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Comparison of Plant Trait Biometrics for Paired Invasive and Non-Invasive Species to Magnetized Seed and Watering Treatments

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ABSTRACT

A greenhouse study evaluated the widely held hypothesis that invasive plant species have a quicker or stronger response to environmental stimuli such as magnetized irrigation water treatments. A second study objective was to test whether the polarity of magnetized water affected the responses for invasive and non-invasive plant species. Six invasive and six non-invasive plant species were stimulated by magnetizing the seeds followed by applying several magnetized water treatments to the germinated seeds. The species were taxonomically paired then the seeds were exposed to three magnetic field treatments, planted, and irrigated with three magnetized water treatments for approximately two months. The electrical conductivity, oxidation reduction potential (ORP), pH of the water, and nine plant biometrics were measured, collected, and analyzed. The study hypothesis was validated when the invasive species showed enhanced responses to the magnetized seed and water treatments. The invasive species had increased growth in seven out of the nine growth biometrics when exposed to the magnetized seed and water treatments. The long exposure time for pretreatment of seeds (six days) and extended exposure time of the water treatments on the magnets (20 h) contributed to the higher growth rates. The average increase in foliar biomass and leaf area for two paired, invasive species was 184 and 182%, respectively, for the combined seed/watering treatments. In comparison the average increase in foliar biomass and leaf area for two paired, non-invasive species was 88 and 111%, respectively, for the combined seed/watering treatments. The physicochemical water properties for the three magnetized water treatments were correlated with plant growth. The combined magnetic seed/watering treatments produced growth rates that substantially exceeded crop growth rates in comparable magnetized irrigation studies.

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1. Introduction

Three interacting biological processes determine the ability of a plant species to become invasive: 1) the ability to propagate and disperse, 2) the ability to exploit resources and escape predation, and 3) the ability to adapt and adjust to the environment. A widely accepted hypothesis is that invasive plant species adapt to environmental stimuli or stressors more readily or more intensely than non-invasive species [1-2]. In this study we evaluated the ability of a plant species to adapt and adjust to an environmental stimulus by measuring plant traits associated with invasiveness. Plant traits used for adaptation and environmental adjustment such as epigenetic inheritance, phenotypic plasticity and genetic variation may enable a species to rapidly alter its phenotype when exposed to environmental stimuli such as nutrients, light, or water resources [3]. To evaluate the adaptive ability of invasive plants a study should be designed that compares taxonomically paired species with known invasive and non-invasive properties.

Invasive plant trait models could be designed by exposing different species to environmental stimuli such as light, water, temperature, or nitrogen to quantify and rank plant traits with invasiveness. Plant studies designed to include both stimuli/stressor factors and invasive/non-invasive species may be able to correlate plasticity potential within a genotype with their associated invasive potential [4-5]. This study was designed to correlate the phenotypic plasticity potential, based on seedling traits, with the known invasive classification for each species when exposed to magnetized seed and watering treatments.

The rationale for using magnetized seed and watering treatments as the environmental stimuli is that numerous studies using these magnetic treatments have shown enhanced plant growth, physiology, crop yields and drought tolerance [6-7]. Zdyrska *et al*. [8] studied the growth of *Lupinus angustifolius* (lupine) plants after a set of magnetized seed treatments. Suarez-Rivero *et al*. [9] studied *Zea mays* growth after 21 days of exposure to static magnets. Ali *et al*. [10] and Chibowski and Szczes [11] published reviews on magnetized water and magnetized irrigation water. Duarte-Diaz *et al*. [12] and Maheshwari and Grewal [13] investigated the effects of magnetized irrigation water on crop growth. Baghel *et al*., [14] found that magnetized irrigation water increased Fv/Fm in water stressed soybean plants. Both Ghanati *et al*. [15] and Hasan *et al*. [16] found that magnetized irrigation water reduced membrane lipid peroxidation which is caused by excess free radical injury. Other crop irrigation studies by Yusuf and Ogunlela [17], Baghel *et al*. [14], and Selim and El-Nady [18] found that magnetized irrigation water improved plant physiological and growth responses under deficit irrigation schedules or used osmotic chemical treatments to create water stress conditions. A study by Mostafazaden-Fard *et al*. [19] found that magnetized irrigation water increased soil moisture by 7.5%. These studies provide substantial evidence that magnetized seeds and irrigation water stimulates plant growth. Invasive species should be able to capitalize on such environmental stimuli treatments in contrast to non-invasive species responses.

The effect of static magnetic fields on water properties is dependent on; 1) field strength (mT), 2) magnet composition or grade, 3) length of exposure time to magnetic field and 4) magnetic field polarity (North and South Pole). The length of exposure time is an important parameter in closed loop systems where water is recirculated between a storage tank and magnetized water lines before being applied to crops. North Pole fields generally increase plant tolerance to biotic and abiotic environmental stressors, while South Pole fields tend to stimulate plant growth [20-22]. Ruzcariccar *et al*. [23] found that shoot growth of *Castenea sativa* in a Petri dish culture, when exposed to North Pole magnetic fields, grew about 18% less than the control culture shoots. They also found that South Pole magnetic fields (20mT, 28-day exposure) increased seedling growth from 86 to 138% of the control seedlings. Potenza *et al*. [24] found that South Pole magnetic fields increased cell growth of *Escherichia coli*. Magnetized seed and plantlet studies also show that South Pole fields stimulate plant growth [23, 25-27].

Despite a long history of investigating energized water properties there is still little consensus on water structure models developed from energized or magnetized water treatments. The physicochemical properties of magnetized water have yet to be correlated with plant growth responses. Recent research suggests that the structure of magnetized water and biological water surrounding cell membranes are analogous [28-31]. Binhi [32] conducted a review of theoretical mechanisms underlying magnetizing water and mentioned that magnetic fields changed the structure of water. At the quantum level of energized water molecules Barnes and Greenebaum [33] found a direct relationship between magnetic field strength and the velocity of the valence electrons which leads to a higher orbital energy state and excited water molecules as field strength increases. Acceleration of valence electrons due to magnetic fields is the probable quantum mechanism by which magnetized water converts into two phases where a fraction of the water forms hexagonal rings and becomes structured water [32, 34]. The water fraction converted into structured water depends on magnetic field strength and exposure time. Ibrahim [35] found that electrical conductivity of water increased with increasing strength of the static magnetic field providing evidence of excited valence electrons in higher orbits. Marais *et al*. [36] and Hakala-Yatkin *et al*. [37] found that magnetic fields reduced photoinhibition in light-stressed plants by reducing the generation of disruptive free radicals. The effects of magnetic fields on free radicals have also been studied by Hansen and Pederson [38] and Wang and Ritz [39]. Szczes *et al*. [40] investigated the effects of magnetic fields on hydrogen bond strength in water. As the strength of hydrogen bonds in water increases under a magnetic field, hexagonal rings of water molecules start self-propagating, which modifies the physicochemical properties of water [41-43].

