seed was treated with 0 or 3.3 g Zn kg\(^{-1}\) as ZnO and combined with no Zn, granular ZnSO\(_4\) applied at 11 kg Zn ha\(^{-1}\) (GRAN), 1.68 kg Zn ha\(^{-1}\) as MicroEssentials (MESZ), 1.1 kg Zn ha\(^{-1}\) as foliar-applied Zn-EDTA (EDTA), and 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 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, seed treated with 3.3 g Zn kg\(^{-1}\) increased seedling tissue-Zn concentration and biomass by 1.6 mg Zn kg\(^{-1}\) and 48 kg ha\(^{-1}\) respectively. Rice receiving GRAN, increased tissue-Zn concentration by 7.6 mg Zn kg\(^{-1}\) above rice not receiving Zn (21.3 mg kg\(^{-1}\)). Low-use-rate Zn fertilizers provide minimal Zn nutrition for rice seedlings and should be avoided on fields where Zn deficiencies are probable.

1 | INTRODUCTION
Zinc deficiency of rice (Oryza sativa L.) has been reported in nearly all rice-producing countries (Alloway, 2009), and all of the rice-producing states in the United States (Gior-dano, 1977). Zinc deficiency typically causes yield losses of 10–60%, but, in severe cases, plant death and stand loss can occur, making Zn deficiency a serious problem for rice production (Norman, Wilson, & Slaton, 2003). 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 (Johnson-Beebout, Lauren, & Duxbury, 2009; Neue, Quijano, Senadhira, & Setter, 1998; Yoshida, Ahn, & Forno, 1973), although Gao, Zou, Fan, Zhang, and Hoffland (2006) and Gior-dano 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, Slaton, Herron, and Lafex (2018) 58% of the soil-sampled land area 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$_4$) (Amrani, Westfall, & Peterson, 1999; Norman, Slaton, & Roberts, 2013; Sharma & Katty, 1986). Recommendations for fertilization with relatively high granular Zn rates have existed since research was first initiated investigating how to prevent crop Zn deficiency (Sommer & 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, Thompson, Place, & Shockley, 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 (Carsky & Reid, 1990; Slaton, Gbur, Wilson, & Norman, 2005; Takkar, Mann, & Randhawa, 1975). 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 & Gilkes, 1993). Development of Zn fertilizers with Zn sources other than ZnSO$_4$ (e.g., Zn oxides, oxyzulfates, lignosulfonates, and synthetic chelates) required granular Zn fertilizer recommendations to 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 40–50% of the total Zn in the water-soluble form (Amrani et al., 1999; Gangloff, Westfall, Peterson, & Mortvedt, 2002; Liscano et al., 2000; Mortvedt, 1992). 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 cost of elemental Zn has increased the price of Zn fertilizers over the past 20 yr. The price of elemental Zn was US$1.12 kg$^{-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). The risks associated with using granular Zn fertilizers coupled with the high cost associated with this Zn-fertilization strategy have led 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 with the standard preplant application of 11 kg Zn ha$^{-1}$ as granular ZnSO$_4$. Alternative, low-use-rate, Zn-fertilization methods include preplant or post-emergence applications of Zn solutions, 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-emergence (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 (Mortvedt & Gilkes, 1993; Norvell & Lindsay, 1969). A foliar application of Zn-EDTA or liquid ZnSO$_4$ to rice at the 2- to 3-leaf stage is a common practice in Arkansas. Slaton, Wilson, Norman, and Gbur (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, Orlowski, and Bond (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 with ZnSO$_4$ (110.5 mg Zn kg$^{-1}$) and Zn-EDTA (104.1 mg Zn kg$^{-1}$). Many product labels suggest using Zn rates lower than the 1.1 to 2.2 kg Zn ha$^{-1}$ commonly recommended by land-grant institutions (Camberato & Maloney, 2012; Norman et al., 2013).
difficult to mix and apply uniformly, and commercially treated seed often contains less Zn than recommended.

