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Effects of Magnetized, Chelated Iron Foliage Treatments on Foliar Physiology, Plant Growth and Drought Tolerance for Two Legume Species

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ABSTRACT

A greenhouse study was conducted to determine the effects of foliar applications of magnetized, chelated liquid iron fertilizer for increasing the drought tolerance of two legumes. Study objectives were to determine the drought tolerance effects of four treatments on foliar gas exchange, soil moisture, and plant growth for velvet bean (*Mucuns pruriens*) and soybean (*Gylcine max*) plants. The four foliage treatments included applications with chelated liquid iron fertilizer (2.5 and 5%) with a conventional boom sprayer, with and without magnets in the spray lines. Physiological measurements were collected before foliar treatments and again after a 24-day deficit irrigation schedule. Physicochemical water properties were measured for each of the foliar treatments. Photosynthesis rates were 5.98, 2.04 and 3.19 µmol/m2/s for the control, non-magnetized and magnetized fertilizer treatments (2.5%), respectively, after completing the deficit irrigation schedule. Instantaneous water use efficiency (IWUE) was 0.60, 0.28 and 1.02 for the control, non-magnetized and magnetized fertilizer treatments (2.5%), respectively, after completing the deficit irrigation schedule. Photosynthesis and IWUE increased 56 and 263% for the magnetized fertilizer treatment (2.5%) compared to the non-magnetized foliar treatment, when averaged across both legume species. Photosynthesis and IWUE increased as electrical conductivity increased and oxidation reduction potential (ORP) decreased in absolute terms. A single foliar application resulted in aberrant physiological responses that are contrary to very widely held plant defense theories involving abiotic stressors. The single application improved the photosynthesis and water use efficiency for water stressed legumes emphasizing the need to better understand the relationships between water quality, plant bioenergetics, and stress physiology. Improved drought tolerance in row crops such as dry beans and soybeans, with a single magnetized fertilizer application, would be cost effective and easily adapted into current cropping systems. Interactions among physicochemical water properties, bioenergetics, plant metabolism, and crop stress physiology need to be further investigated in order to improve the quality of irrigation water to enhance drought tolerance of field crops.

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1. Introduction

Most of the row crops in the USA are rain fed. Such crops would be at risk of lower yields and/or abiotic stress injury under lower rainfall conditions predicted by many climate change models. Drought tolerant research has recently accelerated but has focused on major crops such as maize through development of crop breeding methods or genetically modified varieties [1-2]. Drought stressed crops produce lower yields and have a higher risk of compromised natural defenses against pathogen infections and insect damage [3]. This study evaluated the less known areas of drought tolerance research such as improved plant micro-nutrition and altering physicochemical properties of irrigation water.

Temperate and tropical legumes, such as soybean and velvet bean species, have C3 Calvin cycle pathways which are susceptible to photoinhibition when stressed by excess light, heat, or water stress. Drought tolerant research involving crops with C3 metabolic pathways should address methods that boost plant defenses with nutrient programs, or evaluate plant responses to irrigation with activated or energized water properties [4].

Drought stress may be partially alleviated with micro-nutrient applications [5-7]. Iron (Fe) is a micro-nutrient that is utilized by legumes in several key nitrogenase enzymes, and for ferredoxin which is an electron carrier [5-6]. Legumes also utilize iron in the synthesis of heme in hemoglobin and for nodule formation. Singh *et al*., [8] improved groundnut (*Arachis hypogaea*) yield when grown under drought conditions using a foliar application of iron citrate. Our study evaluated the effects of applying a micro-nutrient fertilizer containing chelated iron in combination with a magnetized sprayer for improving drought tolerance of soybean, which is a temperate legume (*G. max*) and velvet bean, which is a tropical legume (*M. pruriens*). The seedlings were subjected to deficit irrigation under controlled greenhouse conditions to determine their responses to the foliar micronutrient treatments.

One theory for the increased drought tolerance in magnetized crop irrigation involves the putative ability of static magnets attached to the water lines that can energize or alter several physicochemical water properties [9]. The degree of water activation is a function of the strength of the magnets and the exposure time to the magnetic field [10-11]. As magnetic fields interact with the polar water molecules, the energy fields widen the hydrogen bond angles and elevate the valence electrons into higher energy orbits, thereby altering the physicochemical properties of the water [12-15]. Selim and El-Nady [16] found that water use efficiency increased 119% for drought stressed tomato plants that were irrigated with magnetized water. They also found that transpiration rates, water use efficiency, and chlorophyll contents were increased by magnetized seeds and/or irrigation. Also, Tayari and Jamshidi [17] found that irrigation efficiency increased by 30% when greenhouse cucumbers were treated with magnetized irrigation water. Magnetized irrigation water enhanced drought tolerance in crops in several field studies [18–24].

The physicochemical water properties are altered when static magnetic fields widen the hydrogen bond angles and shorten the H-bonds in water [12-15]. As the hydrogen bond angles widen from 104° to 109.5° the water molecules form a tetrahedral design resulting in five (pentagonal) and six (hexagonal) molecular rings that have greater stability and less enthalpy or bonding energy [25-28]. Ice is the solid crystal structure of water, which consists of layers of hexagonal rings of water molecules that form as the hydrogen bonds become rigid. In contrast, bulk water generally contains a variable percentage (20 to 80%) of structured water containing hexagonal rings of water molecules [29]. Binhi [30] conducted a review of theoretical mechanisms underlying magnetizing water and concluded that magnetic fields increase water structure. Also, Barnes and Greenebaum [31] found a direct relationship between magnetic field strength and the velocity of the valence electrons that excites the electrons into a higher orbit. The energized or activated water molecules then realign into structured water containing hexagonal rings [24, 29]. Structured water studies, including magnetized irrigation water that results in partially structured water, have consistently resulted in improved plant physiology or enhanced productivity and yield [32-36].

Chelated iron contains both ionic Fe species (Fe^{2+} and Fe^{3+}) that can also be used to generate slightly structured water. Soluble iron ions create charged polar fields in the bulk water that in turn widens the hydrogen bond angle between water molecules [29]. As the H-bond angle widens into a tetrahedral angle a percentage of the bulk water converts into structured water with hexagonal rings [29]. This study used a combination of chelated iron and magnetized water to increase the ratio of structured to unstructured water in the foliar treatments to improve drought tolerance in two legume species.

The first hypothesis of this study is that the combination of magnetized water with structure-inducing chelated iron would enhance the physicochemical water properties, increasing the ratio of structured water to bulk water. This hypothesis was tested by measuring any changes in the physicochemical water properties after adding chelated iron to the foliar spray solution. The second hypothesis is that a temperate legume species may have different physiological or growth responses than a tropical legume species to the foliar treatments. A third hypothesis is that the foliar treatments will improve foliar physiology biomarkers, soil moisture parameters and seedling growth rates when the two legume species are watered under a deficit irrigation schedule. The overall goal is to further elucidate the interactions between water treatments containing chelated iron and exposed to magnetic fields and plant physiology and growth when grown under deficit irrigation.

2. Material and methods

2.1. Study design

A greenhouse study was conducted at the USDA-APHIS laboratory in Fort Collins, CO to determine the effects of four foliar treatments containing a series of magnetized/non-magnetized, chelated iron solutions on two C3 legumes. The study design was a completely randomized design (CRD). All plants were moved on a semi-weekly basis to avoid shading or temperature effects on plant growth. The study factors consisted of two legume species, two sprayer systems (magnetized and non-magnetized spray booms), two chelated iron fertilizer rates (2.5 and 5% v/v), and three measurement dates.

The two legume species selected were velvet bean (*Mucuns pruriens*) which is a tropical legume, and soybean (*Glycine max*) which is a temperate legume. There were five replicates for the velvet bean plants (VB), and four replicates for the soybean plants (SB) for each of the four application treatments. Spray solutions were analyzed for three water properties. Foliar gas exchange and volumetric soil moisture (m^3/m^3) data was collected to determine the legume responses to two deficit irrigation events.

2.2. Foliar Treatments and Deficit Irrigation Description

Velvet bean and soybean seeds were germinated in plastic trays and allowed to grow into 5-10 cm seedlings. The soil mixture was 50% sand and 50% potting soil (Fafard 4M mix with Sphagnum peat moss and vermiculite). Velvet bean seedlings were 69 days old and the soybeans were 90 days old at the time of the micronutrient fertilizer applications.

Baseline gas exchange measurements and soil moisture data were taken over twelve days for both species. The four foliar treatments were applied and allowed to absorb and translocate into the foliage and vascular system for nine days. Then first deficit irrigation treatment was initiated by reducing soil moisture levels from 0.37 m^3/m^3 down to 0.19 m^3/m^3 over a five-day event. Gas exchange responses and soil moisture data was collected over nineteen days to measure plant responses during the sustained water stress event. Soil moisture was maintained at target levels by monitoring the daily soil moisture losses and replacing the average water loss with a measured volume of water daily.

At the end of the first water stress event all plants were watered up to field capacity again, and then moved into a greenhouse bay with high intensity, height-adjustable grow lights. All plants were adjusted to the light intensity for six days. The greenhouse light intensity was set at 500 $\mu mol/m^2/s^1$, and a 9 h photoperiod, and the temperature was set at 27 C. The second deficit irrigation treatment was initiated over five days after the plants had adapted to the greenhouse conditions. Volumetric soil moisture levels were reduced from an average of 0.31 m^3/m^3 (31%) down to 0.22 m^3/m^3 (22%), and plants could adapt to the new deficit irrigation treatment over five days. Gas exchange responses and soil moisture data were then collected over the next eight days. At the end of the second deficit irrigation event the foliar biomass was harvested to determine the overall leaf retention and plant growth responses to the study treatments.

