

## Objectives

- To establish maize plants with a confirmed Fe or Zn deficiency in their respective trials prior to Fe or Zn foliar application
- To compare the effect of foliarly-applied Pheroid nanoparticle, chelate, and sulfate forms of Fe and Zn on biomass, nutrient uptake and mobilization on Fe and Zn-deficient maize (*Zea mays* L.)

## Introduction

- Fe and Zn are essential to Maize growth and development
- Foliarly-applied micronutrients are commonly used to supply micronutrients to crops (Figure 1).
- The effect of foliarly-applied micronutrients on maize grain yield is inconsistent and often has no effect.
- Nanoparticles have a size between 1 and 100 nm and show properties that are not evident in their bulk counterpart.
- There is an increasing body of literature reporting improved dermal penetration, timed-release, and mobility of the active ingredients in both animal and plant systems when using nanotechnology such as Pheroid nanoparticles (Figure 2).
- Each of these properties would be beneficial in improving the effect of foliarly-applied nutrients, improving nutrient use efficiency and would likely have a key role in moving towards sustainable intensification.

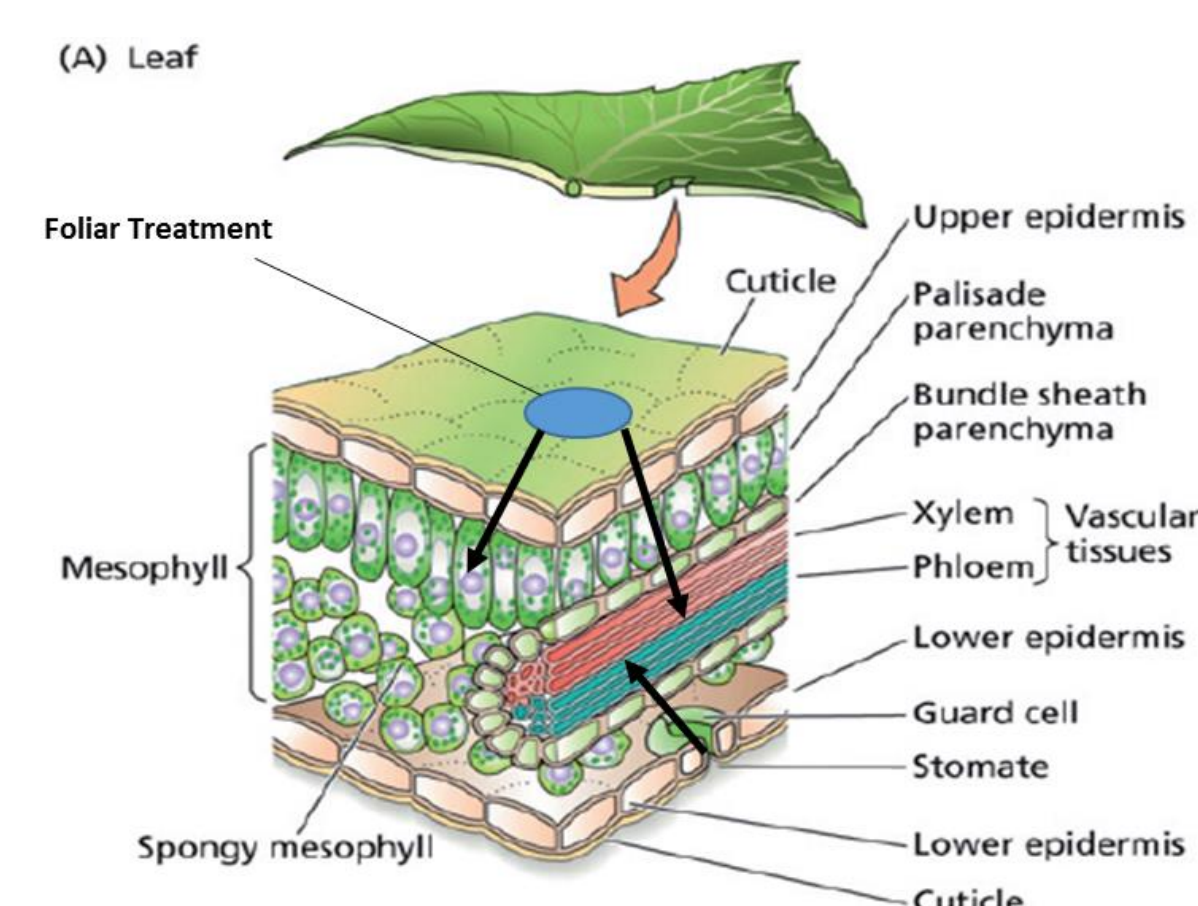


Figure 1. Side view of leaf with a proposed mechanism of foliar micronutrient entry (adapted from Plant Physiology, 4th Edition 2007)

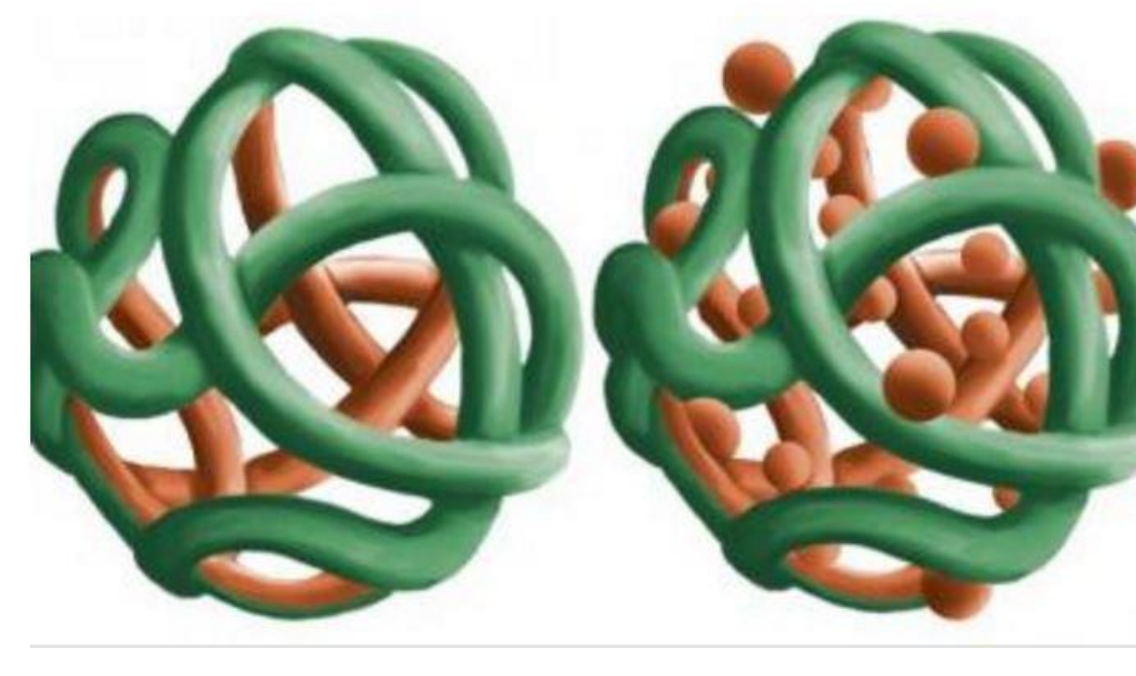


Figure 2. Pheroid nanoparticles are composed of an organic carbon backbone and fatty acids that results in a nano-sponge that can be manipulated to entrap compounds so they can be transported across biological membranes (Grober, 2009).

## 1. Methods – Establishing Zn and Fe Deficient Maize

- Two Hydroponics Greenhouse Trials
  - Trial 1: (-)Fe Nutrient Solution
  - Trial 2: (-)Zn Nutrient Solution
- Modified Hoagland's Solution excluding Fe or Zn in their respective trials (Table 1)
- Experimental Design: RCBD with three replicates (Figure 3)



Figure 3. a) Experimental hydroponics design b) Image of maize seedlings held in the hydroponics solutions