Macronutrient fertilizers can also be coated with ZnO powders, such as Wolftrex Zn-DDP (dry dispersible powder, 620 g Zn kg⁻¹, Compass Minerals), which are marketed to producers claiming enhanced efficiency compared with granular ZnSO₄ due to more uniform Zn distribution because Zn adheres to each macronutrient fertilizer granule. Shaver and Westfall (2008) reported that Wolftrex Zn-DDP did not increase the shoot Zn concentration of greenhouse-grown corn above the tissue-Zn concentration of corn receiving no Zn, whereas ZnSO₄ increased tissue-Zn concentration relative to the no-Zn check, but similar to corn fertilized with 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, Olson, and Daverde (2016) reported that corn fertilized with MicroEssentials SZ (MESZ, The Mosaic Company) yielded 11,680 kg ha⁻¹, which was significantly greater than corn fertilized with a bulk blend of monoammonium phosphate, ammonium sulfate, and ZnSO₄ 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 compared with the standard Zn-fertilization method of preplant soil application of 11 kg Zn ha⁻¹ as ZnSO₄ granules.

2 | MATERIALS AND METHODS

2.1 | Site description

A total of seven field trials were established in 2017 and 2018. Selected soil properties are summarized in Table 1. Each location is identified by the soil series and year (e.g., Calhoun-17) the trial was conducted. All trials having Calloway (finesilty, 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 65 °C, crushed to pass through a sieve with a 2-mm diameter screen, and analyzed for soil pH (1:2 soil/water mixture; Sikora & Kissel, 2014), organic matter by weight loss on ignition (Schulte & Hopkins, 1996), and soil nutrient concentrations extracted with Mehlich-3 solution (Zhang, Hardy, Mylavarapu, & Wang, 2014). The Mehlich-3 extracts were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Arcos-160 SOP, Spectro, NJ).

2.2 | 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’, and ‘LaKast’ rice seed was treated with Zinche ST (325 g Zn kg⁻¹ and 488 g Zn L⁻¹, Drexel Chemical Company) 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 CO₂ pressurized sprayer, and allowed to mix for 10 min to ensure that the Zn evenly coated the rice seed. Rice seeds were also treated with AV-1011 (50% 9,10-anthraquinone, ARKION Life Sciences LLC) 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. Subsamples (n = 3) of treated and untreated seed were digested with concentrated HNO₃ and 30% H₂O₂ to determine the seed-Zn concentration (Jones & 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 (Norman et al., 2013; Slaton et al., 2001).

The six Zn-fertilization methods included: (a) no Zn, (b) granular ZnSO₄ preplant applied at 11 kg Zn ha⁻¹ (GRAN, 355 g Zn kg⁻¹, Winfield Solutions, LLC), (c) MESZ preplant applied at 1.68 kg Zn ha⁻¹ (MESZ; 28% water-soluble [WS] Zn, 120 g N, 175 g P, 100 g S, and 10 g Zn kg⁻¹), (d) 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) applied at the 2-leaf stage (EDTA), and (e and f) 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...
TABLE 1  Selected soil chemical property means (0- to 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

| Soil-year\(^a\) | Soil pH\(^b\) | Soil OM\(^c\) (g kg\(^{-1}\)) | Mehlich-3 extractable soil nutrients\(^d\) mg kg\(^{-1}\) |
|-----------------|---------------|------------------------|--------------------------|
|                 |               |                        | P | K | Ca | Mg | S | Na | Fe | Mn | Zn | Cu | B |
| Calloway-17a    | 6.6           | 22                     | 28 | 79 | 1,335 | 204 | 14 | 48 | 345 | 444 | 3.1 | 0.8 | 0.5 |
| Calhoun-17a     | 7.6           | 21                     | 22 | 77 | 2,002 | 311 | 7  | 33 | 314 | 303 | 2.5 | 1.0 | 0.6 |
| Calhoun-18a     | 7.9           | 20                     | 34 | 105| 2,948 | 406 | 25 | 113| 437 | 203 | 2.0 | 1.4 | 0.4 |
| Calloway-18a    | 7.9           | 21                     | 16 | 47 | 1,968 | 296 | 8  | 68 | 470 | 164 | 1.4 | 1.3 | 0.3 |
| Calloway-18b    | 6.7           | 23                     | 33 | 106| 1,278 | 243 | 12 | 64 | 352 | 263 | 2.1 | 1.4 | 0.2 |
| Calhoun-18b     | 7.9           | 22                     | 61 | 98 | 2,529 | 379 | 14 | 61 | 411 | 281 | 2.2 | 2.1 | 0.3 |
| Sharkey-18      | 7.9           | 26                     | 62 | 268| 5,125 | 829 | 18 | 147| 408 | 67  | 2.2 | 2.2 | 0.8 |