The physicochemical properties of water are also altered by cold plasma or microbubble technologies. Redox properties of cold plasma treated water has been correlated with enhanced plant growth rates [44-46]. Static magnets alter the physicochemical water properties such as oxidation reduction potential (ORP), pH, and electrical conductivity. However, the redox properties of magnetized irrigation water have not been evaluated in plant growth studies. Hassen *et al*. [47] found that static magnets (100 to 200 mT) increased electrical conductivity by 3% but reduced ORP by 16.9%. Yin *et al*. [43] found that tap water exposed to a static magnetic field (500 mT) for 7 h increased electrical conductivity by 1.2% but decreased ORP by 14%. The physicochemical properties of water can be manipulated with energy field treatments and can be optimized for enhanced plant growth. In addition to testing the invasive species adaptability hypothesis, this study also evaluated the effects of physicochemical water properties on seedling growth.

The primary hypothesis of this study was to determine whether invasive plant species adapted more rapidly or intensely to environmental stimuli than taxonomically paired non-invasive species. A second hypothesis is that the magnetized water treatments altered the physicochemical water properties thereby enhancing seedling growth.

2. Materials and Methods

The physicochemical water properties were measured with a multi-meter (ORP/EC/pH meter – model PC 650, Oakton Instruments, Vernon Hills, IL, USA). The water measurements included oxidation reduction potential, electrical conductivity, and pH. The measurements were taken for tap water without magnetic field exposure and tap water placed on static magnets. The static neodymium magnets (5 x 7.6 cm) (K&J Magnets, Inc., Pipersville, PA, USA) were grade N-42 with a measured surface strength of 570 mT. The water was magnetized by adding water to hard plastic containers and placing the containers on top of the magnets for 20 h before taking the water measurements. The physicochemical water measurements are listed in Table **1**.

Table 1: Taxonomically paired plant species based on known invasiveness status. The species were paired at the Family level

Pair	Family	Invasive Species	Non-Invasive Species	Family
1	*Verbenaceae*	*Verbena hastasta*	*Lysimachia vulgarius*	*Primulaceae*
2	*Lythraceae*	*Lythrum salicaria*	*Lythrum virgatum*	*Lythraceae*
3	*Asteraceae*	*Cirsium arvenses*	*Cirsium undulatum*	*Asteraceae*
4	*Asteraceae*	*Chrysanthemum vulgare*	*Erigeron speciosus*	*Asteraceae*
5	*Scrophulariaceae*	*Linaria vulgaris*	*Pensetmom angustifolius*	*Scrophylariaceae*
6	*Poaceae*	*Sorghum halepense*	*Sorghum vulgarae*	*Poaceae*

Twelve plant species were selected for this greenhouse study including six species classified as highly invasive, and six other species classified as non-invasive. The invasive species were taxonomically paired with a non-invasive species at the taxonomic Family level (Table **1**). The exceptions to this pairing method were *L. vulgarius* (yellow loosestrife) which was paired with *V. hastasta* (blue vervain). *V. hastasta* is taxonomically related to loosestrife and is considered non-invasive.

Each plant species had a paired set of magnetized seed and magnetized water treatments. The three magnetized seed treatments were paired by magnetic polarity with the three water treatments. The three sets of magnetized seed treatments were: 1) no magnetic field, 2) North Pole face of magnet, and 3) South Pole face of magnet. The three magnetized water treatments were: 1) no magnetic field, 2) North Pole, and 3) South Pole. The static neodymium magnets (5 x 7.6 cm) used for both the seed and water treatments had a surface strength of 570 mT.

There were 40 seeds per plant species for each of three magnetized water treatments for a total of 120 seeds per species. Seeds were sandpapered to break seed dormancy and stimulate germination rates. Chemical stimulants were also used to promote seed germination which included 26 µl of NPK seaweed extract plus (Stimupro, Robertsdale, AL), 12 µl of Silwet L-77 organo-silicone surfactant (Helena, Collierville, TN), and Dyna Green calcium chloride (Hummert International, Topeka, KS). Seeds and chemicals were added to 10 ml of water, placed in 30 ml plastic cups, and soaked for 24 hours on static magnets. After 24 h of seed soaking on three magnetic treatments (North or South Pole facing magnets, and no magnets for control seeds) the seeds and chemical solutions were germinated in Petri dishes. The Petri dishes contained Super Smoke Plus pink filter paper (CAPE, San Francisco, CA) and seeds were sandwiched between two filter papers. Also, the seeds were dusted with Dampaide power (Seedman, Vancleave, MS, USA) to inhibit fungal infection. The Petri dishes were then placed on top of the static magnets (North or South Pole facing magnets, and no magnets for control seeds) for six days.

Magnetized seeds for each species were planted in plastic trays (Stuewe & Sons, Tangent, OR, USA). Tray cells (6 x 12 cells/tray) were filled with Fafard Growing Mix 4P Professional Formula potting soil (Sungro Horticulture, Agawam, MA, USA). Each cell (~ 30 ml) was planted with one seed at 5 cm depth below the soil surface with the exception of *L. vulgaris* seeds, which were placed on the top of the soil surface to be exposed directly to the sunlight.