	----No Zn Solution†----		---Complete Solution‡---		----No Fe Solution§----	
	mg/liter	µM	mg/liter	µM	mg/liter	µM
Ca	302	7540	302	7540	302	7540
K	283	7240	283	7240	283	7240
Mg	37.8	1550	37.8	1550	37.8	1550
NH <sub>3</sub> -N	321	22900	321	22900	321	22900
NH <sub>4</sub> -N	39.0	2780	39.0	2780	39.0	2780
Cl	65.0	1940	65.0	1940	65.0	1940
S	58.5	1820	58.5	1820	58.5	1820
P	2.00	65	2.00	65	2.00	65
Fe	2.76	49	2.76	49	0	0
Mn	0.974	18	0.974	18	0.974	18
B	0.536	50	0.536	50	0.536	50
Zn	0	0	0.300	4.6	0.300	4.6
Cu	0.076	1.2	0.076	1.2	0.076	1.2
Mo	0.155	1.6	0.155	1.6	0.155	1.6
Na	4.56	200	4.56	200	4.56	200
HEDTA	13.0	47	13.0	47	0	0

† Solution administered in trial 1  
‡ Solution administered in trial 1 and trial 2  
§ Solution administered in trial 2

Table 1. Hydroponics nutrient solution compositions. Specific chemicals and mixing details in Clark, R.B., 1982. (Adapted from Clark, R.B., 1982)

## 1. Results – Establishing Zn and Fe Deficient Maize

### Fe Deficiency

- Fe deficiency was clearly induced
  - Visual signs of deficiency began at V2 (i.e. chlorosis in upper leaves)(Figures 4).
  - V5 foliage biomass was reduced from 8.4g to 2.2g  $p < 0.0001$
  - Root biomass was reduced from 21.1g to 5.1g  $p = 0.0003$
  - V5 Foliage Fe concentration was reduced from 90.7 mg  $kg^{-1}$  to 55.7 mg  $kg^{-1}$  ( $p = 0.20$ )
  - Fe concentration reduced to near the lower level of the Fe sufficiency range (i.e. 50-250 mg  $kg^{-1}$  Fe) for maize less than 0.305m (12in) tall (Mills, et al., 1996).

### Zn Deficiency

- Zn deficiency was limited
  - No visual signs of Zn deficiency (Figure 4).
  - No foliage or root biomass difference at V5
  - V5 foliage Zn concentration was reduced from 78.7 mg  $kg^{-1}$  to 52.0 mg  $kg^{-1}$  ( $p = 0.01$ ).
  - Both Zn concentrations were within the Zn sufficiency range (i.e. 20-60 mg  $kg^{-1}$ ) for maize less than 0.305m (12in) tall (Mills, et al., 1996).
  - V9 foliage Zn concentration was reduced from 40.2 mg  $kg^{-1}$  to 18.0 mg  $kg^{-1}$   $p = 0.008$  which was also near the lower level of the Zn sufficiency range for maize prior to tassel (i.e. 15-60 mg  $kg^{-1}$  Zn).