\(^a\)The lowercase letter by the year (17 or 18) differentiates the sites that shared the same soil series and year.

\(^b\)Soil pH measured in a 1:2 soil/water mixture (Sikora & Kissel, 2014).

\(^c\)OM, organic matter by weight loss on ignition (Schulte & Hopkins, 1996).

\(^d\)Extracted using Mehlich-3 method (Zhang et al., 2014).

TABLE 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 1 for selected site information

| Site-year | Planted   | Foliar Zn application\(^e\) | Flood established | Plant sample | Harvest  | Canopeo measurement |
|-----------|-----------|-----------------------------|-------------------|-------------|----------|---------------------|
| Calloway-17 | 18 Apr. 16 May | 24 May 31 May 9 Sept. 23 May 31 May 7 June – – |
| Calhoun-17 | 3 May 22 May | 31 May 7 June 9 Sept. 31 May 7 June 13 June 21 June – |
| Calhoun-18a | 10 Apr. 15 May | 31 May 12 June 10 Sept. – – – – |
| 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 Sept. 15 May 24 May 31 May 5 June 12 June |
| Calhoun-18b | 24 May 5 June | 20 June 24 May 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 |

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

adherence of the product to granular fertilizer (1 kg Zn-DDP 100 kg\(^{-1}\) fertilzer).

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 Pha\(^{-1}\)) 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). 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, Lorenz, Scott, & Norman, 2013a).

2.3 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.) that uses red-green-blue (RGB) color values (Patrignani & 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).

An iPad (5th generation; 8-megapixel camera; Hon Hai Precision Industry Co., Ltd.) 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 m²) 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, Wilson, & Norman, 2013b).

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 mid-tillering growth stage. The samples were placed in paper bags, oven-dried at 55 °C 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 inductively coupled plasma–atomic emission spectroscopy (ICP-AES, Jones & Case, 1990).

At maturity, a 5-m² 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 H₂O kg⁻¹ grain.

### 2.4 Statistical analyses

Analysis of variance was performed using the GLIMMIX procedure in SAS (v.9.4, SAS Institute). 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 requiring that canopy coverage data be divided by 100. When appropriate, means were separated using Fisher’s protected least significant difference at a significance level of .10.

### 3 | RESULTS AND DISCUSSION

#### 3.1 Canopy coverage

Canopy coverage was affected by the fertilization method main effect (Calloway-17) or the interaction between Zn-seed treatment and fertilization method (Calloway-18b) at only two of the six sites where canopy coverage was measured (Table 3). Interactions between canopy measurement time and Zn-seed treatment rate or fertilization strategy were not significant (e.g., $P > .10$) for any of the six trials. As would be expected, canopy coverage, averaged across Zn-seed treatment and fertilization method, increased with each successive measurement (cumulative GDU) at all six sites (Table 4). At Calloway-17, planting Zn-treated rice seed and fertilizing with MESZ resulted in the numerically greatest canopy coverage, averaged across sample times, but was significantly greater than only the rice that received no Zn, with or without Zn-seed treatment, and rice that received GRAN plus the Zn-seed treatment (data not shown). 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, whereas 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 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, and other Zn sources did not differ from each other or from the no-Zn control. There are at least 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, whereas 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 1). Second, MESZ was the only Zn-fertilizer treatment that included pre-plant 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
TABLE 3 Rice canopy coverage as affected by the main effects of Zn-fertilization method and Zn-seed treatment, for each of the six locations averaged across sample times (n = 3–5)