Table 2: MANOVA model results for four plant trait responses. The four traits were plant height, average leaf width, oven dry biomass, and root/shoot biomass ratio

Source	*p*-value
Invasive class	<.0001
Magnetic polarity	<.0001
Invasive class*magnetic polarity	<.0001

Table 3: Average electrical conductivity, oxidation reduction potential and pH for filtered tap water and filtered tap water exposed to static neodymium magnets (570 mT) for 20 hr. The three physicochemical water properties were tested using the South and North pole magnetic fields

Water treatment	Electrical conductivity (uS/cm) [a]	Oxidation reduction potential (ORP) (mV) [a]	pH [a]
Filtered tap water	140 b	-46 b	7.8 b
South Pole magnetized water	328 a	-76 a	8.3 a
North Pole magnetized water	335 a	-76 a	8.3 a

[a] Levels not connected with the same letter are significantly different.

The greenhouse environmental controls were set at a maximum temperature of 26.67° and a photoperiod of 16 hours of light followed by 8 hours of darkness. Seedlings were watered daily for 64 days with the matched, magnetized water treatment. The daily water treatments were prepared by placing 1L of water on either a North or South Pole static magnet. The control water treatment was not exposed to any magnetic fields. The water containers were exposed to the magnetic fields for 20 h before watering the seedlings with the three water treatments. Static, neodymium magnets (5 x 7.6 cm) used to magnetize the tap water had a surface strength of

570 mT. Once a week an Essential Plus root and plant stimulant (Growth Products, White Plains, NY, USA) was added at a rate of 120 ml per 800 ml for all three water treatments.

At 64 days after planting the seedlings were harvested, root washed, and measured for foliar, root and total biomass, plant height, leaf area, and leaf width. The relative growth rate (g/day) and root/shoot ratio for the seedlings were calculated from the initial dataset. Immediately after harvesting seedling height measurements were taken then each leaf was removed from a seedling and leaf area was measured with a LICOR 3100 leaf area meter (LI-COR Environmental, Lincoln, NE, USA). Seedling samples were then oven dried at 60°C (Yamato Scientific Co. Ltd. Tokyo, Japan) until biomass weights were constant. Foliar and root biomass was weighed to nearest 0.01g (Mettler Toledo, Columbus, OH, USA).

The study factors were combined with the data for the eight plant responses and compiled together with the physicochemical water property data in a master dataset. The data for the water properties were collected independent of the water used to irrigate the plant seedlings. Due to the independence of the two water treatment datasets the compiled dataset was not analyzed with Least Squares Fit models to quantify the water property effects on the plant responses. The two datasets were only analyzed for potential linear relationships between the two quantified variables. The study design only allows the qualitative levels for plant invasive classification and magnetized water properties (control or South Pole polarity water treatments) to analyze the effects of the plant classification and water treatments on plant responses.

Analysis of this study was conducted with JMP software (SAS Institute Inc., Clary, NC, USA). The MANOVA program tested for study factor effects on the seedling responses. Multivariate logistic regression analysis was used to fit the predictive model to four plant traits with strong responses to the water treatments. The four plant traits were 1) total oven dry plant biomass (root + shoot biomass), 2) plant height, 3) average leaf width, and 4) root/shoot ratio. Logistic models were validated by excluding every fifth data point for each treatment while developing the models. The excluded data was included in the validation test to predict the invasiveness class for each species based on the excluded data. Significance was set at $\alpha = 0.05$. Paired species comparison testing was completed for the invasive/non-invasive species to determine the effects of magnetic seed and water treatments and invasive status. The Student's t-test was used to determine any treatment differences.

3. Results

Low seed germination rates prevented data analysis for four out of six paired species, despite planting a high number of seeds (40 seeds) for each specie and seed/magnetized water treatments. Only eight out of twelve planted species germinated and grew into seedlings for an adequate dataset for the multivariate logistic analysis which was used to predict species invasiveness. All species were taxonomically paired (one invasive to one non-invasive), except purple loosestrife which was compared with two non-invasive species (Blue vervain and E. wand loosestrife). SAS cluster analysis was used to test all twelve plant traits for multicollinear problems. Four plant traits were chosen for their high differentiation ability and low collinearity problems. The traits were: 1) Plant height, 2) Average leaf width, 3) Root/shoot ratio, and 4) Root biomass growth per day. Combining the four plant traits into a multi-dimensional analysis could not be viewed in a single graph but was better expressed as a numeric probability table (Table **4**). Although the invasive species had larger foliar and biomass parameters than the non-invasive species, the relative growth responses to the south pole stimuli were mixed with no clear pattern between invasive classes.

Table 4: Analytical results (*p*-value) for *Lythrum salicaria* vs. *Lythrum virgatum* for study factors and their interaction term

Source	Foliar Biomass (g)	Root Biomass (g)	Plant Biomass (g)	Height (cm)	Leaf Length (mm)	Root to Shoot Ratio	Leaf Area (sq cm)	Max Leaf Width (cm)	Ave. Leaf Area (sq cm)
Species	0.0415	0.0029	0.0013	0.0001	<0.0001	0.0052	0.0303	0.0368	<0.0001
Magnetic Polarity	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.9495	<0.0001	<0.0001	<0.0001
Species * Magnetic Polarity	<0.0001	0.1796	0.0118	<0.0001	<0.0001	0.0004	<0.0001	0.0021	0.4922

The multivariate logistic model predicted that 28 out of 40 plants tested were accurately evaluated as invasive based on the four plant traits (Fig. **1**). The model also showed that 30 out of 42 plants were accurately predicted to be non-invasive. The overall accuracy of the predictive model was 70% (28 were predicted invasive out of 40 true invasive plants) for invasive classification. Also, the model had an accuracy of 71% for predicting non-invasive species (30 were predicted non-invasive out of 42 true non-invasive plants). The validation model found that 17 out of 19 plants known to be invasive were predicted to be invasive. The validation model also showed that 15 out of 17 were predicted to be non-invasive, while 2 out of 17 were falsely predicted to be invasive when they were non-invasive. Accuracy of the model improved with inclusion of taxonomically similar species in the developmental dataset. Accuracy is also improved by selecting the best combination of traits that accurately separated species into invasive or non-invasive classes. Single traits generally have low statistical power to differentiate species. However, when traits are combined the synergistic interaction improves their differentiation ability in the overall model.