2.3. Sprayer Description

The CO^2 backpack sprayer (R&D Sprayers, Opelousas, LA) had a six nozzle, 3.4 m boom. The spray pressure was 310 kPa, and the total spray volume was 701 l/ha. The nozzles were TeeJet XR 8003 VS which produce a droplet with a volume median diameter of 285 microns. Nozzles were spaced at 46 cm apart, and nozzle height above the plants was approximately 41 cm. One half of the boom was fitted with three nozzles that were fed with magnetized spray hoses. The other half of the boom was fitted with three nozzles that were fed with non-magnetized spray hoses.

The magnetized spray hoses had cylindrical neodymium magnets inserted into the three sections of hose located between the three nozzles. The magnets (grade N-52) were hollow allowing the spray solution to be magnetized as it passed through each magnetized section of the boom. Magnets were axially magnetized, i.e., magnet poles were at the ends of the magnets, and magnet size was 19 x 19 mm with a 6.4 mm inside diameter. There was a total of 36 magnets in the three hoses in the magnetized half of the boom. Plastic washers were placed between magnets to allow magnetic fields to surround each magnet and penetrate the spray solution at the end of each magnet (Image **1-2**). The magnetic field strength was 560 mT (0.56 Tesla) when measured on the end of one magnet that was at the end of a string of fourteen magnets inserted in a single hose section. Each of the six nozzles was adjusted and calibrated to deliver a flow rate of 18.3 ml/s.

Image 1: Photo of static, neodymium cylinder magnets with plastic washers inserted between each magnet.

Image 2: Photo of static, neodymium cylinder magnets inserted into the sprayer water hose.

2.4. Chelated Iron Fertilizer Description

The liquid fertilizer contained nitrogen, phosphorous, and potassium (NPK) plus micronutrients, a surfactant, and two rates of chelated iron fertilizer. The N-P-K fertilizer (Jack' Classic, JR Peters, Alantown PA) was mixed with water at 7.5 ml/l. The surfactant (Silwet L-77, PhytoTech Labs, Lenexa, KS) was mixed at 0.2% (v/v) for all four treatments. The chelated iron fertilizer (Iron & Soil Acidifer, Green Light, San Antonio TX) contains sulfur – 3.1%, copper – 0.12%, iron – 4.6%, and zinc – 0.12%, and was mixed at either 2.5 or 5% (v/v). The micro-nutrient fertilizer had a Fe concentration of 4.6% that was chelated with a trisodium salt of N-hydroethylenediaminetriacetic acid (HEDTA). The four spray solutions were two magnetic foliar applications with 2.5 or 5% iron fertilizer, and two non-magnetic applications with 2.5 or 5% iron fertilizer.

2.5. Physicochemical Water Measurements

Previous research has shown that electrical conductivity (EC), oxidation reduction potential (ORP), and pH are significant physicochemical water properties that are correlated with beneficial plant responses to abiotic stressors. A portable, multi-meter (Oakton PC 650 ORP/EC/pH meter-Oakton Instruments, Vernon Hills, IL, USA) was used to measure all three water properties. The water properties were measured in a separate lab study. The four spray solutions were mixed in small plastic containers and placed on a neodymium magnet, exposed to the South Pole field (grade N-52) and strength of 525 mT for 10 s. exposure time.

2.6. Plant Measurements

Plant and soil data collection included three measurement times for gas exchange sampling, two soil moisture readings, and a final foliar harvest to determine oven dry stem and foliar biomass. The three gas exchange readings included a baseline/initial measurement before the foliar application and during the first and second deficit irrigation event. The first and second gas exchange measurements started at 17 and 59 days after the foliar spray treatments, respectively. These measurements included photosynthesis (Pn - CO_2 µmol/m^2/s), stomatal conductance (g - mol/m^2/s), transpiration (E – H2O mmol/m^2/s), leaf temperature (Lt-C), vapor pressure deficit based on leaf temperature (vpdl), and internal CO_2 concentration (Ci). Instantaneous water use efficiency (IWUE) was estimated from the photosynthesis/transpiration ratio (mol CO_2 /m^2/s per mol H_2O/m^2/s) for regression analysis. A photosynthesis instrument (LICOR 6400 XT, LI-COR Environmental, Lincoln, NE) was used for all gas exchange readings. During the baseline and first water stress readings the 6400 XT environmental conditions were set for: PAR = 200 µmol/m^2/s^1, CO2 concentration = 400 mg/l, air flow rate = 300 µmol/s, and block temperature = 30 C across all treatments.

2.7. Soil Moisture Methods

Volumetric soil moisture (SM) readings were collected with data loggers and soil moisture and temperature sensors (ECH2O data logger and 5-TM soil sensors, METER Environmental, Pullman WA). During the second water stress test, however, soil moisture data was collected on a 24h basis, at 1h intervals, to estimate evapotranspiration rates for each treatment. Soil evapotranspiration rates (SETR) were estimated by converting volumetric soil moisture (m^3/m^3) into evapotranspiration rates (ml/h), using the average volume of soil in each pot (1,340 ml or 0.00134 m^3) and the average hourly moisture losses calculated from the 24 h data. All plants were watered daily, in the morning, so soil moisture was monitored daily to determine the overall water use/loss between watering cycles. Soil sensors were rotated between the legume species after each set of gas exchange measurements was completed.

2.8. Foliage Iron Content Study

A preliminary study was conducted with soybean plants to determine the foliar uptake rate of chelated iron fertilizer for eight treatments. This study was conducted in conjunction with the above-mentioned study to determine the effects of the sprayer system and micro-nutrient foliar treatments on mineral concentrations in young and mature foliage. This study involved two sprayer systems (magnetic and non-magnetic) and three chelated iron fertilizer rates (5, 10, and 15% Fe). All fertilizer treatments were applied at the same rates and methods mentioned above to maintain the same conditions as the first study. Two months after the soybean plants were treated with the iron fertilizer the foliage was sampled for eleven elements and percent dry leaf tissue matter. The elements were P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, B, and Mo. There were ten plants per treatment, and four leaves were collected from each plant. Leaf sampling included two mature, lower leaves that were fully formed at the time of fertilizer application, and two young, upper leaves that had developed in the two months after the application. The leaves were composited by treatment and leaf age, dried, and analyzed for micronutrient concentrations and percent dry matter tissue. Composited leaf samples were used in this pilot study to save on lab fees, but still give an average for 20 leaves collected per leaf type and foliar treatment. Leaves were analyzed for mineral concentrations using a standard forage analysis procedure at the Colorado State University Soil, Water, and Plant Testing laboratory.

2.9. Analysis

Data was analyzed as a repeated measure study with the same plants measured over time for the baseline and two deficit irrigation events (Table **1**). The first data collection occurred before the plants were treated to provide the baseline plant responses. A table for baseline and treatment means (Table **2**) is listed to compare plant responses after the second deficit irrigation event. The foliar gas exchange data was compiled with the water property data and the soil moisture data. Analyses of gas exchange and soil evapotranspiration data used the SAS JMP (SAS Institute Inc., Clary, NC) Restricted Maximum Likelihood model (REML). The REML model was restricted to two-way interaction terms to take advantage of hidden interactions. The JMP Standard Least Squares model was used to test treatment effects for foliar biomass. Analysis results were deemed to be significant if *p*-values were less than 0.05. Error bars in graphs represent the standard error of model values.

Multivariate and regression analyses were conducted to correlate any relationships between two physicochemical water properties, soil moisture and five physiological responses. Also, regression analysis was utilized to test the relationships between photosynthesis and leaf temperature for baseline data and both water stress events, by sprayer system. Regression analysis was also used to test the relationships between transpiration efficiency and soil moisture for both water stress events, by sprayer system. Oven dried foliar biomass for soybeans was analyzed after the second water stress event using the Tukey test to analyze plant growth trends between the sprayer types.

3. Results

The physicochemical water properties for the four foliar treatments were different for EC and ORP but not for pH (Table **1**). Magnetizing the micronutrient solution increased EC, but decreased the ORP, in negative terms, for the 2.5% Fe solution and increased ORP for the 5.0% Fe solution.

Multivariate analyses tested the correlations between eight continuous variables and three study treatments which were, 1) baseline plant responses, 2) magnetized foliar plant responses, and 3) non-magnetized foliar responses (Table **2**). Correlation analyses was tested across the chelated iron treatments and both legume species. The eight variables included five physiological plant responses (Pn, g, E, Ci, and vpdl), two water property covariates (EC and ORP), and soil moisture. The correlation table paired the eight variables together and listed the correlation strength and probability for each of the three study treatments. The advantage of the correlation table is that it summarizes and highlights all the significant correlations among the study variables and covariates in a single table so that the REML model could be built using only the significant covariates as model terms. For example, the correlation table shows that Pn, g, and E were correlated but had opposite signs when comparing the baseline responses with the magnetized application responses. In other words, Pn, g and E were positively correlated with SM for the baseline plant responses and non-magnetized micronutrient foliar treatments. The positive, or direct relationship, between physiological responses and soil moisture for the baseline and non-magnetized treatments follows the universally accepted pattern of increased physiological responses with increased SM up to soil field capacity. In contrast, the magnetized, micronutrient foliar treatments resulted in an insignificant, but trending toward a negative, or indirect, correlation between Pn, g and E and SM. In other words, the magnetized, chelated iron foliar treatments disassociated, or disconnected the universally accepted, direct relationship between Pn, g and E, and soil moisture.

Table 1: Average electrical conductivity, oxidation reduction potential (ORP), and pH for filtered tap water and four chelated iron solutions. The chelated iron solutions were diluted with filtered tap water to 2.5 and 5% (v/v) and measured for water properties. The second set of chelated iron solutions diluted to 2.5 and 5% were placed on a static magnet for 10 s then measured for three water properties.

