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Application of a Structured Water Generator for Crop Irrigation: Structured Water, Drought Tolerance, and Alteration of Plant Defense Mechanisms to Abiotic Stressors

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Abstract:

A greenhouse study was conducted to enhance drought tolerance in velvet bean plants (*Mucuns pruriens*) using structured irrigation water. The study combined magnetized seed treatments with watering plants with structured water treatments. A closed-loop, water system was custom-built to generate the structured irrigation water. The custom water generator utilized two energy fields (magnetic and ultra-violet radiation) to generate the structured water. The objectives of the study were to: 1) determine the effects of a magnetized seed treatment on velvet bean plants, 2) determine the effects of magnetized water treatments on velvet bean plants, 3) determine the effects of water treated with a hydroxylated water generator on velvet bean plants, 4) determine the effects of three soil moisture levels on velvet bean plants. The plant responses included: 1) foliage gas exchange rates 2) soil moisture, 3) cumulative water volume for each plant, 4) plant water use efficiency, and 5) oven-dry foliage biomass. The foliage gas exchange responses showed that the magnetized seed and structured water treatments disassociated the relationships between photosynthesis, stomatal conductance, transpiration and internal carbon dioxide rates from soil moisture and leaf temperature. The optimal, combined magnetized seed and structured water treatments increased water savings from 32 to 52% over the unstructured water treatments, under the low soil moisture level. The maximum plant water use efficiency was 2.81, which occurred with a structured water treatment under the high soil moisture level. There was a 6.8 % decrease in oven-dry foliage biomass for the optimal magnetized seed and structured water treatment when compared with the control treatment. However, the tradeoff in reduced biomass was compensated with a 41% savings in water usage, 25% reduction in Pn, 34% reduction in stomatal conductance, and a 7% reduction in internal carbon dioxide under the low soil moisture level for the optimal magnetized seed and structured water treatment. The combined seed and water treatments fundamentally alter drought adaptation plant responses to water stress conditions which resulted in a significant reduction in irrigation water usage. The interactions between magnetized seed treatments and structured water treatments on plant stress physiology need to be further investigated to confirm these water conservation findings. Structured water generators should be evaluated for physicochemical water properties and stability of water in soil and plant matrices.

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1. INTRODUCTION

The accelerated use of crop irrigation, landscape and residential lawn irrigation, and hydroponic greenhouses is straining the already limited supply of available fresh water for these non-drinking water uses. Conserving water supplies for irrigation uses has primarily focused on improving irrigation technology using precision, on-demand watering systems and developing drought tolerant crop varieties. Research on the properties of irrigation water has been largely ignored, except for a scattering of obscure studies that have not gained wide recognition [1-6]. These studies show that water properties can be readily manipulated and can conserve irrigation water usage by up to 10 to 20% [7-12]. New research initiatives are needed to broaden irrigation and hydroponic water research to include the interactions among water properties, plant stress physiology, crop health, drought tolerance and agricultural water usage.

The physicochemical properties of irrigation water can be manipulated by several energy-based treatments [13]. Decades of research have shown that bulk water exposed to energy sources that strengthen or shorten the hydrogen bonds in water molecules such as infrared radiation, magnetic fields, sonic waves, mechanical vibrations, or low temperatures will add structure to water [14-20]. As water is exposed to low temperatures, or a range of energy sources, the hydrogen bonds become shorter, and the bond angles widen from 104º to 109.5º. This enables a percentage of the water molecules to form a tetrahedral design resulting in five (pentagonal) and six (hexagonal) molecular rings [18, 20-28]. As the hexagonal rings form, the water becomes more structured with greater stability and less enthalpy or bonding energy [20-28].

Structured water is a semi-crystalline form of water with a higher viscosity, lower density, and surface tension [21, 28, 31]. The physicochemical properties of structured water include increased electrical conductivity and pH [13, 31]. Water conductivity and pH increase with an increasing ratio of structured water to unstructured water due to an increase in delocalized, and quasi-free electrons and protons that form vortices in and around the hexagonal rings of water [32-36]. Structured water is also termed as biological water, bound water, activated water, energized water, coherent domain water, vitalized water, or hexagonal water.

When unstructured, liquid water is exposed to a combination of chemical and/or electromagnetic energy sources, such as ozone or hydrogen peroxide combined with ultra-violet light or magnetic fields, a fraction of the water molecules will decompose into hydroxyl radicals [37-38]. Commercial wastewater systems are available that are based on the hydroxyl generator technology that combine ozone with ultra-violet lamps with wavelengths at 185 nm or shorter [37-38]. The ratio of structured or hexagonal water to bulk water increases as magnetic fields increase in strength. The ratio also depends on the combination of energy sources utilized, the mineral composition, and temperature of the water [16, 25, 40].

A custom-built water system was developed for this study using a combination of three energy sources to generate structured irrigation water. The closed loop water system included a water pump, hose lines, a hydroxyl generator, static magnets, 132 l water tank and a control panel (Image **1**). The hydroxyl generator component utilized ultraviolet lamps to convert water vapor into ozone. This ozonated water was then converted into free radical hydroxyl molecules [38 -40]. The hydroxyl free radicals have an extremely short half-life of approximately 10^{-9} seconds which convert back to water molecules with stronger H-bonds that form into hexagonal water rings [41-43]. Static DC magnets were the third energy source and were placed next to the water tube in the water generator to increase the yield of the hydroxyls being generated, thereby increasing the structured portion of the irrigation water. The closed loop system allowed the water to recirculate and pass through the hydroxyl

Image 1: Photo of custom-built water generator that generated all the water treatments in this study.

generator numerous times. As the exposure time increased the water structure also increased.

The plant species selected for this drought tolerance study was velvet bean (*Mucuns pruriens*). This tropical legume has a C3 Calvin cycle pathway that exhibits photoinhibition defense responses when water stressed. Velvet bean is a fast- growing, twining vine with limited drought tolerance often used as a cover crop [44]. Due to its rapid growth rates, and large leaf area the plants have high foliage gas exchange rates which make it an ideal species for this study. A previous study by Ramsey [45] investigated the drought tolerance of velvet bean plants using a series of magnetized foliar treatments which had promising results.

This study tested the ability of magnetized seeds combined with irrigation water from a custom-built magnetized + hydroxylated water generator to improve the drought tolerance of velvet bean plants. The study objectives include 1) determine the effects of a magnetized seed treatment on velvet bean plants, 2) determine the effects of magnetized water treatments on velvet bean plants, 3) determine the effects of water treated with a hydroxylated water generator on velvet bean plants, and 4) determine the effects of three soil moisture target levels on velvet bean plants.

2. MATERIALS AND METHODS

The greenhouse study was conducted in Fort Collins, CO. Greenhouse parameters were set at ambient light conditions, and a temperature range was set at 26 to 35 C.

The study design involved a factorial model with the four study factors fully crossed with each other in other to test the main effects and all interactions among the study factors. The plant responses were: 1) foliage gas exchange rates, 2) volumetric soil moisture, 3) cumulative water volume for each plant, 4) plant water use efficiency, and 5) oven dry foliage biomass.

2.1. Seed Treatments

The two seed treatments in this study included non-magnetized (NMS) and magnetized (MS) seeds. The control treatment seeds were soaked in water for three h before planting, without exposure to any magnetic fields. The magnetized seeds were soaked in water while placed on a static magnet for three h before planting. A neodymium static magnet (grade N-42) was used with South Pole face of magnet facing up into the seeds. After soaking the seeds were coated with a powder form of *Rhizobium leguminosarum* (N-Dure, INTX Microbials, Kentland, IN) before they were planted. The rhizobium species is specific for legumes and is a gram-negative bacteria used to inoculate legume roots to start nitrogen fixing colonies in root nodules. Each pot was planted with eight coated seeds. All germinated seeds were culled down to the two most vigorous seedlings at the two-cotyledon leaf stage. Two velvet bean plants were grown in each pot until completion of study. All pots were randomly assigned to either seed soaking method.

2.2. Pot and Soil Description

The wood fiber pots had a soil volume of 4.87 l, and a water saucer volume of 800 ml (Western Pulp Products Co. Corvallis, OR). Sixty-four (64) pots were filled with potting soil which was a mix of Canadian sphagnum peat moss, processed pine bark, vermiculite, and perlite mix (Farfard-4-MP, Sun Gro Horticulture, Agawam, MA). A controlled release fertilizer (19-5-6 NPK) (FloriKote CRF, Florikan ESA, Sarasota, FL) was applied at 10 g/pot at 11 days after planting (DAP).

2.3. Magnetic Water Treatments

Two grades (grade N-42 and N-52) of static neodymium magnets were used in this study (K&J Magnets, Inc, Pipersville, PA). The large cylinder magnets (N-42) were 7.6 cm diam x 5.1 cm thick, and the small cylinder magnets (N-52) were 5.1 cm diam x 2.5 cm thick. There are three magnetic field treatments: 1) control, or no magnets on hydroxyl water generator or water hoses (0-MT), 2) two N-42 neodymium magnets placed on the top cover of hydroxyl water generator (2-MT, and 3) two N-42 neodymium magnets placed on the top cover of hydroxyl water generator, and 10 N-52 neodymium magnets placed on the water hose between generator and water tank (10-MT). The ten N-52 magnets were placed on top of a steel U-shaped channel beam with the water hose inserted into the U channel. The magnetized water treatments were combined with or without the hydroxylated water treatments, depending on the assigned water treatment run for that day.

All neodymium magnets had their South Pole facing the water hose or the hydroxyl water generator. The measured strength at the magnet surface was 493 and 510 mT for the N-42 and N-52 neodymium magnets. However, both magnets were placed at different distances from the water hoses or from the hydroxylated water column. The measured strength of the N-42 magnet at 7.6 cm from the water column in the hydroxyl generator was 45 mT. The measured strength of the N-52 magnet at 5 cm from the water hoses was 131 mT. The static magnets were removed or replaced daily, depending on the water treatment assigned to be generated on that day.

2.4. Hydroxylated Water Treatments and Closed Loop System Description

The hydroxylated water generator was a commercial generator (EcoMaster PZ-784, Prozone Water Products, Huntsville, AL). The generator was slightly modified by placing static magnets on the metal surface of the generator, just above the water lines running through the generator. The hydroxyl generator operates by allowing air to enter the unit where two hybrid UVC lamps (287 nm wavelength) convert the air and water vapor into ozone (O_3) and hydroxyls ($OH^·$). Water enters the generator through a venturi injector drawing O_3 and $OH^·$ from the lamp chamber. A static mixer combines water, O_3 and $OH^·$ into a micro-bubble water flow. The ozonated water passes through a quartz water column that is also radiated with the two UVC lamps which converts the ozonated water into hydroxyl radicals. The two hydroxylated water treatments were: 1) hydroxyl water generator turned on (HWT), or 2) hydroxyl water generator turned off (NHWT).