Numeric results of predictive model,
based on validation dataset for eight species

Known invasive status	Count Total % Col % Row %	**Predicted invasive**	**Predicted non-invasive**	**Total counts**
	True invasive	28 34.15 70.00 70.00	12 14.63 28.57 30.00	40 48.78
	True non-invasive	12 14.63 30.00 28.57	30 36.59 71.43 71.43	42 51.22
		40 48.78	42 51.22	82

Figure 1: Numeric table of predictive model results for eight species. Probability ratio is 28 plants out of 40 were accurately predicted to be invasive (highlighted numbers in top row).

The probability of invasiveness was estimated for the eight species using the control and South Pole magnetized treatments, based on the root/shoot ratio trait (Fig. **2**). The single plant trait was chosen to visually show the effects of magnetized seed and watering treatments on the invasive ranking of the seedlings. When the probability of invasiveness was graphed using colored points based on the apriori invasive status of each specie the South Pole treatments were easier to visually differentiate the invasive from the non-invasive species (Fig. **2**). The data points for the probability of invasiveness for the South Pole treatments were grouped closer together showing a smaller variance when compared to the data points for the control root/shoot ratios. The graph offers visual evidence validating the first study hypothesis, i.e., the invasive species appear to adapt quicker or stronger to the South Pole magnetized seed and watering treatments when compared to the non-invasive species. The Tukey multiple range test (Fig. **3**) shows that the mean probability of invasiveness for the invasive species was significantly higher than the non-invasive species.

Due to low seed germination rates, analysis of plant trait differences between the invasive and non-invasive species was limited to two of the six paired species. The paired species evaluated for plant trait differences were: 1) *Chyrsanthemum vulgare* (invasive) with *Erigeron speciosus* (non-invasive), and 2) *Lythrum salicaria* (invasive) with *Lythrum virgatum* (non-invasive). These four species were analyzed using nine plant traits to determine any trait differences associated with invasiveness.

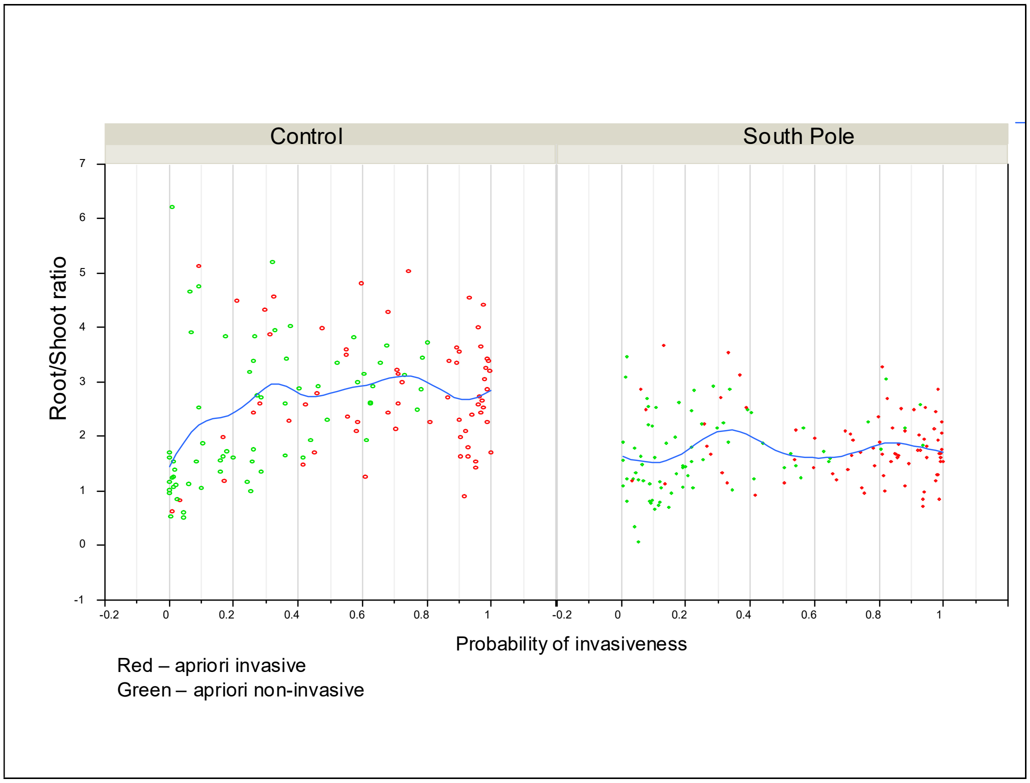

Figure 2: Probability of invasiveness (x-axis) for eight species control and south pole treatments, based the root/shoot ratio (y-axis). Invasive species (red dots) and non-invasive species (green dots) are differentiated by logistic regression.

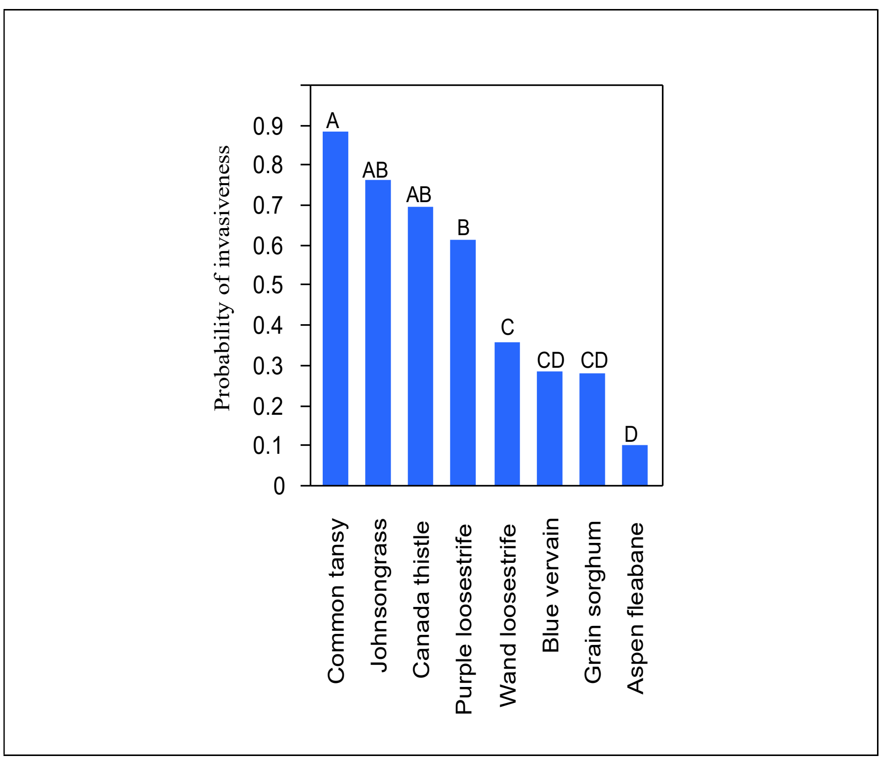

Figure 3: Probability of invasiveness plot for eight species. Note that the overall ranking of invasiveness generally agrees with published invasive properties of each species. Common tansy, Johnsongrass, Canada thistle, and Purple loosestrife are known invasive species, and European wand loosestrife, blue vervain, grain sorghum, and Aspen fleabane are non-invasive.