| Fertilizera | Calloway-17 | Calhoun-17 | Calloway-18a | Calloway-18b | Calhoun-18b | Sharkey-18a |
|-------------|-------------|------------|--------------|--------------|-------------|-------------|
| No Zn       | 39.3 b      | 38.4       | 27.6         | 19.7 b       | 19.8        | 10.4        |
| EDTA        | 47.1 ab     | 37.3       | 29.5         | 19.2 b       | 24.2        | 11.4        |
| DDP1        | 48.4 ab     | 40.5       | 26.1         | 19.5 b       | 15.6        | 12.3        |
| GRAN        | 48.8 ab     | 39.9       | 27.0         | 19.1 b       | 19.3        | 11.3        |
| MESZ        | 57.7 a      | 41.0       | 32.3         | 26.7 a       | 20.7        | 12.8        |
| P value     | .0044       | .8876      | .2665        | .0020        | .2283       | .8730       |

Seed treatmentb

| 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       | .6373 | .2828 | .9815 | .8105 | .7119 | .9704 |
| Interaction P value | .0578 | .9042 | .7089 | .5119 | .6459 | .9529 |

Note: Means within a column followed by different lowercase letters are statistically different at the .10 level. Mean separations were performed on data transformed using a beta distribution. The actual, untransformed means are listed in the table.

aEDTA Ultra-Che Zinc 9% EDTA, Winfield Solutions, LLC; DDP1, WolfTrax, Compass Minerals; GRAN, granular zinc sulfate, Winfield Solutions, LLC; MESZ, MicroEssentials The Mosaic Company.
bZinche ST, Drexel Chemical Company.

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- to 3-leaf stage when canopy coverage averaged 5.7% (ranging from 0.75 to 14.1% among site-years, Table 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 belowground uptake of fertilizer Zn very important. Haslett, Reid, and Rengel (2001) reported that the EDTA chelate offers no advantage or disadvantage for Zn uptake through the leaf compared with inorganic Zn. The organic molecule EDTA enhances Zn mobility in soil increasing the likelihood that the Zn will be taken up by small seedlings (Mortvedt & Gilkes, 1993; Norvell & Lindsay, 1969).

3.2 | Seedling aboveground biomass

The Zn-seed treatment × Zn-fertilization method interaction had no significant effect on aboveground biomass (P = .8514), but aboveground biomass was significantly affected by Zn-fertilization method (Table 5) and Zn-seed treatment (P = .0101). Although the numerical difference was nominal, the aboveground biomass was greater for rice receiving seed-applied Zn (1155 kg ha⁻¹) than without seed-applied Zn (1107 kg ha⁻¹). Slaton et al. (2001) reported that rice total dry matter would be maximized for rice receiving Zn-seed treatments applied at 2.2–5.8 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 and all other Zn-fertilizer methods when averaged across Zn-seed treatment rates (Table 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 with other Zn-fertilizer treatments. Rice receiving Zn-EDTA or GRAN produced greater aboveground biomass than rice in the no-Zn control, DDP1.1, and DDP0.5 treatments. Rice receiving no-Zn, DDP1.1, or DDP0.5 produced similar 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.

3.3 | Tissue-zinc concentration and aboveground zinc content of seedling rice

The Zn-seed treatment rate × Zn-fertilization method interaction had no significant effect on tissue-Zn concentration (P = .6895) or content (P = .8857). However, tissue-Zn concentration and aboveground Zn content were both 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 22.9 to
TABLE 4  Percentage rice canopy coverage, averaged across Zn-seed treatment and fertilization method, 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 | DD10 | Canopy coveragea (%) |
|----------|-------------|-----|------|----------------------|
| Calloway-17 | 23 May | 275 | 14.1 c |
|           | 31 May | 371 | 36.7 b |
|           | 7 June | 472 | 89.5 a |
| P value   |          |     | <.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   |          |     | <.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   |          |     | <.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   |          |     | <.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   |          |     | <.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   |          |     | <.0001 |

Note. Within the same column and location, means followed by different lowercase letters are statistically different at the .01 level. Mean separations were performed on data transformed using a beta distribution. The actual, untransformed means are listed in the table.