Water Description	Electrical Conductivity (uS/cm)[a]	Oxidation Reduction Potential (mV)[a]	pH
Filtered tap water	140	-46	7.8
Chelated iron (2.5%) w/o magnetic field	7,201 A	-83.2 A	8.45 A
Chelated iron (2.5%) with magnetic field	8,943 B	-82.0 B	8.43 A
Chelated iron (5%) w/o magnetic field	14,555 C	-76.3 C	8.30 A
Chelated iron (5%) with magnetic field	16,343 D	-76.9 D	8.32 A

[a] Treatments not connected by the same letter are significantly different.

The correlation table also shows that Ci was positively correlated with Pn, g, E, SM, and EC, but was negatively correlated with ORP for baseline responses (Table **2**). In contrast, Ci was not correlated with Pn, vpdl, SM, EC, and ORP for the magnetized foliar treatments. In addition, vpdl was negatively correlated with Pn, g, Ci and EC and positively correlated with ORP for baseline responses. In contrast, vpdl was positively correlated with EC and SM but negatively correlated with ORP for the magnetized foliar treatments. These findings show that the magnetized foliar treatments either disassociated several variables from the Ci parameter or resulted in an opposite response in vpdl when comparing the baseline results with the magnetized treatments.

The final REML models for Pn, g, and E show that each model that included the date term showed a significant effect between the baseline and deficit irrigation measurements for all three responses (Table **3**). Also, the three REML models show that all four study factors were significant as either primary or interaction terms in the models. The models reveal that plant responses during baseline measurements were generally different from the magnetic sprayer responses when measured during deficit irrigation. Each of the models also had different, two-way interaction terms (Table **3**).

The REML model was also used to predict the responses for three plant and two soil variables, using the baseline and second deficit irrigation data (Figs. **1-5**). Baseline responses for Pn showed a general trend to be equivalent with the second deficit irrigation responses when compared to each legume species (Fig. **1**). However, baseline responses for g were lower than the second deficit irrigation response for velvet bean seedlings and for soybean seedlings treated with chelated Fe (2.5%) (Fig. **2**). Foliar application with the magnetic sprayer and chelated iron increased g, especially for velvet bean seedlings. Baseline responses for WUE were higher than the second deficit irrigation responses (Fig. **3**). Similarly, baseline SM was higher than all the treatments monitored during the second deficit irrigation, and SM averaged 19 and 24% for the magnetic and non-magnetic applications during the second irrigation treatment (Fig. **4**). Finally, the soil evapotranspiration rates were equivalent between both legume species during the second deficit irrigation treatment (Fig. **5**).

Regression analysis was used to discern any differences between baseline and foliar treatment effects on Pn and Lt responses (Fig. **6**). It is widely accepted that there is a negative relationship between Pn the Lt. This relationship was confirmed with the baseline analysis that showed a negative relationship between Pn and Lt for soybean (p-value = 0.004) and velvet bean (p-value = <0.0001) (Fig. **6A**). Analyses of the second deficit irrigation data, however, reveal either no relationship or a negative relationship between Pn and Lt, depending on the sprayer type and rate of chelated Fe (Fig. **6B-C**). Only two out of the eight regression tests showed a negative relationship between Pn and Lt, which paralleled the baseline results. The other six regression tests showed no relationship between Pn and Lt. In other words, 75% of the combined chelated Fe and magnetized sprayer treatments resulted in an unlinked disassociation between photosynthesis and leaf temperature during the second deficit irrigation trial.

Table 2: Correlation strength and correlation *p*-values for electrical conductivity, oxidation reduction potential, soil moisture, photosynthesis, stomatal conductance, internal leaf CO2 concentration, transpiration, and vapor pressure deficit. The magnetic and non-magnetic sprayer treatments were correlated across both the first, and second deficit irrigation events, and across the chelated iron treatments and legume species. Multi-colored numbers in the magnetic sprayer column indicate either a change in sign (+ or -) or change in *p*-value significance when compared to baseline or non-magnetic sprayer values.

Correlation Variables		**Baseline**		**Magnetic Sprayer System**		**Non-Magnetic Sprayer System**	
Variable	**by Variable**	**Correl Strength**	**Correl *p*-Value**	**Correl Strength**	**Correl *p*-Value**	**Correl Strength**	**Correl *p*-Value**
EC	Soil	0.2175	0.0237*	0.1507	0.0934	-0.1768	0.0671
ORP	Soil	-0.23	0.0167*	-0.1507	0.0934	-0.1768	0.0671
ORP	EC	-0.8624	<.0001*	-1	<.0001*	1	<.0001*
Photosyn	Soil	0.2782	0.0036*	-0.0748	0.407	0.3345	0.0004*
Photosyn	EC	0.1222	0.2076	-0.3797	<.0001*	0.078	0.4224
Photosyn	ORP	-0.2453	0.0105*	0.3797	<.0001*	0.078	0.4224
Stom	Soil	0.3539	0.0002*	-0.0764	0.3968	0.5442	<.0001*
Stom	EC	0.2255	0.0189*	-0.1778	0.0472*	0.0004	0.9965
Stom	ORP	-0.3341	0.0004*	0.1778	0.0472*	0.0004	0.9965
Stom	Photosyn	0.8415	<.0001*	0.7238	<.0001*	0.7239	<.0001*
Ci	Soil	0.3877	<.0001*	-0.032	0.7235	-0.1251	0.197
Ci	EC	0.2855	0.0027*	-0.073	0.4187	0.0308	0.7517
Ci	ORP	-0.2853	0.0028*	0.073	0.4187	0.0308	0.7517
Ci	Photosyn	0.5595	<.0001*	0.1194	0.1849	-0.1971	0.0409*
Ci	Stom	0.6668	<.0001*	0.3134	0.0004*	-0.1616	0.0948
Transp	Soil	0.3604	0.0001*	0.0491	0.5867	0.5682	<.0001*
Transp	EC	0.0402	0.6795	-0.1087	0.2274	-0.0312	0.7487
Transp	ORP	-0.1342	0.1662	0.1087	0.2274	-0.0312	0.7487
Transp	Photosyn	0.7913	<.0001*	0.6819	<.0001*	0.724	<.0001*
Transp	Stom	0.8549	<.0001*	0.9004	<.0001*	0.968	<.0001*
Transp	Ci	0.6833	<.0001*	0.3034	0.0006*	-0.1596	0.099
VpdL	Soil	-0.0904	0.3521	0.3401	0.0001*	0.1665	0.0851
VpdL	EC	-0.4893	<.0001*	0.3111	0.0004*	-0.0652	0.5026
VpdL	ORP	0.4235	<.0001*	-0.3111	0.0004*	-0.0652	0.5026
VpdL	Photosyn	-0.4499	<.0001*	-0.2701	0.0023*	0.0038	0.9687
VpdL	Stom	-0.534	<.0001*	-0.4325	<.0001*	0.0598	0.5389
VpdL	Ci	-0.333	0.0004*	-0.1386	0.1232	0.0424	0.6628
VpdL	Transp	-0.1388	0.1519	-0.0513	0.57	0.2895	0.0024*

EC = Electrical conductivity, ORP = Oxidation Reduction Potential, Stom = stomatal conductance, Photosyn = photosynthesis, Soil = soil moisture, Transp = transpiration, Ci = internal CO_2 conc., Vpdl = vapor pressure deficit calculated from leaf temperature.

Table 3: The *p*-values for the final REML model are listed for each study factor and the significant two-way interaction terms for photosynthesis, transpiration, and stomatal conductance plant responses. The model includes the three measurement dates to test for longitudinal effects over time.

Photosynthesis	*p*-Value	Transpiration	*p*-Values	Stomatal Conductance	*p*-Values
Species	0.0059	Species	0.0251	Species	0.0131
Sprayer	0.3397	Sprayer	0.2025	Sprayer	0.9103
Fertilizer	0.2935	Fertilizer	0.6709	Fertilizer	0.3174
Date	<.0001	Date	<.0001	Date	<.0001
Soil moist	0.0025	Soil moist	<.0001	Soil moist	0.0443
Species* Fertilizer	0.0004	Sprayer*Date	0.0016	Spec*Sprayer	0.0099
Species*Date	<.0001	Sprayer*Soil moist	0.0642	Species*Fert	0.0003
Sprayer*Fert	<.0001	na	na	Sprayer*Fert	0.0031
Sprayer*Date	0.0014	na	na	Sprayer*Date	<.0001
				Date*Soil	0.0043

Date = three measurement dates.