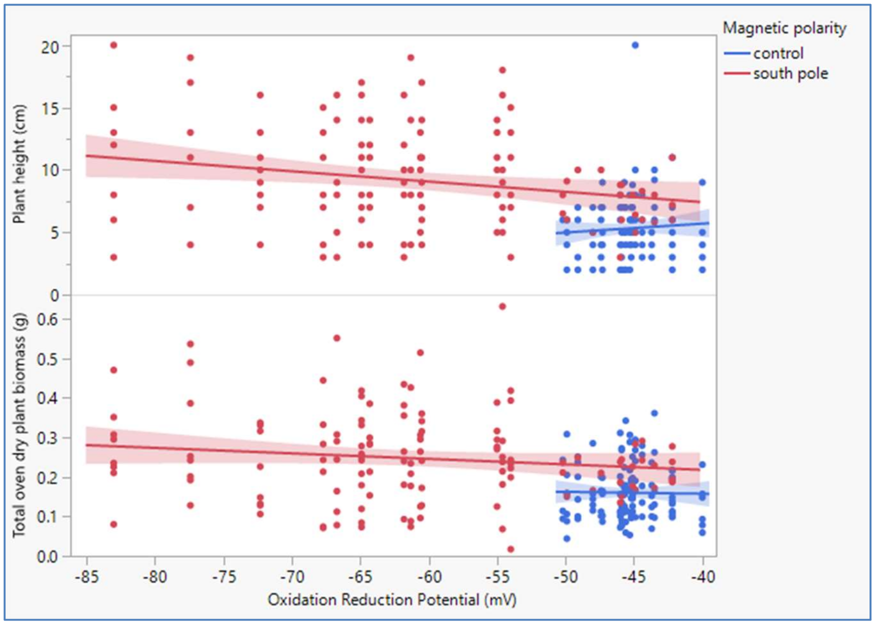

Figure 4: Total oven dry plant biomass and plant height (y-axis) relationship with oxidation reduction potential (x-axis). The regression tests were separated by magnetic polarity of the water treatment (legend).

Analyses of the *Lythrum salicaria* and *Lythrum virgatum* paired species data revealed interactions between species and magnetic seed and seed/water treatments for seven out of eight plant responses (Table **4**). Average plant responses for the four magnetic seed/water treatments are listed by species in Table **6**. For *L. salicaria*, the invasive species, the South Pole seed/water treatments increased foliar biomass (300%), total biomass (200%), plant height (164%), leaf length (146%), leaf area (288%), and maximum leaf width (106%) when compared to the control seed/water treatments. For *L. virgatum*, the non-invasive species, the South Pole seed/water treatments increased foliar biomass (80%), total biomass (142%), plant height (31%), leaf length (61%), leaf area (111%), and maximum leaf width (38%) when compared to the control seed/water treatments. The root/shoot ratio, which is a ratio of two plant biomass responses, was reduced by 39% for *L. salicaria,* but was increased by 67% for *L. virgatum*, when comparing the South Pole magnetized seed/water treatments with the control treatments.

Table 5: Analytical results (*p*-value) for *Chrysanthemum vulgare* vs. *Erigeron speciosus* for study factors and their interaction term

Source	Foliar Biomass (g)	Root Biomass (g)	Plant Biomass (g)	Height (cm)	Leaf Length (mm)	Root to Shoot Ratio	Leaf Area (sq cm)	Max Leaf Width (cm)	Ave. Leaf Area (sq cm)
Species	<0.0001	<0.0001	<0.0001	<0.0001	0.0008	0.8840	<0.0001	<0.0001	<0.0001
Magnetic Polarity	<0.0001	0.2658	0.0277	<0.0001	<0.0001	<0.0001	<0.0001	0.1900	<0.0001
Species * Magnetic Polarity	0.0048	0.3954	0.1063	0.0207	<0.0001	0.0029	0.0163	0.9916	<0.0001

The *Chrysanthemum vulgare* and *Erigeron speciosus* paired species analyses revealed interactions between species and magnetic seed/water treatment for six out of eight plant responses (Table **5**). Average plant responses for the four magnetic seed/water treatments are listed by species in Table **7**. For *C. vulgare*, the invasive species, the South Pole seed/water treatments increased foliar biomass (67%), plant height (54%), leaf length (16%), leaf area (76%), and average leaf width (55%) when compared to the control seed/water treatments. For *E. speciosus*, the non-invasive species, the South Pole seed/water treatments increased foliar biomass (96%), plant height (74%), leaf length (68%), and leaf area (112%) when compared to the control seed/water treatments. Also, the root/shoot ratio was reduced by 69 and 167% for *C. vulgare* and *E. speciosus*, respectively, when comparing the South Pole magnetized seed/water treatments with the control treatments.

Table 6: Average plant responses and Student T test for *L. salicaria* vs. *L. virgatum*, based on the interaction between species and polarity of magnetized seed/water treatments

Species	Magnetic polarity	Foliar Biomass (g)[a]	Root Biomass (g)[b]	Plant Biomass (g)	Leaf Area (cm sq)	Ave. Leaf Width (cm)[b]	Plant Height (cm)	Leaf Length (mm)	Root to Shoot Ratio
L. salicaria	Control	0.03 D	0.1	0.13 C	8.6 D	0.4	5.8 D	19.5 C	3.2 A
L. salicaria	South pole	0.12 A	0.27	0.39 A	33.5 A	0.7	15.3 A	48.0 A	2.3 B
L. virgatum	Control	0.05 C	0.07	0.12 C	12.1 C	0.7	7.5 C	16.9 C	1.5 C
L. virgatum	South pole	0.09 B	0.2	0.29 B	25.5 B	1.0	9.8 B	27.2 B	2.5 AB

[a] Average plant response followed by a Student T test letter. Averages not connected by the same letter are significantly different.