The hydroxylated water generator was a component of the closed loop water system. The closed loop system was run for 30 min. to generate each batch for a specific water treatment. There were six water treatments in this study (3 MWT x 2 HWT = 6 WT), and one water treatment was generated per day due to the time required for each run. Each water treatment was stored in a labeled 19 l container so that it could be used over a three-day period before making a new batch. In other words, each water treatment batch was stored after it was generated, and then used to irrigate the assigned pots over a three-day period before generating a new water treatment batch. The water treatments were generated on a rotating basis. Each water treatment could be run with or without the hydroxylated water generator turned on, or the static magnetics placed on the water hoses or the generator. The water pump capacity was 3.78 l/min. The water tank and total water hose capacity was 118 l. The water turn-over rate for a full water tank was 10.9 times for each 30 min. water treatment run, i.e., for each water treatment the water was exposed to a combination of magnetics and/or hydroxylated water generator 10.9 times before collecting and storing the treated water. The control water (0 MT + NHWT) was run in the closed loop system for 30 min. without the hydroxylated water generator turned on or the magnets placed on the water hoses. All water lines and the water tank were purged between each water treatment run.

2.5. Soil Moisture Levels and Soil Moisture Methods

Three soil moisture levels were selected to simulate water stress or drought conditions to determine the effects of the water treatments on drought stressed plants. The soil moisture level targets were implemented after the seedlings reached the second set of trifoliate leaves at approximately 21 days after planting. The three soil moisture levels were based on volumetric soil moisture (SM) levels which were:

1) low soil moisture (LSM) (10 to 15% v/v)
2) moderate soil moisture (MSM) (15 to 20% v/v SM)
3) high soil moisture (HSM) (20 to 25% soil moisture).

All pots were well watered with tap water between seed planting and the second trifoliate stage for the seedlings to be well established with long roots before starting the water stress stage of the study. All pots were randomly assigned to the three soil moisture levels and water treatments. The first set soil moisture levels were too high, and the plants were not showing any wilting symptoms. At 21 days after planting daily watering was reduced and soil moisture was monitored until the three soil moisture levels were reached.

Daily volumetric soil moisture measurements were collected using a data logger and a soil moisture and temperature sensor (ECH2O EM-50 data logger and 5-TM soil sensor, METER Environmental, Pullman WA). Each pot was measured every morning between 8-10 am using a single datalogger and a 5-TM soil probe, and the data was hand recorded. The sensors were buried so that soil moisture reading was collected at approximately 5-10 cm.

2.6. Daily Soil Moisture Measurements and Watering Methods

Volumetric soil moisture (SM) readings were collected with a data logger and soil moisture sensors (ECH2O data logger and 5-TM soil sensors, METER Environmental, Pullman WA). An equation developed by METER scientists was used to adjust the default algorithm based on mineral soil. Potting soil is virtually organic soil and the raw tensiometer data was converted to percent soil moisture data using an algorithm suited for organic soil. The daily soil moisture measurements were hand recorded and used to estimate the daily water volume needed to maintain each pot at its label or assigned irrigation target. Water volume was adjusted daily for each pot based on its growth rate, and irrigation target.

All 64 pots were watered daily after estimating the water volume to apply to each pot. Treated water stored in the 19 l containers was added to smaller pails that were used to fill either a 500- or 1,000-ml volumetric cylinder, so that precise water volumes per pot could be recorded. The daily water volume was compiled into a single dataset for study factor analyses, or as a covariate in the study analyses.

2.7. Foliage Gas Exchange Methods

A LICOR 6400 XT gas exchange instrument (LICOR Environmental, Lincoln, NE) was used to measure foliage physiology parameters just before the plants were harvested at the end of the study. The foliage measurements included: photosynthesis (Pn), stomatal conductance (g), transpiration (E), instantaneous water use efficiency (IWUE), internal CO_2 (Ci), leaf vapor pressure deficit (vpdl), and leaf temperature (ltemp). 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.

The LICOR 6400 parameters are set as: Leaf ratio = 0.5, PAR = 800 umol/m2/s, block temperature = 35 C, and flow rate = 400, and CO_2 = 400 mg/l. The upper most, fully mature leaves were selected for gas exchange measurements. Three leaves per plant were selected, which were dark green or mature leaves with no disease spots inside the measurement area. Soil moisture data was collected along with the gas exchange parameters and all the parameters were combined into a single dataset so that soil moisture could be tested as a covariate in the data analyses.

2.8. Oven Dry Plant Biomass and Water Use Efficiency Methods

Both plants in each pot were harvested at 61 to 64 days after planting. The aboveground foliage and stems for both plants per pot were combined in one bag for oven drying. The oven dryer is set at 67 C, and the bags were dried until they reached a constant weight when measured over two consecutive days for three randomly selected bags

Average daily biomass growth rates (ABGR) were estimated with the following formula: ((total aboveground, oven dry foliage biomass (g)/2 plants/pot)/number of days between seed planting and plant harvest) = average daily growth rate (g/day). The oven dry foliage biomass for each plant was recorded. The Cumulative Watering Volume (CWV) per pot was calculated from the daily watering dataset, using only watering data between 27 and 52 days after planting. The following formula was used to estimate CWV (ml/plant) = Sum of daily watering volume for each water treatment/ 2 plants per pot. Plant Water Use Efficiency (PWUE) was calculated for each plant as PWUE (g biomass/ml of irrigation water) = total oven dry foliage biomass (g)/cumulative watering volume (ml). A dataset was compiled for each treatment using the average daily growth rate, total oven dry foliage biomass, and plant water use efficiency.

2.9. Pilot Water Stability Study

Physicochemical water properties were measured for several water treatments during the study. However, due to low quality instruments and measurement errors the water property data could not be used. Storage of the treated water over a 3-to-5-day period before watering the plants posed a question whether the treated water retained its structure during storage. Thus, a pilot study was conducted after the study was finished, using a high quality, multi-parameter meter, to collect more accurate data. The objective of the preliminary study was to determine the stability of structured water over six days. The study used an alternative method to generate a specific structured water treatment. The alternative method used a chemical reaction (Fenton reaction) to generate the structured water. The method required mixing hydrogen peroxide with chelated iron, resulting in structured water that was charcoal filtered to remove the iron precipitate. The iron (4% ferric Fe) was chelated with HEDTA (Cannon Packaging, Humboldt, TN). Hydrogen peroxide (35%) was diluted to 10%, and 3-ml of chelated Fe was added to 300 ml of 10% H_2O_2. The solution was placed on a neodymium magnet for 24 h. The neodymium magnet strength at the surface was 560 mT, and the magnet size was 10 x 7.6 x 5 cm. The solution was filtered with activated powdered charcoal to remove the Fe precipitate. Tap water and the structured water solution was measured for electrical conductivity (EC) over a six-day period using a data logger that collected temperature and conductivity at 15 min. time intervals.

The same structured water generated with hydrogen peroxide and chelated iron was used to water two velvet bean plants in this first-stage, pilot study. The objective of watering the two plants was to determine if the water would be stable enough to not react with potting soil, or with any vascular plant tissue after it was transpired from the plant foliage. Two pots were used as the control plants which were watered with filtered tap water. The other two pots were watered with the structured water for. All pots were watered for seven days after the plants reached the fourth set of trifoliate leaves. At the end of seven days all four plants were enclosed in one gallon zip lock bags to collect all the foliage transpiration over two afternoons for four h per day. The zip lock bags collected the water vapor from transpiration as condensate for each covered plant. The condensate was labelled and papered filtered to remove any organic tissue in the condensate. The filtered condensate was measured for electrical conductivity, oxidation reduction potential, and pH values for both water types that had been transported from the soil into the plant vascular system and converted into transpiration vapor. Physicochemical water properties were compared, before watering the plant and for as leaf condensate, to determine whether the structured water remained stable during the entire water transport process.

2.10. Data Analyses

The study design included hidden replication for the statistical modeling analysis. Hidden replication restricts the interaction tests to only two-way interactions. The SAS JMP (SAS Institute Inc., Clary, NC) Design of Experiment (DOE) generated a design with 16 replications of the whole plots which included the hidden replicates. The foliar gas exchange data was analyzed with the SAS JMP Restricted Maximum Likelihood (REML) model. This model included variation among the three leaves as a random variable. The JMP Least Squares Fit (LSF) model was used to test treatment effects for average percent soil moisture, cumulative water volume, oven dry foliage biomass, and plant water use efficiency. Multivariate analysis was conducted to test any correlations among the plant and soil variables. Regression analysis tested relationships among the gas exchange variables and between water usage and plant 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.

The daily watering volumes, soil moisture data, and gas exchange data were collected and compiled into a spreadsheet that list all study factors by pot number. Soil moisture was recorded each morning and used as a covariate in the analysis for all plant responses.

3. RESULTS

The velvet bean plants were allowed to twine up bamboo stakes over the 64-day study. Photos were taken the last week of the study just before the plants were harvested and they showed that the plants were healthy and still vigorously growing (Images **2-4**).

Multivariate analysis of the foliar gas exchange responses correlated six physiological responses and the soil moisture parameter to the 0-MT + NHWT, 2-MT + HWT, and 10-MT + HWT treatments (Tables **1-4**). The correlation strength for the plant and soil parameters is reported for the LSM and MSM soil moisture levels (Tables **1**, **3**), and the correlation probability is reported for the LSM and MSM soil moisture levels in Tables **2** and **4**. The multivariate analysis summarizes the interactions among the plant and soil responses and highlights any changes in these interactions due to the 0-MT + NHWT, 2-MT + HWT, and 10-MT + HWT treatments.

Image 2: Photo of two rows of velvet bean plants on the bench for the control treatment (0-MT + NHWT).

There was a strong negative correlation between Pn and g, Ci, and E for the 0-MT + NHWT treatment and the MSM soil level. However, the correlation reversed to a positive relationship between Pn and g, Ci, and E for the 10-MT + HWT treatment and the MSM soil level

(Table **1**). The correlation probability, however, for the 0-MT + NHWT treatment between Pn and g, Ci, and E was not significant for the MSM soil level. In contrast, the correlation probability for the 10-MT + HWT treatment between Pn and g and E was significant for the MSM soil level (Table **2**).

Image 3: Photo of two rows of velvet bean plants (center of photo) on the bench for the two magnets + hydroxylated water treatment (2-MT + HWT).

Image 4: Photo of two rows of velvet bean plants (center of photo) on the bench for the ten magnets + hydroxylated water treatment (10-MT + HWT).

Also, there was a positive correlation between Pn and vpdl and Itemp for the 0-MT + NHWT treatment and the MSM soil level. This correlation strength was reversed to a negative relationship for the 10-MT + HWT treatment and the MSM soil level (Table **1**). The correlation probability between Pn and vpdl and Itemp for the 0-MT + NHWT and 10-MT + HWT treatment was not significant (Table **2**).

The correlation tables revealed there was a strong positive and significant relationship between Ci and g for the 0-MT + NHWT treatment at the MSM level (Tables **1-2**). Also, there was a strong positive and significant relationship between Ci and g and E for the 0-MT + NHWT treatment at the LSM level (Tables **3-4**). The tables also show that there was no relationship between Ci and g or E, vpdl and Itemp for the 10-MT + HWT, at the LSM soil moisture level (Table **4**).