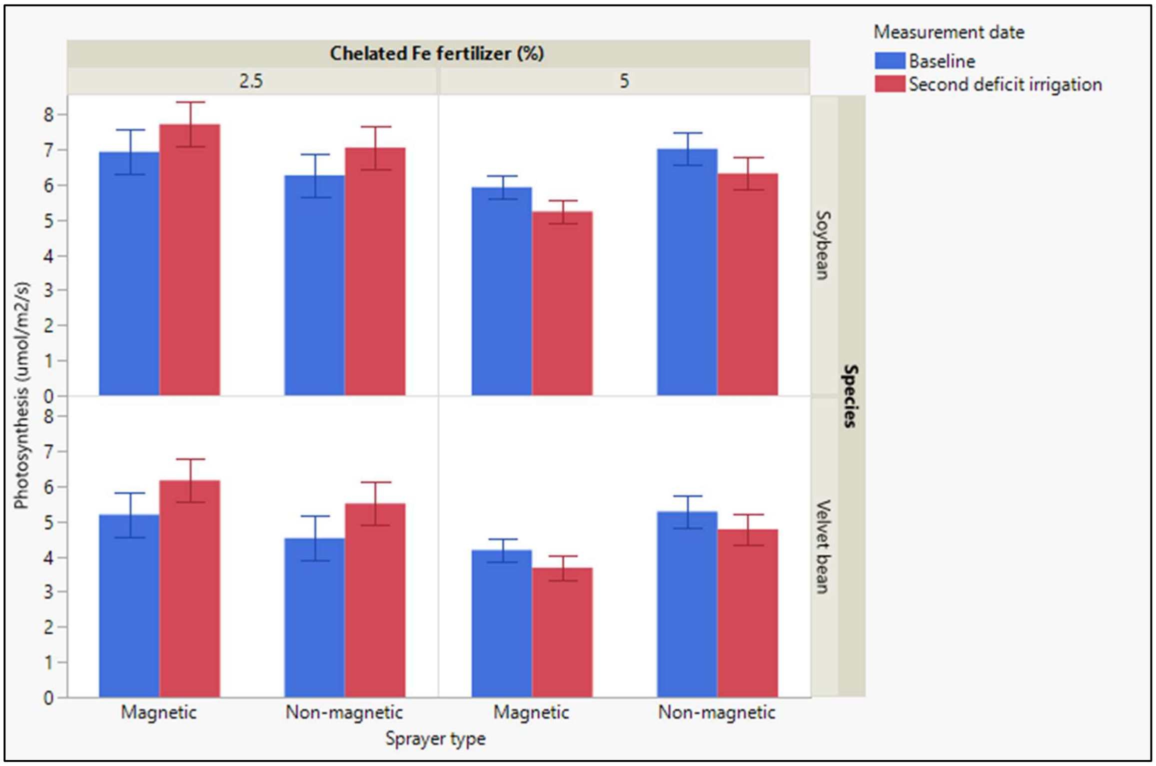

Figure 1: Photosynthesis rates, based on the REML model, are shown by sprayer type (lower x-axis) legume species (right y-axis) and percent chelated iron (upper x-axis) for the baseline and second deficit irrigation events (legend).

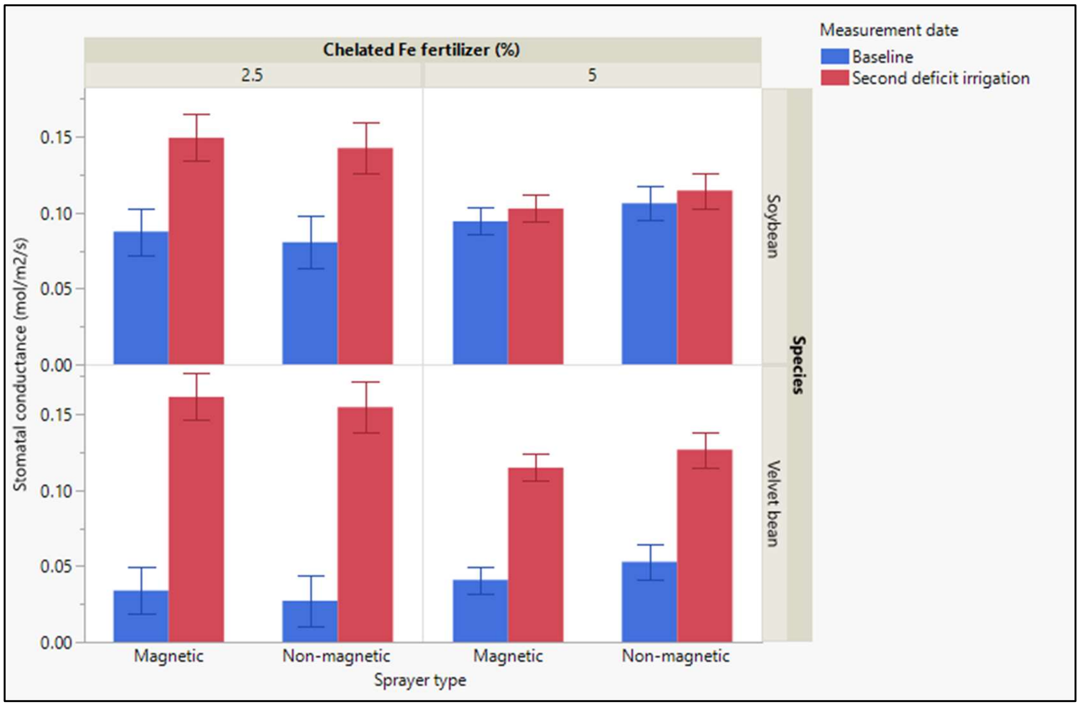

Figure 2: Stomatal conductance rates, based on the REML model, are shown by sprayer type (lower x-axis) legume species (right y-axis) and percent chelated iron (upper x-axis) for the baseline and second deficit irrigation events (legend).

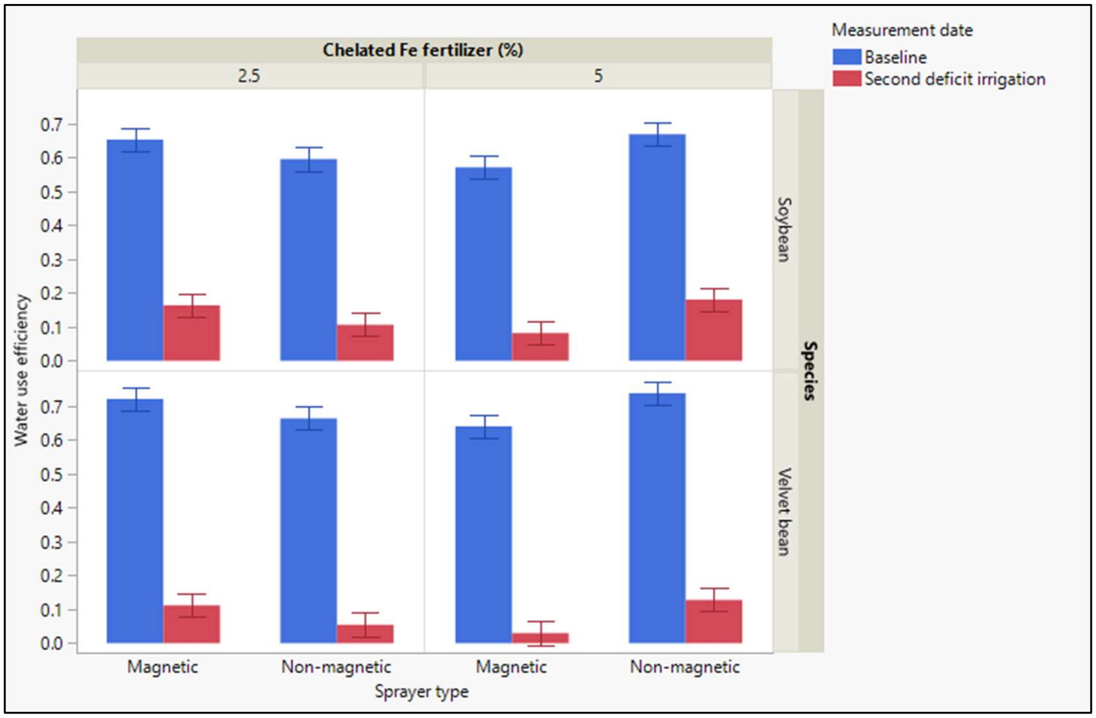

Figure 3: Instantaneous water use efficiency (IWUE) rates, based on the REML model, are shown by sprayer type (lower x-axis) legume species (right y-axis) and percent chelated iron (upper x-axis) for the baseline and second deficit irrigation events (legend).

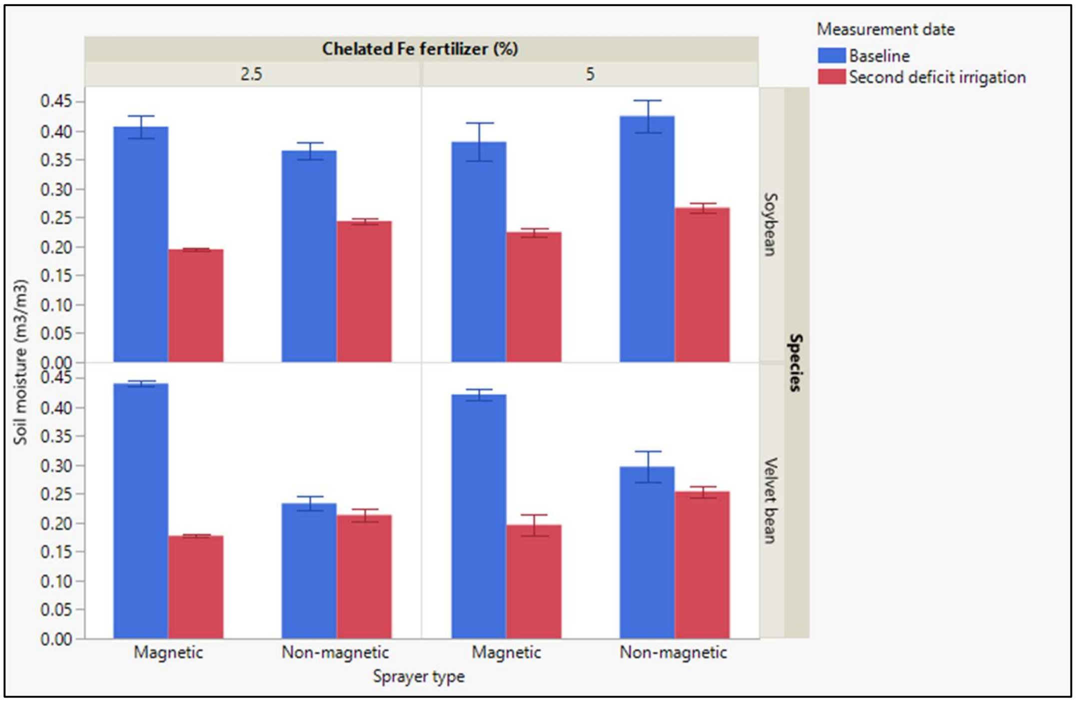

Figure 4: Soil moisture levels, based on the REML model, are shown by sprayer type (lower x-axis) legume species (right y-axis) and percent chelated iron (upper x-axis) for the baseline and second deficit irrigation events (legend).