[b] There were no interaction terms for these two parameters. Only the species and magnetic water treatment terms were significant which prohibited Student T tests across both study factors.

Table 7: Average plant responses and Student T test for *C. vulgare* vs. *E. speciosus*, based on the interaction between species and polarity of magnetized seed/water treatments

Species	Magnet polarity	Foliar Biomass (g)[a]	Root Biomass (g)	Plant Biomass (g)	Leaf Area (cm sq)	Ave. Leaf Width (cm)	Plant Height (cm)	Leaf Length (mm)	Root to Shoot Ratio
C. vulgare	Control	0.06 B	0.17 A	0.23 B	12.6 B	0.6 B	4.8 B	21.1 B	2.7 B
C. vulgare	South pole	0.11 A	0.17 A	0.27 A	22.2 A	0.9 A	7.4 A	24.4 A	1.6 C
E. speciosus	Control	0.02 D	0.07 B	0.09 C	5.8 C	0.4 C	2.3 D	14.9 C	3.2 A
E. speciosus	South pole	0.05 C	0.05 B	0.10 C	12.3 B	0.5 C	4.0 C	25.1 A	1.2 D

[a] Average plant response followed by a Student T test letter. Averages not connected by the same letter are significantly different.

The physicochemical water properties were altered after 20 h exposure to static magnetic fields (Table **3**). The electrical conductivity was not different among the three magnetized water treatments. However, oxidation reduction potential increased in absolute terms for both magnetic water treatments, i.e., ORP became more negative for both the North and South Pole water treatments. Also, pH increased for both the North and South Pole magnetized water. There was a linear relationship between ORP and total oven dry plant biomass, plant height, average leaf width and the root/shoot ratio (Figs. **4** and **5**). The linear relationship shows that the variability in the South Pole water treatments was much smaller than for the control water treatments. There was a linear relationship between pH and total oven dry plant biomass, plant height, average leaf width and the root/shoot ratio (Fig. **6**). Both ORP and pH had a linear relationship with the four plant traits that showed the strongest responses to the water treatments. There was a direct relationship between increasing pH and plant growth and an "indirect" relationship between ORP and plant growth, i.e., as ORP became more negative the plant growth increased for the South Pole water treatments. The relationships showed no evidence of being curvilinear along the range of ORP and pH concentrations tested in this study. Both the physicochemical water measurements and the magnetized irrigation water used the same static magnets for the same exposure time of 20 h ensuring that irrigation water treatments had the same water properties.

4. Discussion

Low germination rates limited data analysis to two paired species. In addition, the low germination rates for all the North Pole seed/water treatments also prevented data analysis for all the species in this set of treatments. Pretreatment of the seeds for six days may have contributed to excessive seed spoilage or infection resulting in low germination rates across all plant species.

Analyses of the plant traits revealed significant, two-way interactions between species and magnetic seed/water treatments for six and seven out of eight plant responses for the *Chyrsanthemum vulgare - Erigeron* *speciosus* and *Lythrum salicaria* - *Lythrum virgatum* paired species, respectively. The interaction term in the MANOVA model shows that the four species responded differently, based on their invasiveness, to the magnetized seed/water treatments. Therefore, the results were reported for the interaction effects associated with the two sets of paired species.

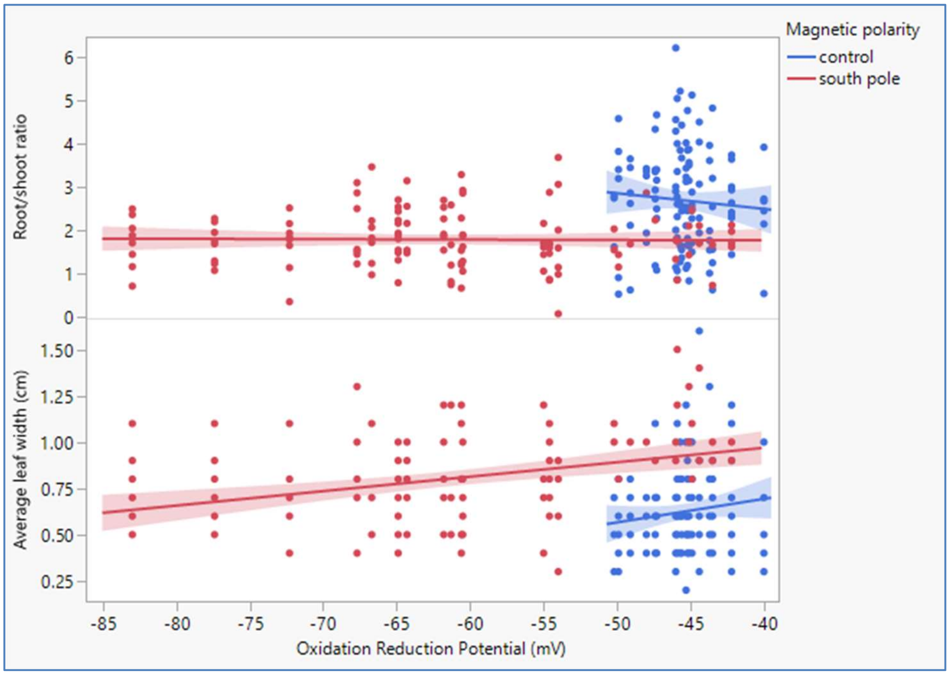

Figure 5: Average leaf width and root/shoot ratio (y-axis) relationship with oxidation reduction potential (x-axis). The regression tests were separated by magnetic polarity of the water treatment (legend).