The REML model results for Pn, g, and E are listed in Table **5**. The three models show slightly different model terms. The Pn, g, and E models had 2, 2, and 3 two-way interaction terms, respectively. Soil moisture was included as a covariate in the g and E models because it improved the model outcome. The REML predicted parameters for g, Pn, E, Itemp, vpdl, and WUE are reported for the LSM soil moisture level (Table **6**).

The REML model predicted lower g, Pn, and E values for the 10-MT + HWT as compared to the 0-MT + NHWT, for the magnetized seed treatments, under the LSM soil moisture level. However, Itemp and vpdl increased for the 10-MT + HWT as compared to the 0-MT + NHWT, for the magnetized seed treatments. There was a 37.5% decrease in Pn for the 10-MT + HWT as compared to the 0-MT + NHWT, for the magnetized seed treatments under the LSM soil moisture level.

The three REML models for gas exchange responses show that only the Pn model includes the seed treatment as a model term. Although only Pn was affected by the seed treatment, carbon assimilation or photosynthesis is also strongly affected by stomatal conductance and transpiration under water stress conditions. Therefore, a table was created to report the percent change for Pn, g, E, Ci, Itemp, and IWUE for the three soil moisture levels relative to the 0-MT + NHWT treatment for both seed treatments and the 10-MT + HWT water treatment (Table **7**). Five out of six gas exchange responses were reduced for both seed treatments combined with the 10-MT + HWT structured water treatment under all three soil moisture levels. Only E increased 37% for the LSM soil moisture level, and IWUE increased 148% for the HSM soil moisture level for the magnetized seed treatment. The non-magnetized seed treatment showed a similar pattern for increased E for the LSM and MSM soil moisture levels, and increased IWUE (4%) for the HSM soil moisture levels. Plant foliage responses to the structured water treatment (10-MT + HWT) were generally reduced for both seed treatments.

Table 1: Multivariate Correlation Strength among Gas Exchange and Soil Parameters for the MSM Soil Moisture Level

Parameter[a]	Water trt[b]	SM	IWUE	Pn	g	Ci	E	vpdl	ltemp
SM	Two	1	0.0746	0.3953	-0.4397	-0.0561	-0.4576	-0.0574	-0.6137
SM	Ten	1	0.229	0.42	0.5305	0.5054	0.3478	-0.9771	-0.9238
IWUE	Control	0	1	0.9897	-0.9998	-0.9989	-0.974	0.966	0.9034
IWUE	Two	0.0746	1	-0.3135	0.6003	-0.9987	0.5663	-0.203	-0.1755
IWUE	Ten	0.229	1	0.0173	-0.3635	-0.7104	-0.4896	-0.3021	-0.5124
Pn	Control	0	0.9897	1	-0.9864	-0.9954	-0.9315	0.993	0.9554
Pn	Two	0.3953	-0.3135	1	-0.5615	0.2971	-0.5124	-0.5085	-0.7692
Pn	Ten	0.42	0.0173	1	0.883	0.3109	0.8555	-0.4772	-0.4211
G	Control	0	-0.9998	-0.9864	1	0.9976	0.9786	-0.9602	-0.894
G	Two	-0.4397	0.6003	-0.5615	1	-0.62	0.9943	0.1624	0.4767
G	Ten	0.5305	-0.3635	0.883	1	0.715	0.9784	-0.5356	-0.3828
Ci	Control	0	-0.9989	-0.9954	0.9976	1	0.962	-0.9772	-0.9228
Ci	Two	-0.0561	-0.9987	0.2971	-0.62	1	-0.5906	0.1967	0.1682
Ci	Ten	0.5054	-0.7104	0.3109	0.715	1	0.6988	-0.4556	-0.2347
E	Control	0	-0.974	-0.9315	0.9786	0.962	1	-0.8822	-0.7827
E	Two	-0.4576	0.5663	-0.5124	0.9943	-0.5906	1	0.198	0.4882
E	Ten	0.3478	-0.4896	0.8555	0.9784	0.6988	1	-0.3552	-0.1925
vpdl	Control	0	0.966	0.993	-0.9602	-0.9772	-0.8822	1	0.9836
vpdl	Two	-0.0574	-0.203	-0.5085	0.1624	0.1967	0.198	1	0.7635
vpdl	Ten	-0.9771	-0.3021	-0.4772	-0.5356	-0.4556	-0.3552	1	0.9659
ltemp	Control	0	0.9034	0.9554	-0.894	-0.9228	-0.7827	0.9836	1
ltemp	Two	-0.6137	-0.1755	-0.7692	0.4767	0.1682	0.4882	0.7635	1
ltemp	Ten	-0.9238	-0.5124	-0.4211	-0.3828	-0.2347	-0.1925	0.9659	1

[a] SM = soil moisture, IWUE = instantaneous water use efficiency, Pn = photosynthesis, g = stomatal conductance, Ci = internal CO2, E = transpiration, vpdl = vapor pressure deficit for leaf, ltemp = leaf temperature.
[b] Control = 0-MT+ NHWT, Two = 2-MT+HWT, Ten = 10MT-HWT.

Table 2: Multivariate Correlation Probabilities among Gas Exchange and Soil Parameters for the MSM Soil Moisture Level

Parameter[a]	Water trt[b]	SM	IWUE	Pn	g	Ci	E	vpdl	ltemp
SM	Two	<.0001	0.8883	0.438	0.383	0.916	0.3615	0.914	0.1951
SM	Ten	<.0001	0.6625	0.407	0.2789	0.3064	0.4993	0.0008	0.0085
IWUE	Control	1	<.0001	0.0914	0.0136	0.0304	0.1456	0.1665	0.2822
IWUE	Two	0.8883	<.0001	0.5451	0.2077	<.0001	0.2414	0.6997	0.7394
IWUE	Ten	0.6625	<.0001	0.9741	0.4787	0.1137	0.3242	0.5607	0.2987
Pn	Control	1	0.0914	<.0001	0.105	0.061	0.237	0.0752	0.1908
Pn	Two	0.438	0.5451	<.0001	0.2463	0.5675	0.2986	0.3029	0.0738
Pn	Ten	0.407	0.9741	<.0001	0.0197	0.5486	0.0298	0.3385	0.4056
g	Control	1	0.0136	0.105	<.0001	0.044	0.132	0.1801	0.2958
g	Two	0.383	0.2077	0.2463	<.0001	0.1891	<.0001	0.7585	0.3392
g	Ten	0.2789	0.4787	0.0197	<.0001	0.1102	0.0007	0.2734	0.4538

(Table 2). Continued.

Parametera	Water trtb	SM	IWUE	Pn	g	Ci	E	vpdl	ltemp
Ci	Control	1	0.0304	0.061	0.044	<.0001	0.176	0.1361	0.2517
Ci	Two	0.916	<.0001	0.5675	0.1891	<.0001	0.2171	0.7087	0.75
Ci	Ten	0.3064	0.1137	0.5486	0.1102	<.0001	0.1224	0.3639	0.6545
E	Control	1	0.1456	0.237	0.132	0.176	<.0001	0.3121	0.4277
E	Two	0.3615	0.2414	0.2986	<.0001	0.2171	<.0001	0.7069	0.3259
E	Ten	0.4993	0.3242	0.0298	0.0007	0.1224	<.0001	0.4895	0.7147
vpdl	Control	1	0.1665	0.0752	0.1801	0.1361	0.3121	<.0001	0.1156
vpdl	Two	0.914	0.6997	0.3029	0.7585	0.7087	0.7069	<.0001	0.0773
vpdl	Ten	0.0008	0.5607	0.3385	0.2734	0.3639	0.4895	<.0001	0.0017
ltemp	Control	1	0.2822	0.1908	0.2958	0.2517	0.4277	0.1156	<.0001
ltemp	Two	0.1951	0.7394	0.0738	0.3392	0.75	0.3259	0.0773	<.0001
ltemp	Ten	0.0085	0.2987	0.4056	0.4538	0.6545	0.7147	0.0017	<.0001

[a]SM = soil moisture, IWUE = instantaneous water use efficiency, Pn = photosynthesis, g = stomatal conductance, Ci = internal CO2, E = transpiration, vpdl = vapor pressure deficit for leaf, ltemp = leaf temperature.
[b]Control = 0-MT+ NHWT, Two = 2-MT+HWT, Ten = 10MT-HWT.

Table 3: Multivariate Correlation Strength among Gas Exchange and Soil Parameters for the LSM Soil Moisture Level

Parameter[a]	Water trt[b]	SM	IWUE	Pn	g	Ci	E	vpdl	ltemp
SM	Control	1	0.746	0.0425	-0.6375	-0.6721	-0.7039	0.0339	0.8891
SM	Two	1	-0.312	-0.877	0.2789	0.328	0.2642	-0.2605	0.2109
IWUE	Control	0.746	1	-0.1704	-0.9027	-0.9888	-0.9297	0.5021	0.8889
IWUE	Two	-0.312	1	0.5915	-0.9552	-0.9865	-0.9234	0.708	-0.6246
IWUE	Ten	0	1	0.8342	0.4927	-0.996	0.4439	-0.8122	0.9484
Pn	Control	0.0425	-0.1704	1	0.4232	0.1689	0.4435	-0.1181	0.0212
Pn	Two	-0.877	0.5915	1	-0.5155	-0.5751	-0.5269	0.2999	-0.1917
Pn	Ten	0	0.8342	1	0.8909	-0.7814	0.8644	-0.9992	0.6165
g	Control	-0.6375	-0.9027	0.4232	1	0.9139	0.9867	-0.6501	-0.8493
g	Two	0.2789	-0.9552	-0.5155	1	0.9138	0.989	-0.5706	0.7844
g	Ten	0	0.4927	0.8909	1	-0.4126	0.9985	-0.9078	0.1915
Ci	Control	-0.6721	-0.9888	0.1689	0.9139	1	0.9193	-0.62	-0.8764
Ci	Two	0.328	-0.9865	-0.5751	0.9138	1	0.8625	-0.8083	0.5737
Ci	Ten	0	-0.996	-0.7814	-0.4126	1	-0.3617	0.7566	-0.9731
E	Control	-0.7039	-0.9297	0.4435	0.9867	0.9193	1	-0.5282	-0.8522
E	Two	0.2642	-0.9234	-0.5269	0.989	0.8625	1	-0.4495	0.759
E	Ten	0	0.4439	0.8644	0.9985	-0.3617	1	-0.8832	0.137
vpdl	Control	0.0339	0.5021	-0.1181	-0.6501	-0.62	-0.5282	1	0.4833
vpdl	Two	-0.2605	0.708	0.2999	-0.5706	-0.8083	-0.4495	1	-0.3845
vpdl	Ten	0	-0.8122	-0.9992	-0.9078	0.7566	-0.8832	1	-0.5855
ltemp	Control	0.8891	0.8889	0.0212	-0.8493	-0.8764	-0.8522	0.4833	1
ltemp	Two	0.2109	-0.6246	-0.1917	0.7844	0.5737	0.759	-0.3845	1
ltemp	Ten	0	0.9484	0.6165	0.1915	-0.9731	0.137	-0.5855	1

[a]SM = soil moisture, IWUE = instantaneous water use efficiency, Pn = photosynthesis, g = stomatal conductance, Ci = internal CO2, E = transpiration, vpdl = vapor pressure deficit for leaf, ltemp = leaf temperature.
[b]Control = 0-MT+ NHWT, Two = 2-MT+HWT, Ten = 10MT-HWT.