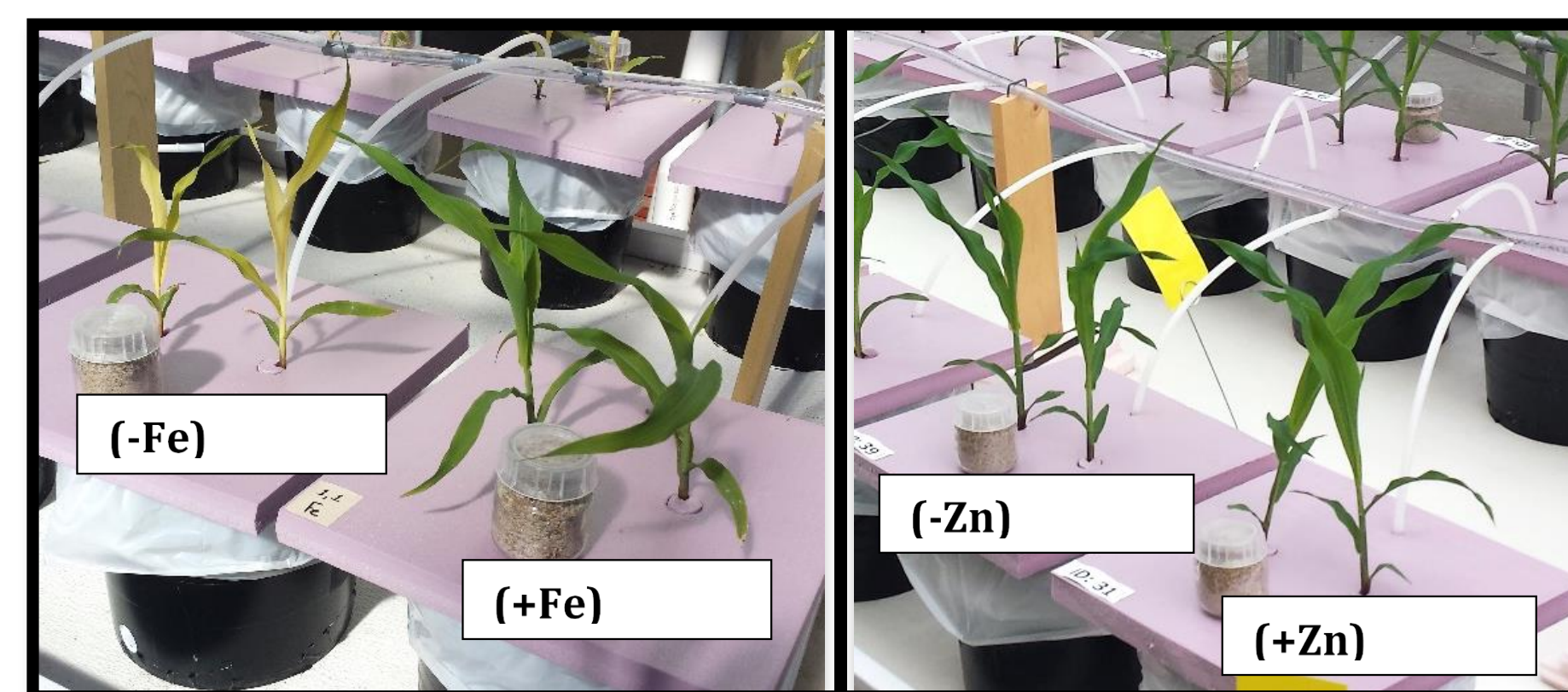


Figure 4. V3-V4 maize plants, prior to spraying, grown in trial 1 (i.e. (-) Fe Scenario) and trial 2 (i.e. (-) Zn Scenario). There were visual signs of Fe deficiency as evident by chlorosis in the upper leaves but no visual sign of Zn deficiency.

## 2. Methods – Testing Foliarly-Applied Zn and Fe

- Foliar treatments were applied at V5 in a spray chamber (Research Track Sprayer; DeVries, Hollandale, MN).
- Two rates of Fe and Zn were applied at the upper and lower level of industry recommendations (0.11; 0.22 kg Fe  $ha^{-1}$  and 0.45; 0.90 kg Zn  $ha^{-1}$ ) in chelated (HEDTA), sulfate and nanoparticle (Pheroid) forms.
  - 9 Treatments (Table 2)
- Foliage and root biomass was collected at V5 and V9, dried, weighed and analyzed for nutrient concentrations at Midwest Laboratories.
- Leaf samples collected at V9 were split into upper and lower samples to evaluate mobility of the applied nutrient.