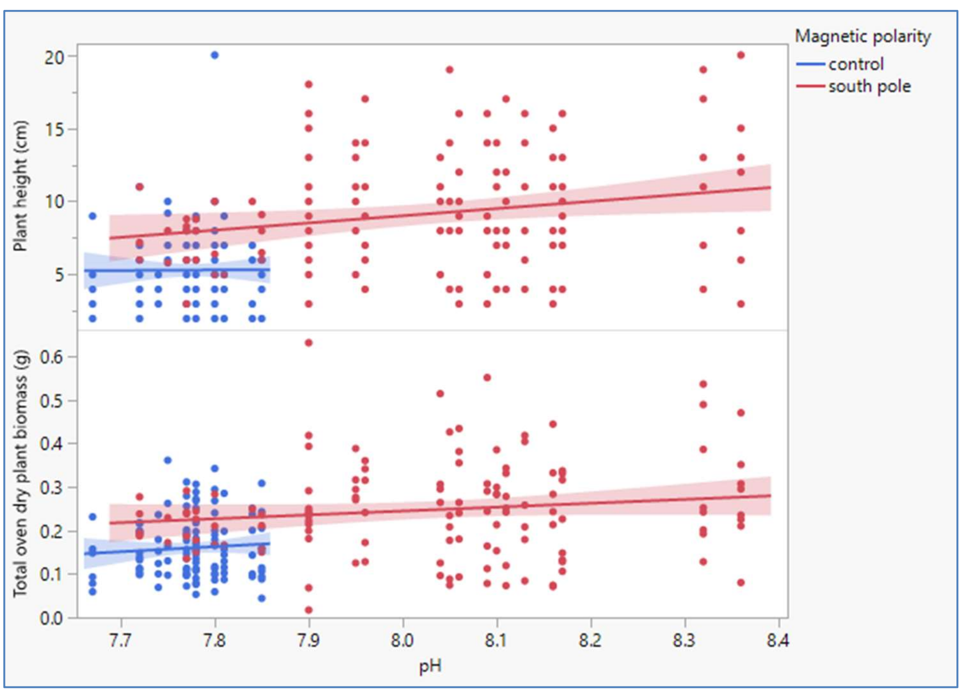

Figure 6: Total oven dry plant biomass and plant height (y-axis) relationship with pH (x-axis). The regression tests were separated by magnetic polarity of the water treatment (legend).

Comparison of plant responses to invasiveness ranking for *Lythrum salicaria* - *Lythrum virgatum* shows that the non-invasive species (*L. virgatum*) had a larger foliar biomass, leaf area, maximum leaf width, and plant height than *L. salicaria*. A comparison of the same responses for *Chyrsanthemum vulgare – Erigeron speciosus* species shows that the invasive species (*C. vulgare*) always had larger plant traits than its non-invasive counterpart. Pretreatment of the seeds and irrigating the seedlings with magnetized (South Pole) water substantially increased six out of eight pant responses for the *Lythrum salicaria* - *Lythrum virgatum* paired species. Also, pretreatment of the seeds and irrigating the seedlings with magnetized (South Pole) water substantially increased four out of eight pant responses for the *Chyrsanthemum vulgare – Erigeron speciosus* paired species. These combined results indicate that invasive plant species had stimulated growth traits when compared to the paired, non-invasive species.

The Tukey test shows that the probability of invasiveness for the invasive species was significantly higher than the non-invasive species. These results show evidence that invasive species tend to adapt quicker or stronger to environmental stimuli. Also, the invasiveness ranking among the species generally concurs with the invasive ranking in the published literature. Ecologists generally agree that plant species exhibit invasive properties over a broad spectrum, i.e., invasiveness is not a discrete entity but a continuous gradient between completely non-invasive and highly invasive. The probability of invasiveness gradient among eight species mimics the continuous invasive gradient found in nature.

The study hypothesis that invasive species adapt quicker or stronger than non-invasive species was confirmed by the results of this study. In addition, a limited number of morphological plant traits were able to differentiate between the two classes (invasive and non-invasive) in this study. Other study factors may be included to improve the ability of the predictive models to separate species into justifiable invasive classes. Such study factors may include use of additional plant traits, including seed production, phenology, physiology biomarkers, and herbivory parameters. Also, within a vegetation form such as forbs or grasses, a study design could include a wide diversity of morphologic and functional forms. Finally, additional stressor/stimuli treatments may be needed to capture a wide range of adaptation abilities for the selected species.

The root/shoot ratio trait was the sole exception to the accelerated growth due to the South Pole seed/magnetized water treatments. This ratio was reduced by the South Pole seed/magnetized water treatments in three of the four species, with *L. virgatum* being the exception. The reduced ratio resulted from increased foliar growth in both invasive species and *E. speciosus*, relative to root growth. An explanation for this root/shoot response is that the more negative ORP and higher pH of the magnetized water improved the water solubility of soil minerals and hydrophobic molecules [48-49]. A magnetized irrigation water study by Mashhour and Abd-Elhady [50] found that micro-nutrient uptake by roots was increased with the magnetized irrigation treatments. These two studies indicate that enhanced nutrient uptake using magnetized irrigation water may redirect plant resource allocation to the foliage due to increased root uptake efficiency.

The combination of magnetic pretreatment of seeds, followed by South Pole magnetized water resulted in an average increase of 184 and 182% in foliar biomass and leaf area for the two invasive species. In comparison, the foliar biomass and leaf area increased by an average of 88 and 111% for the non-invasive species using the same seed/magnetized water treatments (Images **1** – **3**). Recent research shows that magnetized water, using static magnetics attached to the irrigation lines, increased crop biomass and crop yields [10-11, 51-53]. The increase in crop biomass and yield results from these four magnetized irrigation studies was 74% and 91%, respectively, when averaged across all crop species, magnetic strength, and length of watering dates [15, 51-53]. The average magnetic field strength and irrigation time for these four studies was 881 mT and 61 days, respectively. In comparison, the magnetic field strength and plant watering time for this study was 570 mT and 64 days, respectively. The long exposure time for the magnetized seed treatment (six days) and the extended water exposure time on the static magnets (20 h) may partially explain the higher growth rates in this study as compared to the four magnetized irrigation studies mentioned above. The magnetized irrigation systems in the four referenced studies were designed so that static magnets were attached to the water lines which limited the water exposure time to the magnetic field. The plant dry biomass increased an average of 174 and 74% for this study and the four referenced studies, respectively [15, 51-53]. The combination of plant species, magnetic seed treatment, length of time of seed treatment (six days), polarity of magnetized seed and water treatments (South Pole polarity), and length of water exposure time to the magnetic field (20 h) all contributed to increased plant biomass in this study. In contrast the four magnetized irrigation studies mentioned above did not include this combination of study factors which may explain the lower biomass results.