Table 4: Multivariate Correlation Probabilities among Gas Exchange and Soil Parameters for the LSM Soil Moisture Level

Parameter[a]	Water trt[b]	SM	IWUE	Pn	g	Ci	E	vpdl	ltemp
SM	Control	<.0001	0.0886	0.9363	0.1733	0.1437	0.1185	0.9492	0.0178
SM	Two	<.0001	0.5472	0.0218	0.5925	0.5257	0.613	0.618	0.6883
IWUE	Control	0.0886	<.0001	0.7469	0.0137	0.0002	0.0072	0.3102	0.0178
IWUE	Two	0.5472	<.0001	0.2162	0.003	0.0003	0.0086	0.1154	0.1849
IWUE	Ten	1	<.0001	0.3718	0.672	0.0572	0.7072	0.3965	0.2053
Pn	Control	0.9363	0.7469	<.0001	0.4031	0.7491	0.3784	0.8236	0.9683
Pn	Two	0.0218	0.2162	<.0001	0.2953	0.2325	0.2828	0.5636	0.716
Pn	Ten	1	0.3718	<.0001	0.3002	0.429	0.3354	0.0247	0.5771
g	Control	0.1733	0.0137	0.4031	<.0001	0.0108	0.0003	0.1623	0.0324
g	Two	0.5925	0.003	0.2953	<.0001	0.0108	0.0002	0.237	0.0647
g	Ten	1	0.672	0.3002	<.0001	0.7292	0.0352	0.2755	0.8773
Ci	Control	0.1437	0.0002	0.7491	0.0108	<.0001	0.0095	0.1891	0.022
Ci	Two	0.5257	0.0003	0.2325	0.0108	<.0001	0.027	0.0516	0.2338
Ci	Ten	1	0.0572	0.429	0.7292	<.0001	0.7644	0.4537	0.1481
E	Control	0.1185	0.0072	0.3784	0.0003	0.0095	<.0001	0.2813	0.0312
E	Two	0.613	0.0086	0.2828	0.0002	0.027	<.0001	0.3712	0.0801
E	Ten	1	0.7072	0.3354	0.0352	0.7644	<.0001	0.3107	0.9125
vpdl	Control	0.9492	0.3102	0.8236	0.1623	0.1891	0.2813	<.0001	0.3314
vpdl	Two	0.618	0.1154	0.5636	0.237	0.0516	0.3712	<.0001	0.4516
vpdl	Ten	1	0.3965	0.0247	0.2755	0.4537	0.3107	<.0001	0.6018
ltemp	Control	0.0178	0.0178	0.9683	0.0324	0.022	0.0312	0.3314	<.0001
ltemp	Two	0.6883	0.1849	0.716	0.0647	0.2338	0.0801	0.4516	<.0001
ltemp	Ten	1	0.2053	0.5771	0.8773	0.1481	0.9125	0.6018	<.0001

[a] SM = soil moisture, IWUE = instantaneous water use efficiency, Pn = photosynthesis, g = stomatal conductance, Ci = internal CO2, E = transpiration, vpdl = vapor pressure deficit for leaf, ltemp = leaf temperature.
[b] Control = 0-MT+ NHWT, Two = 2-MT+HWT, Ten = 10MT-HWT.

Table 5: Description of the REML Model Terms and their *p*-Values for Three Gas Exchange Responses

Photosynthesis model	
Source	**Prob > F**
Magnetized seed	0.0295*
Mag Water	<.0001*
Hydroxyl Generator	0.7232
Soil moisture level	<.0001*
Mag Water*Soil moist level	0.0849
Hydroxyl Generator*Soil moist level	0.0001*
Stomatal conductance model	
Source	**Prob > F**
Mag Water	<.0001*
Hydroxyl Generator	0.2288
Soil moisture covariate term	0.0256*
Mag Water* Hydroxyl Generator	0.0134*
Hydroxyl Generator*Soil moist covariate	0.0455*

(Table 5). Continued.

Transpiration model	
Source	**Prob > F**
Mag Water	<.0001*
Hydroxyl Generator	0.1001
Soil moisture covariate term	0.2220
Mag Water* Hydroxyl Generator	0.0261*
Mag Water*Soil moist covariate	0.0007*
Hydroxyl Generator*Soil moist covariate	0.0720

Table 6: Predicted Gas Exchange Parameters, Based on the REML Model, for the LSM Soil Moisture Level

Mag seed[a]	Mag water	Hydroxyl Generator	g (mol m-2 s-1)	Pn (µmol m-2 s-1)	E (mol m-2 s-1)	Leaf temp (C)	vpdl (kPa)	WUE
No	0-MT	NHWT	0.031	9.947	0.887	35.312	1.697	0.373
No	2-MT	HWT	0.198	10.591	4.218	33.314	1.737	0.285
No	10-MT	HWT	0.037	6.190	3.026	36.098	3.103	0.510
Yes	0-MT	NHWT	0.186	10.708	4.409	35.023	1.588	1.239
Yes	0-MT	HWT	0.195	10.919	4.018	33.797	1.968	1.010
Yes	2-MT	NHWT	-0.059	10.649	-1.107	35.111	1.249	0.340
Yes	2-MT	HWT	0.067	10.860	1.106	33.885	1.628	0.111
Yes	10-MT	NHWT	-0.014	6.485	0.146	36.243	2.614	-0.015
Yes	10-MT	HWT	-0.023	6.696	1.296	35.017	2.994	-0.244

[a] Mag seed = magnetized seed, Mag water = magnetized water.

Table 7: Six Gas Exchange Responses for the 10-MT + HWT Treatment, Reported by Seed Treatment and Moisture Level. The Gas Exchange Responses are Reported as the Percent Reduction Relative to the Relative to the 0-MT + NHWT Treatment

Gas exchange response	Magnetized seed under LSM soil moisture level	Magnetized seed under MSM soil moisture level	Magnetized seed under HSM soil moisture level	Non-Magnetized seed under LSM soil moisture level	Non-Magnetized seed under MSM soil moisture level	Non-Magnetized seed under HSM soil moisture level
	% Reduction in gas exchange responses[a]					
Pn	25	45	21	16	32	24
g	34	51	72	30	24	56
E	-37	23	72	-36	-37	46
Ci	7	8	69	13	1	28
ltemp	0	7	13	1	4	6
IWUE	47	33	-148	39	49	-4

[a] Percent reduction = ((0-MT + NHWT-10-MT + HWT)/ 0-MT + NHWT)* 100.

Four regression tests were conducted to provide more detail about the relationships between the gas exchange, soil, and leaf parameters. The first regression test was conducted for Pn, g, and E over ltemp for the magnetized seed and structured water treatments, for the LSM soil moisture level (Figure **1**). Magnetizing the seeds reduced the effects of ltemp on Pn and g, i.e., increased ltemp had almost no effect on g for the 0-MT + NHWT and 10-MT + HWT treatments when the seeds were magnetized. Also, Pn was much lower for the 10-MT + HWT treatment, when compared to the 0-MT + NHWT and 2-MT+HWT treatments for both seed treatments.

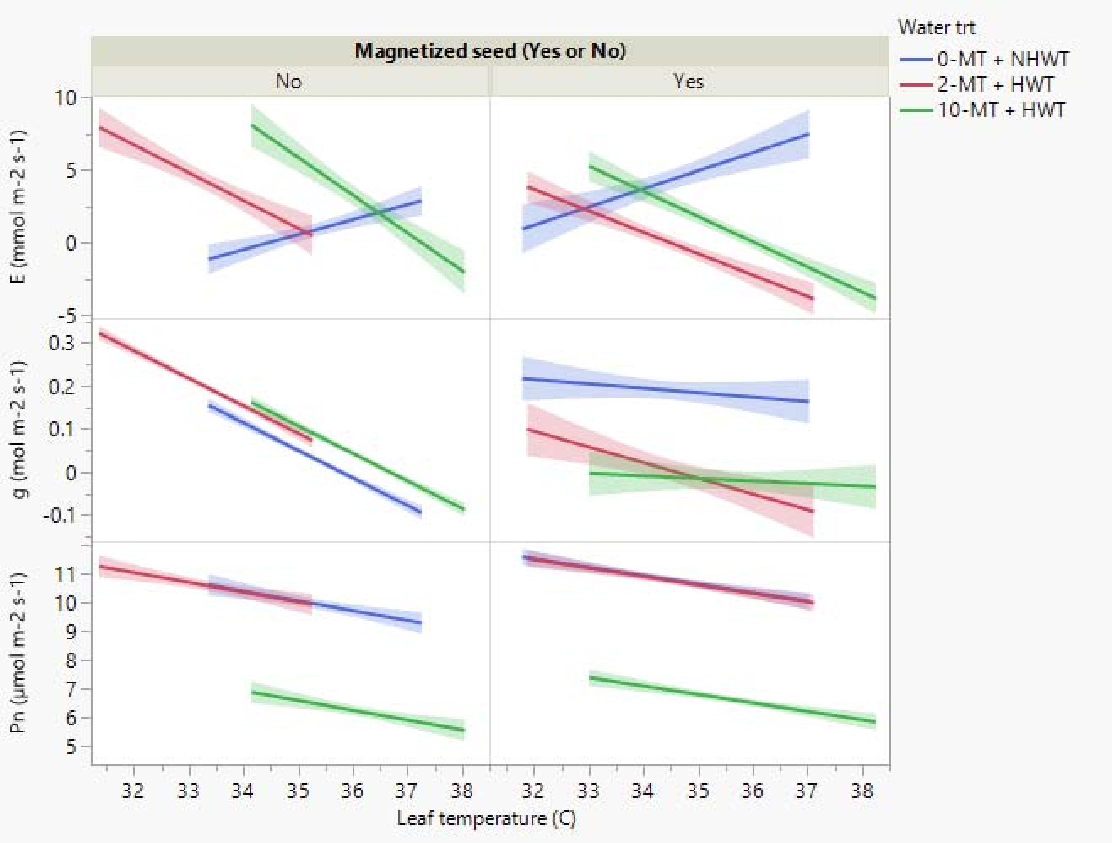

Figure 1: Regression plots for Pn, g, and E (y-axis) over leaf temperature (x-axis) by magnetized seed treatment (upper x-axis) and water generator treatments (legend). The regression is based on REML predicted values for the LSM soil moisture level.