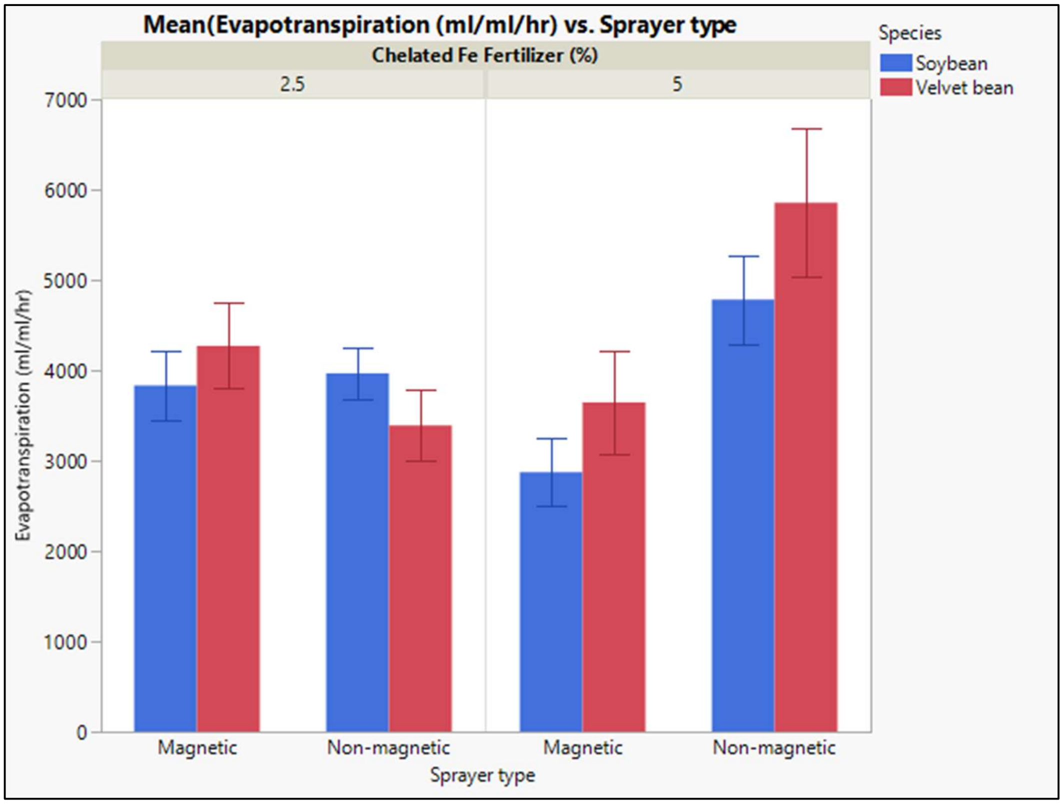

Figure 5: Soil evapotranspiration rates, based on the REML model, are shown for second deficit irrigation for the sprayer type (lower x-axis) and percent chelated iron (upper x-axis) and legume species (legend).

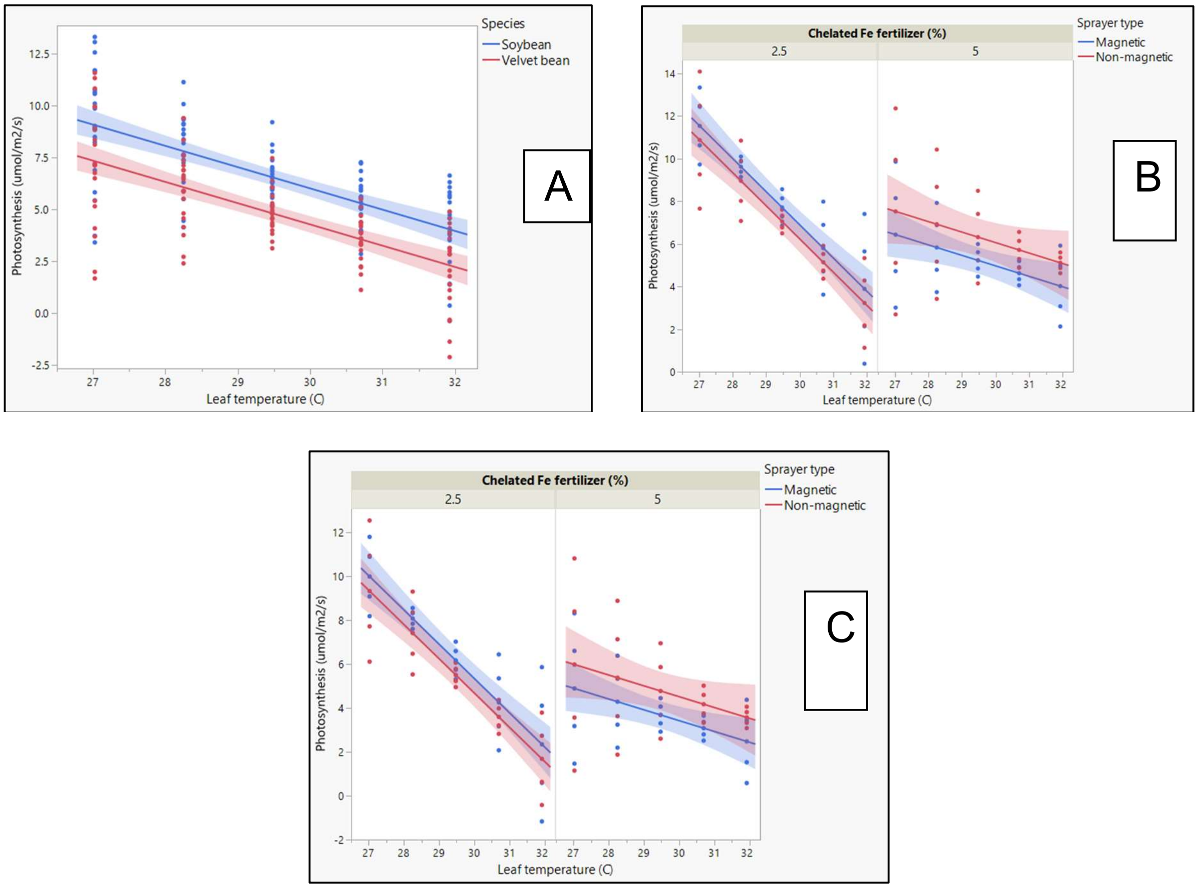

Figure 6: Regression relationship between photosynthesis and leaf temperature during baseline measurements for soybean (p-value = 0.0046) and velvet bean (p-value = <0.0001) (**A**). The second deficit irrigation relationship between Pn and Lt is graphed for soybean (**B**) and velvet bean (**C**). The soybean *p*-value for magnetic and non-magnetic chelated Fe (2.5%) was 0.7809 and 0.0121, respectively (**B**). The soybean *p*-value for magnetic and non-magnetic chelated Fe (5%) was 0.0067 and 0.0802, respectively (**B**). The velvet bean *p*-value for magnetic and non-magnetic chelated Fe (2.5%) was 0.1800 and 0.08903, respectively (**C**). The velvet bean *p*-value for magnetic and non-magnetic chelated Fe (5%) was 0.4569 and 0.1171, respectively (**C**).

During the second deficit irrigation treatment soil moisture was reduced by 54 and 25% for the magnetic and non-magnetic treatments as averaged across both legume species, respectively, when compared to baseline measurements. Despite the reduction in soil moisture foliar transpiration increased during this stress event due to high light intensity and temperatures. Transpiration was correlated with all six variables listed in Table **3**.

Vapor pressure deficit is a driver for transpiration due to the difference between the actual vapor pressure and the saturation vapor pressure at a set temperature. Given sufficient soil moisture there is typically a positive relationship between vpdl and transpiration. During the second deficit irrigation event the correlation between transpiration and vpdl was 0.0466 and 0.3986 for the non-magnetic and magnetic applications, respectively. Despite the 54% reduction in soil moisture, transpiration increased 46% for magnetic applications. In contrast, soil moisture decreased 25% for the non-magnetic treatment and yet transpiration only increased 35% during the second water stress. Increased transpiration following a 25-54% reduction in soil moisture is counter intuitive unless the plants were still adapting to the higher temperature and light intensity during the second deficit irrigation event. These results do not answer the question of how long transpiration rates can be sustained before low soil moisture levels eventually cause leaf wilting and desiccation.

Soil evapotranspiration rates during the second water stress event were affected by sprayer type and chelated iron concentration (Table **2**). When iron was added at 2.5% there was no effect on soil evapotranspiration rates. However, when iron was added at 5% the evapotranspiration rates were 4.4 and 6.7 ml/h for the magnetic and non-magnetic treatments, respectively. The 34% reduction in the soil evapotranspiration rate for the magnetic application, relative to the non-magnetic application, does not support the increased transpiration rates for the magnetic treatments mentioned in the last paragraph. The soil surface in each pot was not covered to reduce evaporation loss. Thus, soil moisture losses were a combination of transpiration and soil surface evaporation losses. The increased transpiration rates mentioned in the previous paragraph appear to conflict with the lower evapotranspiration rates for the magnetic application. This anomaly may be due to inaccurate measurements, measurement timing in relation to the plant adaption process, or a substantial shift in the plant's natural defense activities against abiotic injury from excessive light and temperature conditions.

Analysis of foliar biomass showed that only species influenced plant growth rates. These results may be partially explained by the smaller sample size (n= 8 or 10) due to no interactions among species with other study factors. Six plants died during the second water stress, so mortality reduced the sample size even further. In addition, the insect scale infestation on the velvet bean resulted in unintentional secondary stress that reduced the growth rates for this species. The oven dry foliar biomass with seed pods included for soybean plants was 9.8 and 11.4 g for the non-magnetic and magnetic treatments (Fig. **7**). The 16% difference in foliage biomass was not significant, but this difference indicates that plant growth could be increased for the magnetic treatments if the sample size were larger.