See Table 4.

TABLE 5  Rice aboveground biomass, tissue-Zn concentration, and Zn content at the mid-tillering growth stage and grain yield as affected by Zn-fertilization method, averaged across Zn-seed treatment rates (n = 2) and trials (n = 7)

| Fertilizer | Biomass (kg ha\(^{-1}\)) | Tissue-Zn (mg kg\(^{-1}\)) | Zn-content (g ha\(^{-1}\)) | Grain yield (kg ha\(^{-1}\)) |
|------------|---------------------------|----------------------------|-----------------------------|-----------------------------|
| no-Zn      | 1,063 c                   | 21.3 d                    | 22.6 c                      | 9,982                       |
| EDTA       | 1,141 b                   | 24.7 b                    | 27.1 b                      | 10,048                      |
| DDP0.5     | 1,018 c                   | 22.0 cd                   | 22.0 c                      | 9,878                       |
| DDP1.1     | 1,098 c                   | 22.4 c                    | 24.2 c                      | 9,939                       |
| GRAN       | 1,172 b                   | 28.9 a                    | 32.9 a                      | 10,016                      |
| MESZ       | 1,294 a                   | 23.1 c                    | 30.7 ab                     | 9,939                       |

Note. Within each column, means followed by different lowercase letters are statistically different at the .10 level. Mean separations were performed on data transformed using a gamma distribution. The actual, untransformed means are listed in the table.

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 Zn concentration was significantly affected by Zn fertilization method (Table 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 DDP1.1 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 DDP1.1 treatments. Although EDTA and MESZ significantly increased tissue-Zn concentrations above the no-Zn control the increase in tissue-Zn concentration was nominal (1.8–3.4 mg Zn kg\(^{-1}\)). For aboveground Zn content, rice fertilized with GRAN and MESZ had equal Zn contents that were greater than rice receiving no Zn, DDP1.1, and DDP0.5.

The average rice tissue-Zn concentrations for all treatments (Table 5) were above the 15–20 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 trials 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 percentage WSNZ contained in a fertilizer is an important indicator of plant-available Zn (Amrani et al., 1999; Gangloff et al., 2002; Liscano et al., 2000; Mortvedt, 1992) and could explain why
Zn-DDP (11% WSZn) did not increase tissue-Zn concentration, whereas GRAN (90% WSZn), EDTA (100% WSZn), and MESZ (28% WSZn) did affect tissue-Zn concentrations.

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 with inorganic-Zn fertilizer sources. For example, a fertilizer with a manufacturer’s claimed efficiency ratio of 10:1 would suggest 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 with 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 compared with 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.

3.4 Grain yield

Grain yield was not affected by Zn-seed treatment (P = .1123), Zn-fertilization method or by their interaction (P = .6737, Table 5). The Zn-seed treatment main effect did not significantly increase grain yield in this study but the P value of .1123 was nearly significant suggesting there was a numerical yield increase for rice receiving the seed-applied Zn (10.014 kg ha$^{-1}$) to yield more than rice receiving no seed Zn (9.920 kg ha$^{-1}$). Slaton et al. (2001) and Rush (1972) both 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 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 & 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.

4 CONCLUSIONS

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 regard to the product’s (and its use rate) ability to provide sufficient Zn nutrition to seedling rice. 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, the only low-use-rate Zn strategies providing a benefit to seedling rice that merit use by growers were a recommended rate of Zn applied to the rice seed, preplant-applied MESZ, and a timely post-emergence application of a Zn-EDTA solution.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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