The second regression test was conducted for Pn, g, and ltemp over IWUE, for the magnetized seed and structured water treatments, for the LSM soil moisture level (Figure **2**). Magnetizing the seeds increased IWUE for Pn and g, but decreased IWUE for the non-magnetized seeds, for the LSM soil moisture level. Increasing ltemp had almost no effect on IWUE for all three water treatments. Finally, Pn was much lower for the 10-MT + HWT treatment, when compared to the 0-MT + NHWT and 2-MT+HWT treatments for both seed treatments.

The third regression test was conducted for Pn, g, and ltemp over vpdl, for the magnetized seed and structured water treatments, for the LSM soil moisture level (Figure **3**). Magnetizing the seeds broadened the vpdl range for Pn, g, and ltemp when compared to the non-magnetized seed treatments. Also, ltemp has a strong correlation with vpdl, with almost no variation in the relationship. The magnetized seed treatment combined with the 10-MT + HWT treatment shows that both ltemp and vpdl were in the higher ranges when compared to the other water treatments, for the LSM soil moisture level (Figure **3**).

The final regression shows the relationship between E and soil moisture data that was collected during the gas exchange measurements at the LSM soil moisture level. The 0-MT + NHWT treatment shows a direct relationship between E and soil moisture. However, both the 2-MT and 10-MT + HWT structured water treatments had an inverse relationship between E and soil moisture for both seed treatments. The inverse relationship is contrary to well established stress physiology findings that plants transpire less as soil moisture decreases.

The average percent soil moisture was graphed to show the daily temporal dynamics and general range patterns for the three target soil moisture levels, from 13 to 52 days after the initiation of the study (Figure **5**). The average soil moisture was 12, 18, and 23% for the LSM, MSM, and HSM soil moisture levels. The three target soil moisture levels were kept within a narrow range due to the daily monitoring and water schedule, when averaged across magnetized seed and structured water treatments. The drop in soil moisture from 17 to 24 days after planting was due to readjusting soil moisture levels to induce higher water stress levels in the plants. The higher water stress

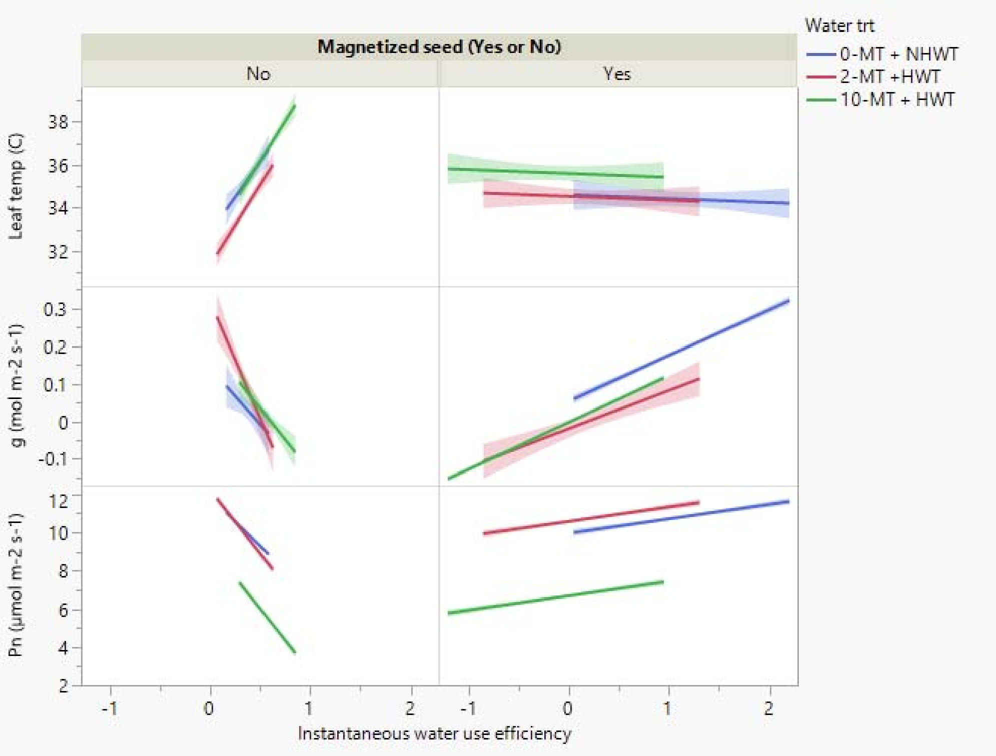

Figure 2: Regression plots for Pn, g, and leaf temperature (y-axis) over IWUE (x-axis) by magnetized seed treatment (upper x-axis) and water generator treatments (legend). The regression is based on REML predicted values for the LSM soil moisture level.

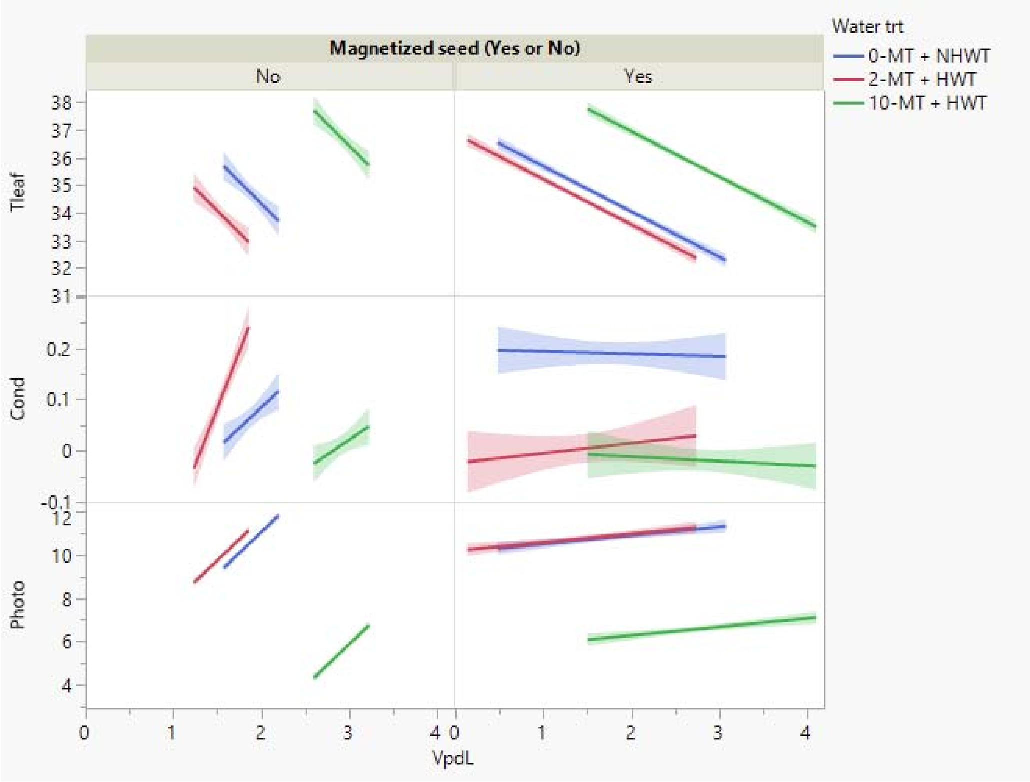

Figure 3: Regression plots for Pn, g, and leaf temperature (y-axis) over vapor pressure deficit for leaves (x-axis) by magnetized seed treatment (upper x-axis) and water generator treatments (legend). The regression is based on REML predicted values for the LSM soil moisture level.

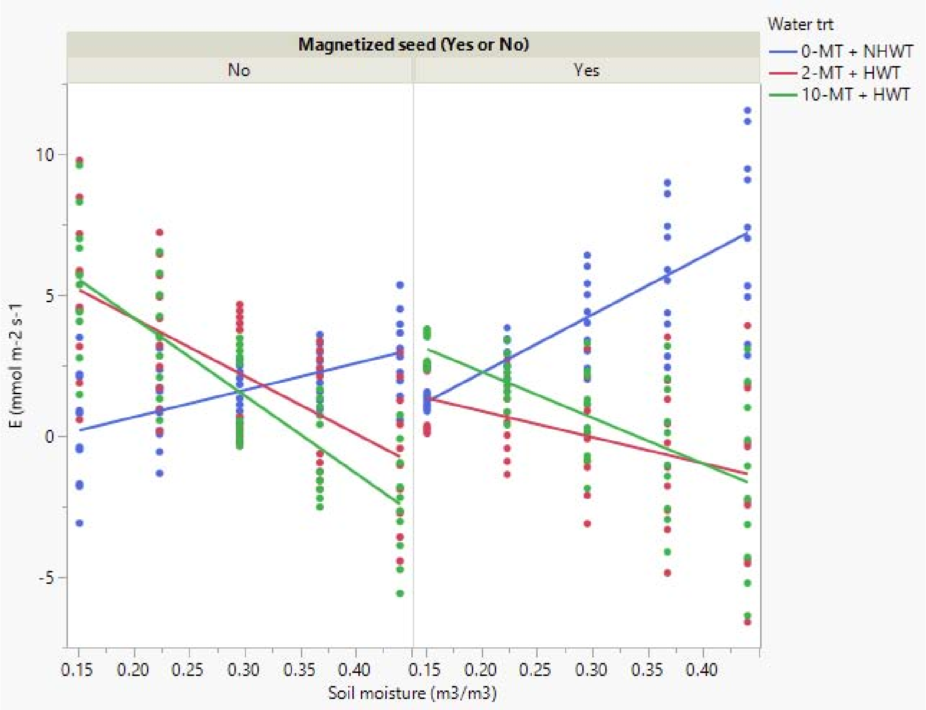

Figure 4: Regression plot for E (y-axis) over soil moisture (x-axis) by magnetized seed treatment (upper x-axis) and water generator treatments (legend). The regression is based on REML predicted values for the LSM soil moisture level.

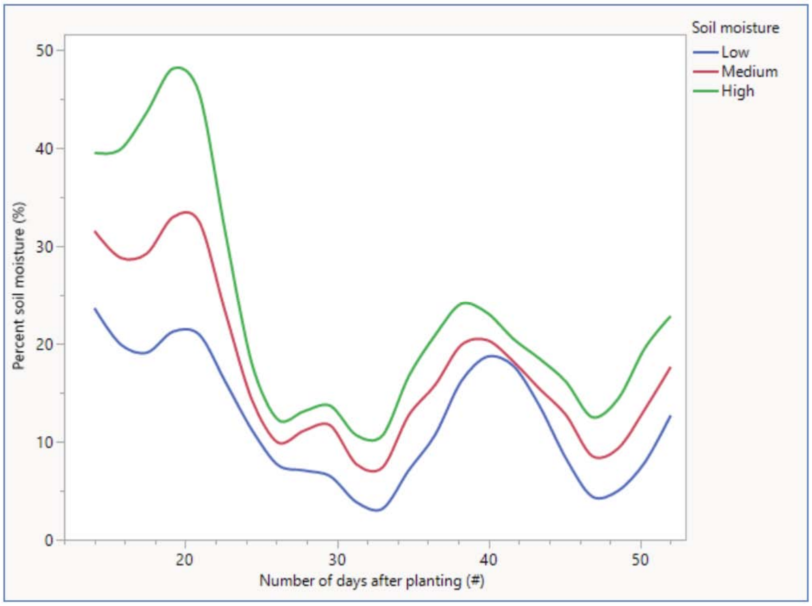

Figure 5: Average daily percent soil moisture measured between 13 and 52 days of the study. A statistical smoother curve plotted soil moisture over time for the three soil moisture levels (legend) and was averaged across the magnetized seed and water treatments. Soil moisture was reduced to the adjusted soil moisture levels after 24th day.

levels ensured that the magnetized seed and structured water treatments were tested on truly water stressed plants, that simulated drought conditions.