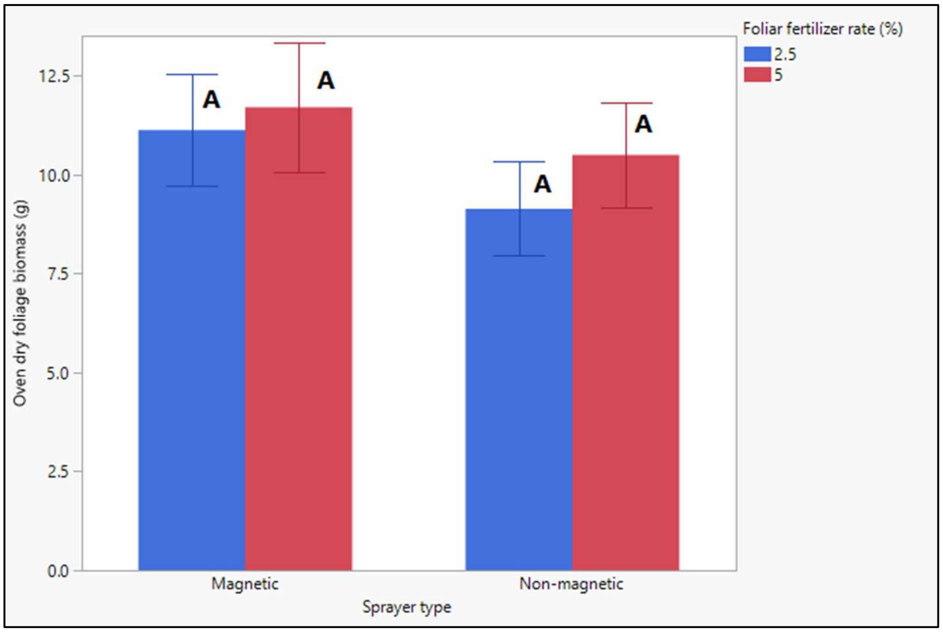

Figure 7: Average oven dry biomass for foliage, stems, and fruit for soybean plants at the end of second deficit irrigation event. (p-value = 0.2764)

The preliminary soybean study, involving micronutrient concentrations in leaf tissue, resulted in foliar iron concentrations of 171 and 1332 mg/kg, in the old leaves, for the 0 and 15% chelated iron treatments, respectively, two months after application (Fig. **8**). In other words, there was a seven-fold increase in foliar iron concentrations with increasing chelated iron rates. The stair step pattern in iron concentrations in the older leaves shows that chelated iron is readily taken up by the foliage but is not readily transported to newer leaves up to 60 days after treatment. The lack of evidence for transported Fe into younger leaves contrasts with a study by Burton *et al*. [37], which found that chelated heme proteins were safely transported from sources to sinks in soybeans, depending on plant developmental needs. The second gas exchange measurements collected in the first study were taken from the uppermost younger leaves at about 60 days after treatment. Improvement in the gas exchange rates due to the magnetized fertilizer is indirect evidence that at least partial redistribution of heme proteins into younger leaves may have occurred as the older leaves desiccated and dropped off the plants. It remains to be seen if heme proteins are redistributed during water stress periods, and whether their relocation is sufficient to provide a concomitant decrease in photoinhibition responses.

The preliminary study also analyzed mature and young soybean leaves for B, Cu, Mo, Zn, Ca, K, Mg, P, and percent dry tissue (Figs. **9–11**). In general, the micronutrient concentrations did not increase in a stair step pattern as the chelated iron concentrations increased for either the magnetic or non-magnetic treatments. The percent dry tissue does appear to decrease for the young leaves as the chelated iron concentration increases for the magnetic treatment, except for the highest rate of 15% Fe (Fig. **11**).

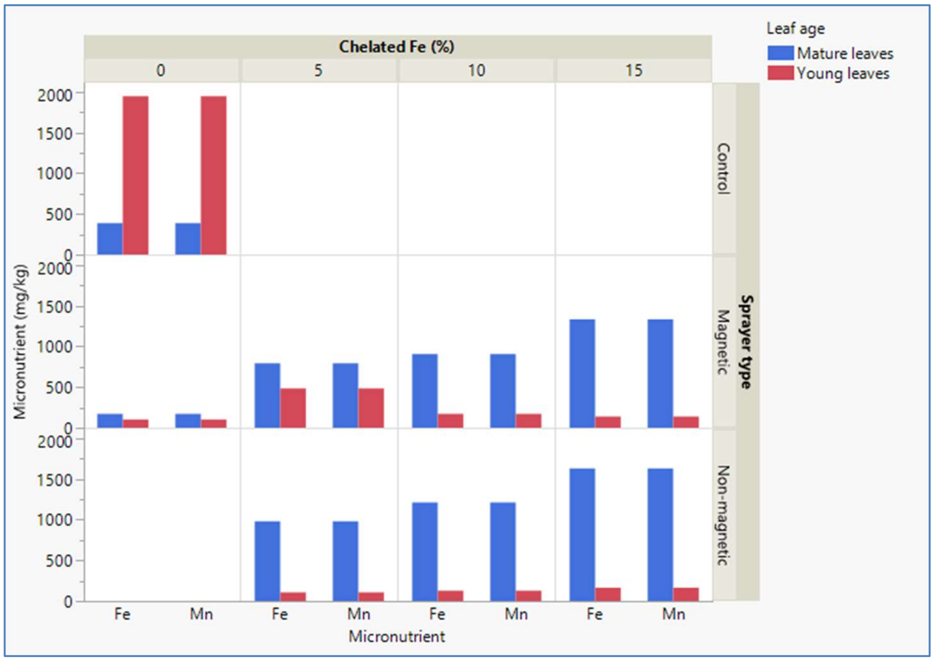

Figure 8: Micronutrient concentration in foliage in the preliminary study at two months after the foliage applications. Concentrations for Fe, and Mn (x-axis) by leaf age (legend), sprayer type (right y-axis) and chelated iron fertilizer rates (upper x-axis).

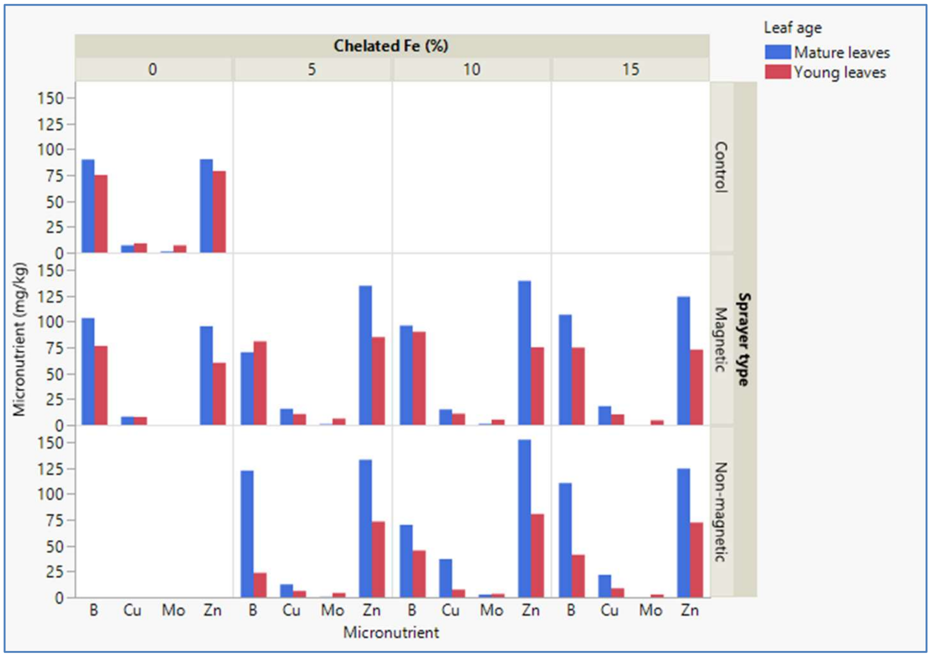

Figure 9: Micronutrient concentration in foliage in the preliminary study at two months after the foliage applications. Concentrations for B, Cu, Mo, and Zn (x-axis) by leaf age (legend), sprayer type (right y-axis) and chelated iron fertilizer rates (upper x-axis).

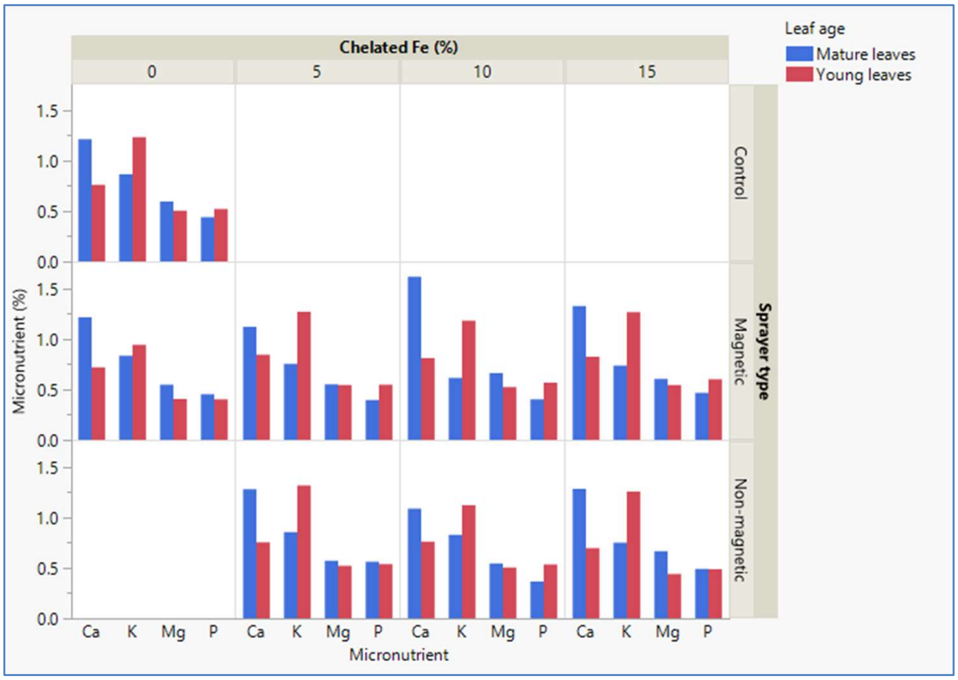

Figure 10: Micronutrient concentration in foliage in the preliminary study at two months after the foliage applications. Percent concentration for Ca, K and Mg (x-axis) by leaf age (legend), sprayer type (right y-axis) and chelated iron fertilizer rates (upper x-axis).