-----Trial 1 (Fe Deficiency Scenario)-----			-----Trial 2 (Zn Deficiency Scenario)-----		
Foliar Treatment	Hydroponics Nutrient Solution	Hydroponics Nutrient Solution	Foliar Treatment	Hydroponics Nutrient Solution	Hydroponics Nutrient Solution
Control No foliar trt applied	Complete Solution	Complete Solution	Control No foliar trt applied	Complete Solution	Complete Solution
Control No foliar trt applied	(-)Fe	(-)Fe	Control No foliar trt applied	(-)Zn	(-)Zn
Pheroid Nanoparticle only Foliar rate 1	(-)Fe	(-)Fe	Pheroid Nanoparticle only Foliar rate 1	(-)Zn	(-)Zn
Fe Pheroid Nanoparticle Foliar rate 1§	(-)Fe	(-)Fe	Zn Pheroid Nanoparticle Foliar rate 1‡	(-)Zn	(-)Zn
Fe Pheroid Nanoparticle Foliar rate 2¶	(-)Fe	(-)Fe	Zn Pheroid Nanoparticle Foliar rate 2††	(-)Zn	(-)Zn
FeHEDTA# Foliar rate 1	(-)Fe	(-)Fe	ZnEDTA§§ Foliar rate 1	(-)Zn	(-)Zn
FeHEDTA# Foliar rate 2	(-)Fe	(-)Fe	ZnEDTA§§ Foliar rate 2	(-)Zn	(-)Zn
Fe Sulfate¶¶ Foliar rate 1	(-)Fe	(-)Fe	Zn Sulfate¶¶ Foliar rate 1	(-)Zn	(-)Zn
Fe Sulfate¶¶ Foliar rate 2	(-)Fe	(-)Fe	Zn Sulfate¶¶ Foliar rate 2	(-)Zn	(-)Zn

† All treatments were applied to individual plants at a speed of 3.7kph (2.3mpb) and height of 0.3m above the canopy with a band width of 0.38m (15in.) in a spray chamber

‡ The pheroid nanoparticle application rate for all pheroid containing treatments was 120mg/ha (1.07\*10<sup>-4</sup> lbs/A)

§ Rate 1 Fe concentrations were 750 ppm 0.11 kg Fe  $ha^{-1}$  (0.1 lbs Fe/A) and 120mg/ha pheroid nanoparticle

¶ Rate 2 Fe concentrations were 1500 ppm 0.22 kg Fe  $ha^{-1}$  (0.2 lbs Fe/A) and 120mg/ha pheroid nanoparticle

# 4.5% FeHEDTA (iron-hydroxyethylene-diaminetriacetate) in addition to proprietary surfactants, saccharides, and antifouling solvents ComSorb

|| 6.0% Iron(II) Sulfate in addition to proprietary surfactants, saccharides, and antifouling solvents ComSorb

‡‡ Rate 1 Zn concentrations were 3,000 ppm or 0.45 kg Zn  $ha^{-1}$  (0.4 lbs Zn/A) and 120mg/ha pheroid nanoparticle

†† Rate 2 Zn concentrations were 6,000 ppm or 0.90 kg Zn  $ha^{-1}$  (0.8 lbs Zn/A) and 120mg/ha pheroid nanoparticle

§§ 6.0% ZnEDTA (zinc-ethylene-diaminetriacetate) and contains proprietary surfactants, saccharides, and antifouling solvents ComSorb

¶¶ 6.0% Zn Sulfate and contains proprietary surfactants, saccharides, and antifouling solvents ComSorb

Table 2. Treatments applied at V5 in trial 1 (Fe deficiency scenario) and trial 2 (Zn deficiency scenario)†

## 2. Results –Foliarly –Applied Forms of Zn and Fe

### Effect on Biomass

- Foliar applications of Fe or Zn in all forms had no effect on new growth or root biomass at V9.
  - The Pheroid only treatment also had no effect
- Comparisons between Fe containing treatments showed that Fe-Sulfate rate 2 had the greatest change in foliage biomass which was greater than FeHEDTA rate 2.
- Comparisons between Zn containing treatments showed no difference in their effect on foliage or root biomass.

### Effect on Leaf Nutrient Concentrations

- There were significant treatment effects on foliage Fe and Zn concentrations in their respective trials.
- Increased Fe and Zn foliage concentration corresponded to increased application rates, regardless of form.
- The Pheroid only had no effect on concentration.

### Fe and Zn Mobility

Mobility of the foliarly-applied nutrient to new growth leaves was assessed by measuring the Fe and Zn concentrations in the top new-growth (un-treated) leaves.

- No increase in Fe or Zn concentrations were observed in the top new-growth leaves.
  - This was evidence for limited or no mobility provided by the Pheroid, chelate and sulfate forms.
- Leaves treated with foliar Fe all had some re-greening especially for rate 2 (Figure 5).
- No visual signs of foliar Zn treatment effects (i.e. re-greening) were observed except leaf burn which was evident for all treatments.
- Foliar Fe-Sulfate re-greened in speckling patterns localized to the droplet. FeHEDTA and Fe-Pheroid also had speckling patterns but had smoother re-greening patterns across the entire leaf surface (Figure 3). This may be suggestive of localized mobilization.