The effects of the magnetized seed and structured water treatments on percent soil moisture was tested with the Least Squared Fit model (Table **8**). The model included all four study factors and three two-way interaction terms. Average percent soil moisture was reported for magnetized and non-magnetized seed treatments by the three other study factors (Tables **9-10**). The magnetized seed combined with the 18 structured water treatments show that the percent soil moisture was higher in the 2-MT + HWT and 10-MT +

Table 8: Description of the Least Square Fit Model Terms and *p*-Values for Soil Moisture (m^3/m^3) when Averaged between 13 and 52 Days after Planting

Source	Prob > F
Magnetized seed	0.2155
Magnetized water	0.0009*
Hydroxyl Generator	0.9677
Soil moisture level	<.0001*
Mag seed* Soil moisture level	<.0001*
Mag water* Soil moisture level	<.0001*
Hydroxyl generator* Soil moisture level	0.0004*

Table 9: Average Percent Soil Moisture, Based on Volumetric Data (m^3/m^3), for the Magnetized Seed Treatment, by Structured Water Treatment, Hydroxyl Generator Status, and Soil Moisture Level. Soil Moisture was Averaged between 13 to 52 Days after Planting

Structured water trt	Hydroxyl generator	Soil moisture Level (%)	Percent soil moisture (%)	Relative change[a]
0-MT + NHWT	No	10 -15	5	0
0-MT + NHWT	No	15-20	14	0
0-MT + NHWT	No	20-25	16	0
0-MT + HWT	Yes	10 -15	7	0
0-MT + HWT	Yes	15-20	12	0
0-MT + HWT	Yes	20-25	16	0
2-MT + NHWT	No	10 -15	10	100
2-MT + NHWT	No	15-20	14	0
2-MT + NHWT	No	20-25	21	31
2-MT + HWT	Yes	10 -15	14	100
2-MT + HWT	Yes	15-20	10	-17
2-MT + HWT	Yes	20-25	23	44
10-MT + NHWT	No	10 -15	16	220
10-MT + NHWT	No	15-20	14	0
10-MT + NHWT	No	20-25	14	-13
10-MT + HWT	Yes	10 -15	19	171
10-MT + HWT	Yes	15-20	10	-17
10-MT + HWT	Yes	20-25	16	0

[a] Relative change = (0-MT + NHWT-2 or 10-MT + NHWT)/ 0-MT + NHWT) x 100) for each associated hydroxyl generator and soil moisture level.

HWT treatments than in the associated magnetized seed treatments with 0-MT + NHWT water treatment under the LSM soil moisture level (Table **9**). The non-magnetized seed treatments resulted in an even larger increase in percent soil moisture for the 2-MT + HWT and 10-MT + HWT treatments than in the associated magnetized seed treatments with 0-MT + NHWT water treatment under the LSM soil moisture level (Table **10**). For both seed treatments the structured water treatments (2-MT + HWT and 10-MT + HWT) increased the LSM soil moisture levels, due to lower transpiration rates (Figures **1**, **4**).

Analysis of the cumulative water volume per pot for each treatment shows that only the magnetized water factor and the soil moisture level were terms in the final model (Table **11**). The two other study factors (seed and HWT factors) were not in the final model due to the

Table 10: Average Volumetric Soil Moisture (m^3/m^3) for Non-Magnetized Seed Treatment by Structured Water Treatment, Hydroxyl Generator Status, and Soil Moisture Level. Soil Moisture was Averaged between 13 to 52 Days after Planting

Structured water trt	Hydroxyl generator	Soil moisture Level (%)	Percent soil moisture (%)	Relative change[a]
0-MT + NHWT	No	10 -15	2	0
0-MT + NHWT	No	15-20	21	0
0-MT + NHWT	No	20-25	16	0
0-MT + HWT	Yes	10 -15	5	0
0-MT + HWT	Yes	15-20	16	0
0-MT + HWT	Yes	20-25	19	0
2-MT + NHWT	No	10 -15	7	250
2-MT + NHWT	No	15-20	19	-10
2-MT + NHWT	No	20-25	21	31
2-MT + HWT	Yes	10 -15	10	100
2-MT + HWT	Yes	15-20	14	-13
2-MT + HWT	Yes	20-25	23	21
10-MT + NHWT	No	10 -15	14	600
10-MT + NHWT	No	15-20	19	-10
10-MT + NHWT	No	20-25	14	-13
10-MT + HWT	Yes	10 -15	16	220
10-MT + HWT	Yes	15-20	16	0
10-MT + HWT	Yes	20-25	16	-16

[a] Relative change = (0-MT + NHWT-2 or 10-MT + NHWT)/ 0-MT + NHWT) x 100) for each associated hydroxyl generator and soil moisture level.

small sample size for each treatment. However, since the cumulative water volume per plant is such an important measurement in this study, the total water volumes were reported for all four study factors to fully understand the effects of the factors on water usage (Table **12**). Based on the cumulative water volumes, the optimal water saving treatments were either magnetized or non-magnetized seed treatment combined with the 10-MT + HWT structured water treatment (Table **12**). The relative change for total water volume used represents the water use savings in the last two columns in Table **12**. The water use savings ranged from 41 to 65%, depending on the three soil moisture levels, for the 10-MT +HWT structured water treatment. The cumulative water volume for the 10- MT +HWT water structure is in general agreement with the percent soil moisture findings, i.e., the 10-MT + HWT treatment increased percent soil moisture when compared to the 0-MT + NHWT water treatment (Tables **9-10**). The structured water treatments used more water than the 0-MT + NHWT treatment in three out of six water treatments (Table **12**).

Table 11: Description of the Least Squares Fit Model Terms and *p*-Values for the Cumulative Water Volume Added Per Plant. Daily Water Volumes Per Plant were Summed between 27 and 52 Days after Planting to Determine the Cumulative Water Volume

Source	Prob > F
Magnetized water	0.0001*
Soil moisture level	<.0001*
Magnetized*Soil moisture level	<.0001*

The cumulative water volume relationship with oven dry foliage biomass was visually evaluated for three study factors (Figure **6**). The regression lines for the 10-MT water treatments showed an inverse relationship between water usage and foliage biomass for magnetized seed treatments across the three soil moisture levels. The 0-MT water treatments showed a moderately strong direct relationship between water usage and foliage biomass. This fundamental relationship is universally accepted and is the rationale

Table 12: Cumulative, Total Water Volume Per Plant Reported for Magnetized Water Treatment, Hydroxyl Treatment and Three Soil Moisture Levels. The Total Water Volume was Reported for Daily Watering between 13 to 52 Days of the Study

Water trt	Hydroxyl Generator	Soil moisture level (%)	Sum for Mag seed water volume added (ml) per plants	Sum for Non-Mag seed water volume added (ml) per plant	Water savings for magnet seed[a]	Water savings for non-magnet seed[a]
0-MT	No	10 -15	20000	20950	0.0	0.0
0-MT	No	15-20	11800	11250	0.0	0.0
0-MT	No	20-25	15600	14950	0.0	0.0
0-MT	Yes	10 -15	18150	20150	0.0	0.0
0-MT	Yes	15-20	24450	22600	0.0	0.0
0-MT	Yes	20-25	30550	30400	0.0	0.0
2-MT	No	10 -15	14500	15450	-27.5	-26.3
2-MT	No	15-20	23450	19550	98.7	73.8
2-MT	No	20-25	25850	26700	65.7	78.6
2-MT	Yes	10 -15	13300	14600	-26.7	-27.5
2-MT	Yes	15-20	22100	21900	-9.6	-3.1
2-MT	Yes	20-25	23900	23700	-21.8	-22.0
10-MT	No	10 -15	19450	16700	-2.8	-20.3
10-MT	No	15-20	20100	20650	70.3	83.6
10-MT	No	20-25	19950	21450	27.9	43.5
10-MT	Yes	10 -15	10650	8900	-41.3	-55.8
10-MT	Yes	15-20	19650	18350	-19.6	-18.8
10-MT	Yes	20-25	10600	12500	-65.3	-58.9

[a] Water savings is based on relative change between water treatments and control water treatment. Relative change = (0-MT-2 or 10-MT)/ 0-MT) x 100) for each associated hydroxyl generator, magnetized seed treatment and soil moisture level.

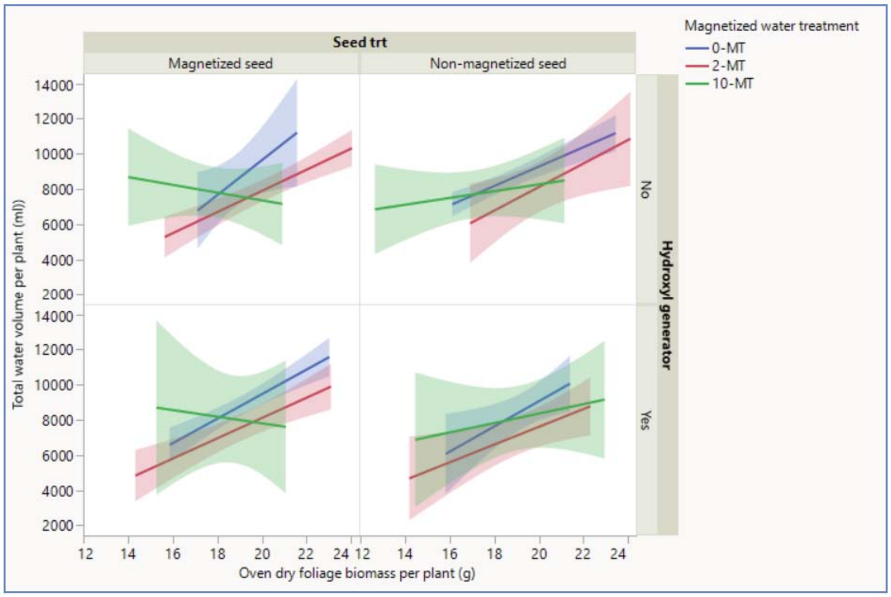

Figure 6: Cumulative water volume (CWV) usage relationship with foliage biomass by structured water treatments. Total water volume reported by seed treatment (upper x-axis), hydroxyl generator (right y-axis) and magnetized water treatments (legend). Regression was averaged across soil moisture levels. Note that the regression line for 10-MT declined for magnetized seed treatments and both hydroxyl treatments, across all three soil moisture levels.