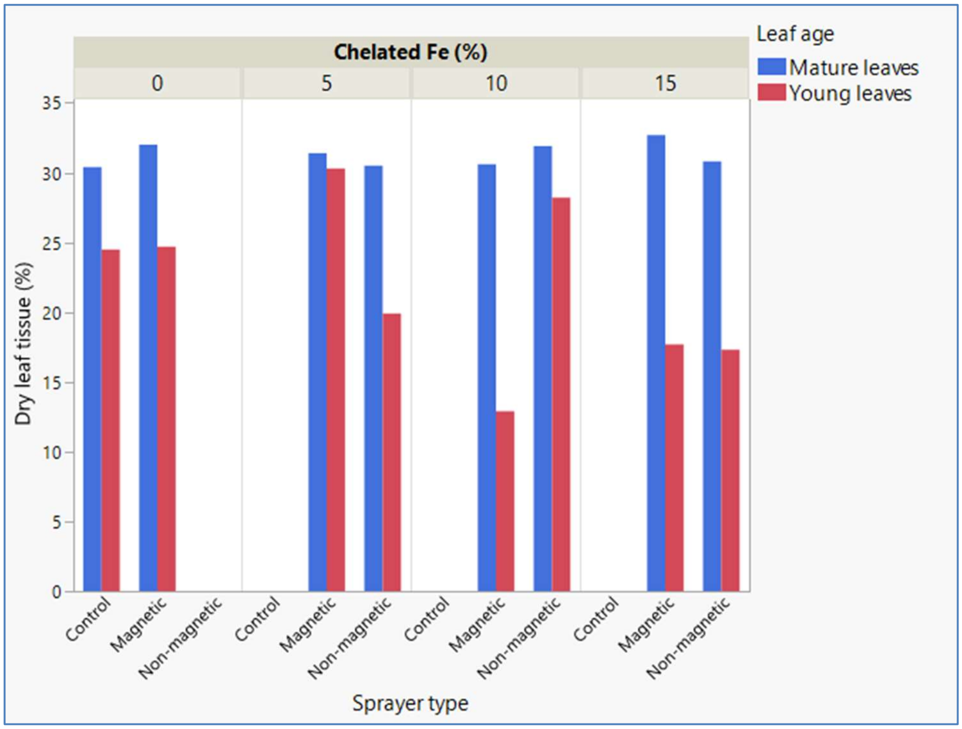

Figure 11: Percent dry leaf tissue in foliage in the preliminary study at two months after the foliage applications. Dry leaf tissue by and leaf age (legend), sprayer type (lower x-axis), and percent chelated iron (upper x-axis).

As the foliar treatments increased in electrical conductivity and increased in the absolute value of lORPl the plant responses for Pn, IWUE, and e also tended to increase for the chelated Fe at 2.5% (Figs. **12–14**). Photosynthesis increased with increasing EC and ORP when 2.5% chelated Fe was applied but remained flat or declined when 5.0% chelated Fe was applied (Fig. **12**). Water use efficiency increased for velvet bean, or remained flat for soybean, when 2.5% chelated Fe was applied but declined when 5.0% chelated Fe was applied (Fig. **13**). Transpiration increased for velvet bean, or remained flat for soybean, when 2.5% chelated Fe was applied but increased when 5.0% chelated Fe was applied (Fig. **14**).

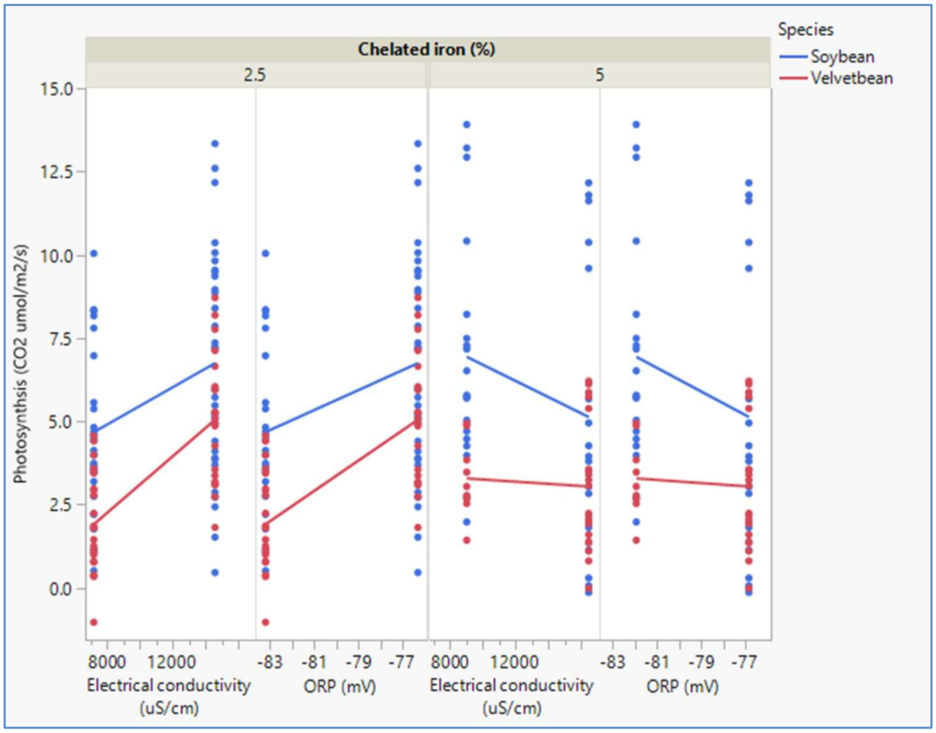

Figure 12: Relationship between photosynthesis and the electrical conductance and oxidation reduction potential of the spray solutions, for percent chelated iron (upper x-axis) and legume species (legend).

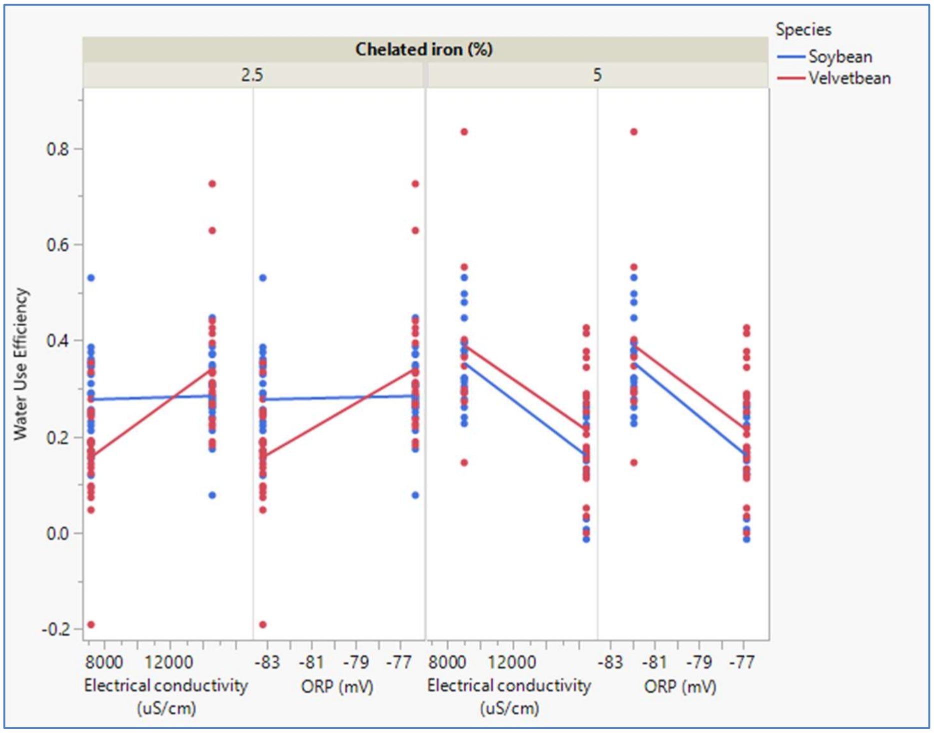

Figure 13: Relationship between IWUE and the electrical conductance and oxidation reduction potential of the spray solutions, for percent chelated iron (upper x-axis) and legume species (legend).