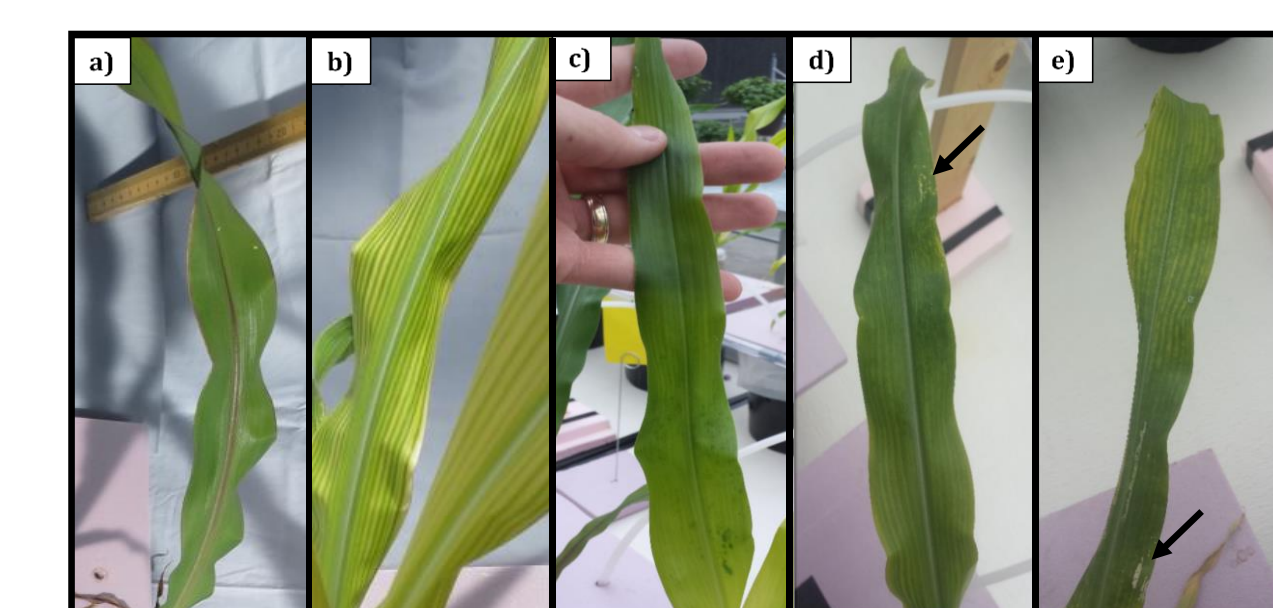


Figure 5. Examples of leaf re-greening characteristics of the fifth leaf of maize plants, grown in trial 1, 10 days post foliar Fe treatment applications. No visual signs of re-greening due to the foliar Zn treatments were observed in trial 2. All images were taken from the same statistical block and each of the treated images received "rate 2" (0.22 kg Fe  $ha^{-1}$ ). Arrows indicate areas with leaf "burn." a) "Complete Control" b) "Deficient Control(-Fe)" c) "Fe-Pheroid Rate 2" d) "FeHEDTA Rate 2" e) "Fe-Sulfate Rate 2"

## Conclusions

- This hydroponics design proved effective for comparing foliar nutrient treatments under their respective nutrient deficiency scenario.
- Biomass was used as an indicator of the effect on grain yield. There is no evidence that foliar Zn or Fe in any form would have increased grain yield.
- Although we did not see any advantage of Pheroid nanoparticles, the theoretical benefits of nanomaterials (i.e. enhanced dermal penetration, timed release, and mobilization of the applied nutrients to metabolically active cellular components) should continue to be investigated for foliar applications of plant nutrients and other topical treatments (i.e. herbicides and insecticides).

## Acknowledgements

The authors would like to thank the Nebraska Corn Board and Winfield Solutions for providing funding for this research.