Table 13: Description of the Least Squares Fit Model Terms and *p*-Values for Plant Water Use Efficiency (PWUE). The LS Model Included the Cumulative Water Volume as a Covariate Term

Source	Prob > F
Magnetized water treatment	<.0001*
Soil moisture level	<.0001*
Cumulative water volume per plant	<.0001*
Soil moisture level* Cumulative water volume per plant	0.0088*

Table 14: Plant WUE and Relative Change in PWUE, by Water Treatment and Soil Moisture Level

Water treatment	Soil moisture level (%)	Plant WUE (g biomass/l H2O))	Relative change in WUE efficiency (%)[a]
0-MT	10 -15	2.13	0.0
0-MT	15-20	2.58	0.0
0-MT	20-25	2.72	0.0
2-MT	10 -15	2.23	4.6
2-MT	15-20	2.68	3.8
2-MT	20-25	2.81	3.6
10-MT	10 -15	1.98	-7.2
10-MT	15-20	2.43	-5.9
10-MT	20-25	2.56	-5.6

[a] Relative change in WUE = (0-MT-2 or 10-MT + NHWT)/ 0-MT) x 100) for each associated soil moisture level.

why approximately 58 million crop acres are irrigated annually in the USA.

Analysis of PWUE data reveals that only two study factors and one covariate term, cumulative water volume, affected water use efficiency (Table **13**). Predicted PWUE values are reported for the two study factors, as well as the relative change in PWUE (Table **13**). Plant water use efficiency increased for the three 2-MT structured water treatments but decreased for the three 10-MT structured water treatments for each of three soil moisture levels (Table **14**). The maximum PWUE (2.81) was achieved with the 2-MT + HWT structured water treatment under the HSM soil moisture level. In comparison, Baligar *et al*., [44] found that "total" WUE for a velvet bean cover crop was 1.84 when grown under full sunlight (PPFD = 400 µmol/m2/s), which was the same sunlight conditions for the vines growing on stakes in a greenhouse.

The biomass analysis (ABGR biomass) shows that growth rates were affected by the magnetized water treatments and the soil moisture levels (Table **15**). The maximum ABGR and foliage biomass was 0.35 g/day and 22.2 g/plant, respectively, which was achieved with the 2-MT + HWT structured water treatment under the HSM soil moisture level (Table **16**). The second highest ABGR and foliage biomass was 0.35 g/day and 21.8 g/plant for the 0-MT + NHWT water treatment under the HSM soil moisture level.

Table 15: Description of the Least Squares Fit Model Terms and p-Values for the Relative Growth Rate (g/day) and Foliage Biomass (g). The LS Model only had Two Significant Terms.

Source	Prob > F
Magnetized water treatment	0.0009*
Soil moisture level	<.0001*

The pilot study included two different trials. The first trial evaluated the ability of structured water to remain stable over six days. The second watering trial was conducted to determine whether the water remained stable after watering plants in potting soil and collecting the vapor condensate from the plant foliage. The pilot study revealed that the structured water remained stable over a six-day period when the water was measured under hot (> 32 C) greenhouse conditions (Figure **7**). Electrical conductivity of the structured water paralleled the sine wave pattern of the greenhouse diurnal temperatures over the six-day measurement period (Figure **7**).

Table 16: Average Daily Foliage Biomass Growth Rate and Oven-Dry, Foliage Biomass Per Plant. Based on the LSF Model Significant Model Terms but Averaged Across Seed Treatments and Hydroxyl Generator Treatments

Water trt	Soil moisture level (%)	Average daily biomass growth rate (g/day)	Foliage oven dry biomass (g)
0-MT	10 -15	0.26	16.3
0-MT	15-20	0.32	19.9
0-MT	20-25	0.35	21.8
2-MT	10 -15	0.26	16.8
2-MT	15-20	0.32	20.4
2-MT	20-25	0.35	22.2
10-MT	10 -15	0.24	14.8
10-MT	15-20	0.30	18.4
10-MT	20-25	0.33	20.3

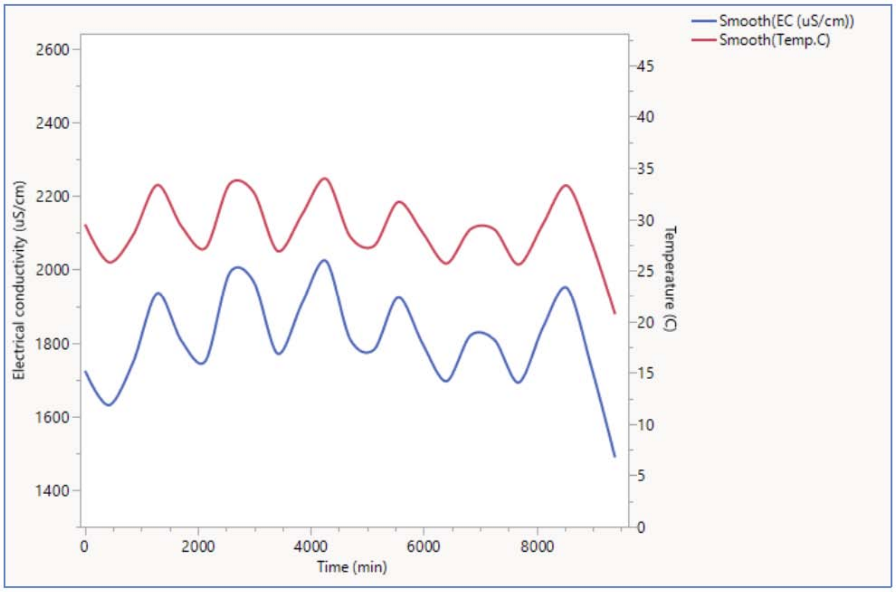

Figure 7: Statistical smoother lines show the relationships between structured water electrical conductivity (left y-axis) and temperature (right y-axis) over time (10 min time intervals) on x-axis. Smoother lines described in legend.

Table 17: Water Properties of Structured and Filtered Tap Water before and after Watering Velvet Bean Plants and Collecting Foliage Transpiration

Water property	SW before[a]	SW after	TW before	TW after
EC (uS/cm)	1,349	172	323	565
ORP (mV)	-56	-9	-43	-7
pH	7.9	7.1	7.8	7.2

[a] SW = structured water and TW = filtered tap water.

4. DISCUSSION

At 21 days after planting, daily watering was reduced, and soil moisture levels were monitored until the three irrigation target levels were reached (Figure **4**). Leaf wilting symptoms were monitored each morning during this time, and short-term wilting appeared when volumetric soil moisture levels were below 10% (v/v). Wilted leaves generally returned to full turgor after watering each morning which indicated that the plants were under moderate water stress for the low, and medium water irrigation targets. The soil volume in 3.8 l pots had limited water holding capacity which made it a challenge to maintain even semi-stable soil moisture conditions. At the end of the study the velvet bean plants ranged in height from 3 to 4 m and had an estimated leaf area that ranged from 3,000 to 5,000 cm^2 (Images **2-4**). The large plants, under the high irrigation schedule, readily transpired up to 1,000 to 1,500 ml of water each day even when they were watered daily. As the plants grew to the top of the 2.5 m stakes, the daily water volume gradually increased to adjust for higher transpiration rates and to maintain the target soil moisture levels.

The study design had variable sample numbers for the different study variables. For example, the gas exchange measurements had the highest sample number (n=12), and the cumulative water volume had the lowest sample number (n=2). Sample size is crucial in narrowing the error bars or confidence limits in regression analysis. The REML and LSF models included 16 statistical replications due to limiting the factorial analysis to two-way interactions. However, all regression tests relied on the typically smaller sample size for each measured response. For example, the CWV and biomass regression had a sample size of n=4 and thus had large confidence intervals (Figure **6**). Also, correlation tests can show that a relationship is strong and yet not significant, or conversely a relationship is weak but significant. Sample size is a key determinate in testing for correlation probabilities, i.e., small samples can easily generate strong correlations by chance and only by testing the *p*-values that may reveal any significant relationships.

Field studies involving soybean crops under water stress show that photosynthesis, stomatal conductance, and yield were all reduced or temporarily shut down [46-50]. Drought tolerance studies have shown that photosynthesis and transpiration decrease under water and heat stress when applied separately or as combination treatments [51-52]. Heat and water stress cause partial or complete stomatal closure, depending on the severity of the stress [51-52]. Partial stomatal closure limits gas exchange and thereby lowers Ci which in turn lowers Pn [48-52]. In this study there was a strong positive and significant relationship between Ci and g for the 0-MT + NHWT treatment at the MSM level. Also, there was a strong positive and significant relationship between Ci and g for the 0-MT + NHWT treatment at the LSM level which substantiates that Ci is directly related to g flux rates. In contrast, there was no relationship between Ci and g or E, vpdl and ltemp for the 10-MT + HWT treatment at the LSM soil moisture level. In other words, stomatal conductance and transpiration had no effect on internal CO_2 concentrations for the 10-MT + HWT treatment, under the LSM soil moisture level, which is contrary to widely accepted stress physiology principles (Table **4**).

A water stress study with legumes by Reynolds-Henne *et al.*, [53] showed that legumes respond to moderate temperature stress by closing stomata or causing irregular conductance but increasing stomatal conductance under high temperature stress. In this study high leaf temperature lowered and flattened g rates under the LSM soil treatment and the 10-MT + HWT treatment (Figure **1**). The gas exchange results of this study are contrary to the findings in the Reynolds-Henne drought study with legumes [53] but validated the gas exchange findings in a legume-based drought tolerance study by Ramsey [45].

The relationships between Pn, g and E were further explored for ltemp, IWUE and vpdl, for LSM soil moisture treatment (Figures **1-3**). In all three graphs Pn was visually lower for the 10-MT +HWT when compared to the control or 2-MT+HWT, for both non-magnetized and magnetized seeds. In other words, ltemp, IWUE and vpdl had virtually no effect on Pn for the 10-MT+HWT treatment. Also, E increased with ltemp for the control treatment, but decreased for both 2-MT+HWT and 10-MT+HWT under LSM moisture levels. The graphs also show that the magnetized seed treatments tended to flatten out the responses of Pn, g and E in relation with ltemp, IWUE and vpdl for the LSM treatment (Figures **1-3**). Finally, IWUE decreased with increasing Pn and g for the non-magnetized seeds, but IWUE increased with increasing Pn and g for the magnetized seeds. The inverse relationship between soil moisture and E for the magnetized seed combined with the 10-MT water treatments shows that the combined treatments can unlink or disassociate the natural stressed plant responses to low soil moisture conditions. The combined seed and water treatments fundamentally alter drought adaptation plant responses to water stress conditions which resulted in a significant reduction in irrigation water usage.