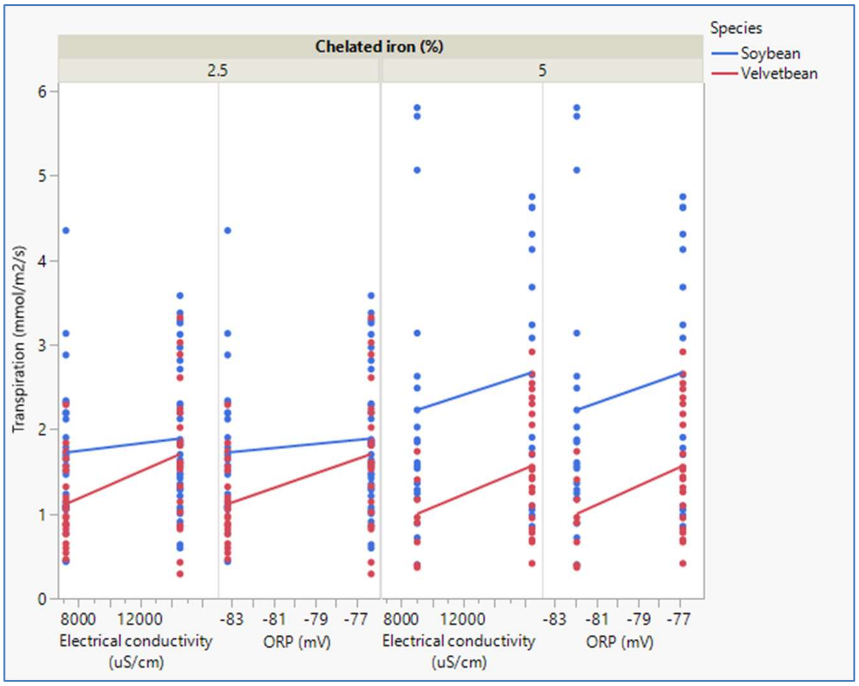

Figure 14: Relationship between transpiration and the electrical conductance and oxidation reduction potential of the spray solutions, for percent chelated iron (upper x-axis) and legume species (legend).

4. Discussion

Plant measurements taken before and after the foliar applications were analyzed in this study to minimize the inherent variation in physiology and growth among individual plants, as compared to a study with separate control plants. Repeated measures analysis revealed that foliar treatments affected the physiological responses for both legumes. In other words, plant measurement parameters were different when analyzed for their baseline (pretreatment responses) and the deficit irrigation responses (post-treatment responses) which indicates significant treatment effects. This study consisted of five factors that included a total of eleven levels (2 x 2 x 2 x 3 x 2) which were crossed among these factors. Only the main effects and relevant two-way interactions were reported in Table **3**. Interaction analysis was only reported for the most relevant results to minimize misunderstanding of all the inter-relationships and to summarize the effects of the main study factors. In general, the results from this study revealed that the plant responses validated the study hypotheses.

Selim and El-Nady [16] found that the calculated water use efficiency increased 118% for tomato plants that were under a deficit irrigation schedule and treated with magnetized seeds plus magnetized irrigation water when compared with non-magnetized seeds and non-magnetized irrigation. In comparison, this study had a 263% increase in instantaneous WUE when compared to the control response for a single application of magnetized iron fertilizer (2.5% Fe). The Selim and El-Nady study also found that dry biomass increased 123, 17, and 38% when soil moisture was 80, 60, and 40% of field capacity for the magnetized seeds plus magnetized irrigation treatment when compared to control plant biomass when irrigated for 70 days [16]. Another magnetized seed and magnetized irrigation water study increased oven dry foliar biomass an average of 136% for four species that were watered for two months [38]. In contrast, this study included a single, foliar application of a magnetized, micronutrient treatment which resulted in a 16% increase in aboveground, dry biomass. The foliar treated soybean plants were also water stressed by reducing the soil moisture down to 19% for 24 days. These three studies involved different sets of treatments with magnetized seeds, magnetized irrigation watering schedules, or using a single magnetized foliar treatment with surprising comparable biomass results for the two irrigation studies [16, 38]. This study resulted in a much smaller increase in oven dried foliar biomass due to the single foliar treatment. However, all three studies indicate that magnetized irrigation or foliar treatments generally increase biomass even under deficit irrigation treatments. Our study found that oven dried soybean foliar and pod biomass for the magnetized application was 16% higher, though not statistically significant, when compared to the non-magnetic fertilizer treatment when soil moisture averaged 19% during 24 days of deficit irrigation. Although analysis revealed no increase in growth for the magnetic applications at the whole plant level, this is more likely the cause of too small a sample size than no magnetized fertilizer effect on legume growth.

In concert with the plant growth responses, three primary physiological responses also increased after the magnetized fertilizer applications when plants were subjected to the second water stress. Stomatal conductance, transpiration, and internal carbon dioxide also increased by 31, 46, and 24%, respectively for the magnetized fertilizer applications when compared to their baseline measurements. Field studies involving soybean crops under water stress revealed that photosynthesis, stomatal conductance, and yield all decreased, as widely accepted responses to drought conditions [39–41]. In contrast, our study found that g, E, and Ci increased under water stress conditions. Correlation analysis shows that the magnetized foliar treatments disassociated the relationship between Pn, g, and E with SM. This indicates that soil moisture levels had minimal to no effect on these gas exchange parameters when treated with magnetized fertilizer, i.e., they were minimally or not regulated by soil moisture during water stress. In comparison, the three gas exchange parameters remained positively correlated with soil moisture levels for the non-magnetized treatments. The lack of correlation between Pn, g, and E with SM may indicate that other environmental factors became more important than soil moisture levels. The lack of correlation for the magnetized foliar treatments may also indicate that plant defense pathways such as photo-inhibition were reduced or redirected. If this hypothesis could be further validated, then such foliar treatments could be used to redirect plant resources from plant defense pathways to plant growth pathways.

Analysis of transpiration, soil evapotranspiration and soil moisture reveal a mixed set of results. The magnetized fertilizer applications had significantly lower soil moisture levels than the non-magnetic applications during the second water stress. These results agree with the increased transpiration levels for the magnetic treatments. However, the soil evapotranspiration results show that the magnetized treatment with 5% chelated iron had 34% less soil moisture loss on an hourly basis than the same non-magnetic treatment. Soil moisture losses in this study could be attributed to either soil surface evaporation or plant transpiration rates, and there is no way of discerning which factor was the primary cause for soil moisture losses.

Oxidation reduction potential measures the ability of a molecule or chemical to exchange electrons with another molecule. Oxidation is the loss of electrons and has a positive ORP value, and reduction is a gain of electrons with a negative ORP [42]. Oxidation reduction potential is measured as a single voltage in millivolts (mV). The ability of water to exchange protons is measured as $-\log_{10}$ (H+), or pH. The negative ORP values and > 7 pH values for the foliar treatments show that the solutions can receive both electrons and protons. The electrical conductivity of water is dependent on the mineral ion concentration and the concentration of hexagonal water rings. The effects of the chelated Fe and magnetic fields on electrical conductivity are listed in Table **1**. The addition of the magnetic field increased the solution conductivity for both Fe concentrations. The delocalized electrons in the hexagonal water rings conduct electricity and therefore are an indirect measurement of the concentration of structured water in a solution [43–44]. As the foliar treatments increased in electrical conductivity and there was a general increase in Pn, IWUE, and e for chelated Fe (2.5%). This is indirect evidence that as electrical conductivity increased by magnetic field exposure, the structured water ratio also increased which resulted in enhanced physiological responses to the foliar treatments. In summary, the putative effects of the structured water in the foliar treatments improved drought tolerance for both legumes in this study.

5. Conclusions

This study shows that a single spray application to the foliage can significantly alter the foliar physiology of legumes and enhance drought tolerance under deficit irrigation conditions. The combination of chelated iron spray solutions applied with a magnetized sprayer reversed or switched several physiological responses to low soil moisture allowing the plants to conserve their resources for plant growth. The ability of the foliar treatments to disassociate photosynthesis from leaf temperature implies that photoinhibition was reduced thereby reducing plant injury to sustained water stress conditions. Finally, this study shows that at least two physicochemical water properties affected the foliar physiology of the legumes. The physicochemical water properties were altered by combining chelated iron and application with a magnetized sprayer, which in turn enhanced the drought tolerance of the legumes. The ability of a single foliar application with enhanced water properties to dissociate physiological relationships highlights the importance of water quality and physicochemical properties on long term plant responses to abiotic stressors.

Bioenergetics and quantum biophysics may offer possible explanations why magnetized water treatments may disassociate predictable plant physiological responses during water stress conditions. Magnetized water has been sufficiently energized to generate water containing a fraction of structured water [25, 29]. Structured water contains hexagonal rings of water molecules with high-grade energy and low entropy properties [29, 43-44]. Cell and higher levels of organization have increased coherence and energy levels due to increased levels of structured water. The exchange of electrons and protons due to the delocalized quantum vortices associated with the hexagonal water rings provide several quantum mechanisms to increase cell efficiency and energy levels [43–45, 51]. These mechanisms include 1) redox potential generated from biological, or structured water that coat all organelles and cell membranes [43, 46], 2) single electron combustion of O_2 into water [44, 47-49], and 3) proton (H+) exchange or transfer [50-51]. The stored energy potential in structured water that coats membranes may reach as high as – 200 mV, depending on the depth of the redox pile [43, 45]. Single electron combustion of O_2 is possible under normal physiological conditions due to high energy properties of structured water. Approximately 180 kcal/mole is released when O2 is completely reduced to two water molecules, which is possible due to electron transfer from delocalized electron vortices in hexagonal water rings [44, 46-48]. Proton availability and flux rates are both mediated by the concentration of hexagonal water rings, which in turn recycles ADP back into ATP [50-51]. In summary, any increase in structured water would also likely enhance the overall metabolic efficiency, coherence and signaling, as well as improve cell energy levels in crop plants [52]. This study offers evidence that foliar treatments that may slightly improve structured water levels within the foliage also appear to disassociate the expected plant defense/physiological responses to water stress thereby alleviating the normal tradeoffs between resources allocated to plant defenses and plant growth.

Structured water properties can be indirectly measured by using a multi-meter to measure physicochemical water properties such as oxidation reduction potential, electrical conductivity, and pH [44, 53-54]. These physicochemical water properties should be included in magnetized irrigation water studies to relate these properties to the plant or crop responses to the irrigation treatments. The interactions among water properties, bioenergetics, plant metabolism, and crop stress physiology need to be further investigated in order to improve the quality of irrigation water. Recent studies involving magnetized irrigation water strongly suggest that physicochemical water properties are a critical component of irrigation water quality parameters for improving drought tolerance or increasing disease resistance in crops.

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