Water savings due to the combined effects of the magnetized seed and structured water treatments were calculated from the cumulative water volume per plant for selected treatments (Table **12**). The best water saving treatment, at the LSM moisture level, was the combination of magnetized seeds and 10-MT + HWT structured water treatment with a 41% water savings. The highest water savings was 65% for the magnetized seed and 10-MT + HWT treatment at the HSM soil moisture level. In comparison smart irrigation controller systems can achieve from 30 to 50% water savings [54]. The water savings resulting from this study (40 to 60%) is unprecedented. Further studies with combined seed and structured water treatments are needed to confirm these findings. It may be possible to combine smart irrigation systems with magnetized seed and structured water generator systems to significantly reduce water usage in crops, lawn, and landscape irrigation. Magnetized irrigation water studies have indirectly reported water savings by reporting crop yields equivalent to the control crop yields even when watered under deficit irrigation schedules [6-11].

Structured water has antioxidant properties due to the quasi-free, delocalized electrons that circle around the hexagonal rings of water. The delocalized electrons can readily quench excess generation of free radicals produced along the electron transfer chain during photosynthesis in heat and/or water stressed plants [20-23, 25]. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidases (GPxs are widely recognized in quenching reactive oxygen species (ROS) that are generated in the electron transfer chain in photosynthesis. However, the ROS quenching ability of structured water has still to be recognized as important in plant physiology and water property interactions in drought tolerant crop research. If structured water can be generated so that it remains stable even after exposure in soil environments and plant vascular systems, then it holds promise to reduce ROS damage during photosynthesis in water stressed crops.

Water use efficiency is defined as the amount of carbon assimilated as biomass or grain produced per unit of water used by the crop [55-58]. Gas exchange measurements are used to calculate IWUE at the leaf level, which is a function of light intensity on the leaf, Pn, g, E and vpdl. IWUE is calculated as the net photosynthetic rate (Pn) divided by transpiration rate (E), based on equivalent gas exchange units [55]. Under water stress conditions IWUE increases due to the higher reduction in Pn compared to smaller reduction of E or g. This study included both IWUE and PWUE estimates. The PWUE results were incongruous with the water savings results for the 10-MT + HWT water treatments. Although PWUE is widely accepted as a parameter to evaluate drought tolerant crop research, the parameter is broadly defined, and other parameters have been offered that mitigate some of its deficiencies [55-58].

There is an ongoing debate on the conceptual differences among water use efficiency, water productivity, and water savings definitions [55-58]. Several water researchers claim that water productivity is the correct term to use when describing the ratio of units of crop biomass or yield over units of water used to grow that biomass. Water use efficiency they argue is a dimension-less percentage, without any units. This study retained the older definition of water use efficiency that utilized the ratio of units of biomass over units of water used. However, both water parameters (water productivity or water use efficiency) assume that there is a moderate to strong direct relationship between crop biomass or yield and water usage. This fundamental assumption, however, would be misleading if applied to plant biomass growth and structured water usage found in this study. In this study, there was an inverse relationship between soil moisture and E for the magnetized seed and 10-MT water treatments (Figure **4**). Also, this study shows that plant biomass was not related to total water volume usage for the 10-MT + HWT structured water treatments across three soil moisture levels (Figure **6**). The PWUE results from this study are clearly contrary with the relative water savings results, i.e., PWUE had a negative value but there was a large water savings for the 10-MT + HWT water treatments. Due to the putative lack of relationship between biomass/yield and water usage it may be more appropriate to include other parameters such as relative water savings when comparing structured water irrigation research with conventional irrigation methods. Relative water savings is calculated as the percent difference in water usage between two irrigation methods. Relative water savings would more accurately portray the full benefits of structured water irrigation than water use efficiency. The water parameters used to compare irrigation methods or systems should be reexamined for their basic assumptions and potential deficiencies when evaluating structured water systems with conventional systems.

Soil moisture field capacity and wilting point for mixtures of peat moss were investigated by Londra *et al.*, [59]. They found that a mixture of sphagnum peat moss (75% v/v) and perlite (25% v/v) had an 26% and 20% capacity for easily available water and difficult available water, respectively. The potting soil in the Londra study was very similar to the potting soil used in this study. The field capacity for the 75/25 moss/perlite mixture was approximately 75 to 80% and the non-available water capacity was 12.3% soil moisture. In this study the daily leaf wilting patterns were observed between 13 and 21 days after planting and compared to the soil moisture data collected each morning. Leaf wilting occurred in the early morning when soil moisture levels were most depleted and reached as low as 8-10% (v/v). The wilted leaves returned to normal within an hour or so after the mid-morning watering. Visual observation of the wilting patterns, combined with the soil moisture data, ensured that the plants under the low and moderate irrigation targets were subjected from moderate to mild water stress conditions for the duration of the study. The high-water capacity of the peat moss-based soil (75-80% v/v) helped the plants by making water available over most of the daytime hours of the study. Also, daily watering ensured that soil moisture levels remained with a narrow range (Figure **5**) for each of the irrigation targets over the study duration.

The first pilot study investigated the stability of structured water under >30 C greenhouse conditions. The degree of water structure was estimated by measuring the electrical conductivity of the treated water. The pilot study showed that the water was structured with a conductivity approximately five-fold higher than filtered tap water. Also, the study revealed that the diurnal conductivity patterns paralleled the greenhouse temperature patterns and that conductivity was stable with no signs of diminishing over the six-day measurement period (Figure **7**). A review article by Lindinger [28] reports that a method of generating structured water was stable for up to 2.5 months.

The second phase of the pilot study evaluated the stability of the structured water in a potting soil and plant transpiration test. The study results show that electrical conductivity decreased for the structured water, but increased for the filtered, tap water treatment (Table **17**). These results show that transpiration vapor collected as leaf condensate consists of free water with fewer H-bonds which allows the water molecules to rapidly vaporize inside the leaves. Structured water has a higher latent heat of vaporization, therefore free water with fewer H-bonds will vaporize before structured water.

Rascio [60] reviewed the relationships between bound water in plants and abiotic stresses. He reported that drought tolerant ferns and durum wheat had a high affinity for bound water on cell membranes [61]. He also postulated about the importance of bound water inducing drought resistance in plants by preventing cell dehydration under water stress conditions. Also, Kuroki *et al*., [62] found that water in resurrection plants (*Haberlea rhodopensis*) formed different molecular structures due to the number of H-bonds formed inside cells. The plant species could readily transition between the different water structures by reducing or increasing the H-bond numbers. They found that this plant species adjusted to extreme dehydration by increasing the number of H-bonds in water [62]. As the H-bonds increased, the structured or highly bound water increased in plant tissue thereby preventing cell damage. The plant's ability to rapidly adjust the level of bound water to match a wide range of soil moisture levels is probably present to a lesser degree in many drought tolerant plants [60-61]. The mechanism plants use to convert sunlight and free water into structured water so that plants can rapidly adjust to low soil moisture conditions is not fully understood yet. However, it is clear from magnetized irrigation water studies that plants can absorb and uptake partially structured water to supplement its own reserves and increase its drought tolerance levels under low soil moisture conditions [28, 64-71]. All plant species have a range in capacity to adjust bound water levels to mitigate harsh environmental conditions. They also have the ability to transition bound water back to free water when soil moisture increases. It appears that the well-watered plants in the pilot study were able to transition the structured water back to free water with a lower latent heat of vaporization that allows the free water to rapidly vaporize and reduce leaf temperatures. Future drought tolerance studies should include measurements for free, lightly bound and highly bound water inside plant tissue to assess the agility of the plant species to adjust their drought tolerance ability.

Oven dry biomass slightly decreased for the 10-MT+HWT treatment but increased for the 2-MT+HWT treatment when compared to the controls (Table **7**). The gas exchange results showed that Pn was significantly lower for the 10-MT+HWT treatments under the LSM soil treatment. The lower Pn and lower water usage for the 10-MT+HWT treatments suggests that less photosynthate was needed to maintain respiration rates and ensure sufficient growth rates. If leaf turgor can be maintained under water stress conditions, then less photosynthate resources are needed for plant biomass to provide plant structure and support. This hypothesis is supported in a magnetized irrigation study by Shabana and Abdelhady [52]. They found that the cell water retention capacity increased with the magnetized watering treatment grown under salinity conditions. Partially structured water appears to maintain leaf turgor in salt stressed plants that may have had an osmotic gradient favoring extracellular water content [52]. In addition, a previous drought tolerance study by Ramsey [45] shows that a single foliage application with a magnetized sprayer, using a chelated iron solution, reduces the dry tissue biomass for immature foliage, when compared to the control treatment. In this present study, the oven dry foliage biomass is not related to water usage for the 10-MT-HWT treatments for the three soil moisture levels (Figure **6**). These three study results suggest that strongly structured water may allow plants to use less photosynthate for structural tissue. If bound water in plants can be increased with irrigated structured water, then leaf turgor may also be maintained or enhanced even under drought or other abiotic stress conditions.

There is a paucity of articles showing the effects of structured water on crop growth and yields [1-6]. A non-replicated, pilot study by Smirnov *et al*., [63] evaluated the effects of a five-day, water stress experiment on tomato and parsley sprouts irrigated with tap water and structured water. They published photographs that showed that the structured watered sprouts were more vigorous and resistant to water stress than the tap watered sprouts. Another plant study by Smirnov [63] found that their activated water treatments increased biomass by 24 and 22% for potato and giant red radish plants after 21 to 25 days from seed germination. A review article by Martin [2] reported that non-replicated field studies with sugarcane crops irrigated with partially structured water using KELEA ceramic tubes increased yields by 12.9% over non-structured irrigation water. In comparison this study revealed a tradeoff between growth and water usage. This study had a 6.8 % decrease in oven dry foliage biomass for the best magnetized seed and structured water treatment, compared to the control treatment. The biomass loss was more than compensated with a 41% savings in water usage, 25% reduction in Pn, 34% reduction in stomatal conductance, and a 7% reduction in internal carbon dioxide under the LSM soil moisture level for the combined treatments (Tables **6**, **12**).

CONCLUSION

The nascent research field for evaluating structured water to irrigate crops shows promise in saving water resources and improving drought tolerance in crops [1-7, 64-71]. Research involving magnetized water also reveals that partially structured water may improve disease resistance in crops [72-76]. The underlying mechanisms for improving drought tolerance and/or disease resistance will involve more advanced basic and applied research into seed and structured water treatments and their interactive effects on animal and plant health [13, 20-21, 28, 31-33]. Different forms of structured water have been studied for decades under other water research [77-82].

Future research is needed to determine the effects of water generator parameters such as time of exposure, number of water treatment passes, strength or intensity of magnetic fields and water hydroxylation methods on the quality and stability of structured water. Also, further research is needed to determine whether physicochemical properties of structured irrigation water are related to improved drought tolerance and disease resistance. These physicochemical water properties can be measured with a multi-meter [83-84]. Full scale irrigation systems need to be designed to include magnetized seed and structured water treatments. Magnetized seeds combined with a structured water irrigation system could be evaluated for improved drought tolerance for drip irrigated crops and for water and cost savings when compared to a conventional system water usage. The interactions among magnetized seed treatments, water properties, redox biology, plant metabolism, water productivity/conservation and crop stress physiology should be further investigated given the promising results from this study.

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