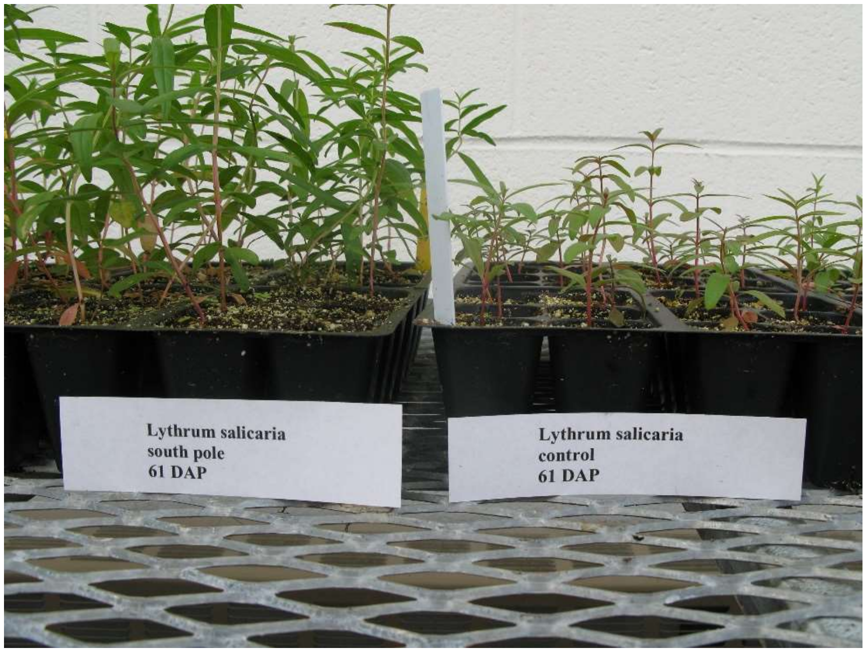

Image 1: Photo of the invasive species *Lythrum salicaria* (Purple loosestrife) at 61 days after seed planting. In left tray the seeds and seedlings were exposed to South pole magnetized treatments versus the control seedlings in right tray.

Image 2: Photo of the non-invasive species *Lythrum virgatum* (European wand loosestrife) at 61 days after seed planting. In left tray the seeds and seedlings were exposed to South pole magnetized treatments versus the control seedlings in right tray.

Image 3: Photo of the non- invasive species *Sorghum bicolor* (Sorghum) at 61 days after seed planting. In left tray the seeds and seedling are the control seedlings and right tray contains seedlings exposed to the South pole magnetized treatments.

Magnetic fields modify the physicochemical properties of water such as oxidation reduction potential, electrical conductivity, and pH. Amor *et al*. [54] found that static magnets (290 mT) slightly increased water pH values after 14 h of exposure. Yin *et al*. [55] found that static magnets (500 mT) decreased ORP of tap water from 122 to 105 mV. They also found that tap water pH increased from 6.4 to 7.1 after 12 h exposure time. Yamashita *et al*. [56] also found that static magnetic fields caused fluctuations in oxidation reduction potential of water, up to 60 mV when measured over several days. Other researchers such as Azad and Ishikawa [57] evaluated alternative energized water treatments and found that a 30 min. ceramic-based water treatment reduced ORP by 155 mV and increased pH by 0.95 compared to the untreated water. Nobuo *et al*. [58] evaluated the effects of electrolyzed water on leek growth. The electrolyzed acidic water had a pH of 2.6, and an electrical conductivity of 500 – 3,000 μS/cm. The electrolyzed alkaline water had a pH of 11.9, and an electrical conductivity of 2,000 - 2, 500 μS/cm. They found that watering leek seedling with acidized and alkaline water on alternate weeks resulted in the maximum growth (39%) based on oven dry biomass.

Magnetic fields energize water molecules by increasing the velocity of the valence electrons which in turn strengthens the hydrogen bonds between water molecules [33, 59]. Exposing water molecules to energy fields energizes the molecules, resulting in increased hydrogen bond strength, and transforming unstructured water into water containing structured and non-structured water domains [60–63]. Magnetized water treatments strengthen the hydrogen bonds among water molecules which causes the formation of "chains of water molecules" or the putative structure of hexagonal rings of water molecules [10, 30, 64].

One method of confirming that magnetized water increases the percentage of structured, or hexagonal water rings is by measuring the electrical conductivity of water. The conductivity of water is dependent on the mineral ion concentration and the concentration of hexagonal water rings. The delocalized electrons in the hexagonal water rings conduct electricity [65 – 68]. In this study, filtered tap water acted as the reference sample that established the initial conductivity properties. After applying a magnetic field (450 mT) for 20 h the electrical conductivity increased from 1.4 to 7.1% for South and North pole treatments, respectively (Table **3**). This slight increase in water conductivity is in general agreement with other energy treatments for water. Hassan *et al*., [47] found that magnetized water (450 mT) increased conductivity by 3%. Ibrahim [35] found that static magnets increased conductivity by 4, 8, and 14-fold, depending on the magnet strength. Yin *et al*. [43] increased magnetized water conductivity (500 mT) by 1 and 4.7% for tap and distilled water, respectively. These studies provide substantial evidence that magnetic fields increase water structure and conductivity. Exposing water to two different energy fields increased conductivity up to 22-fold (Ramsey, unpublished data).

Crop irrigation with structured water is still in the nascent stages, but the published studies involving such water treatments have shown promise [69-71]. Holster [72] evaluated the effects of structured water on reviving severely wilted cut flowers (*Hypochoeris radicata*, catsear dandelions). They found that 46 and 66% of the wilted cut flowers were revived when watered with unstructured and structured water, respectively. Ptok [73] found that structured water increased alfalfa sprout (*Medicago sativa*) growth by 15% compared with sprouts watered with tap water. Korotov [74] found that structured water increased potato root growth by 2-fold over the control watered plant. Numerous case studies of naturally occurring structured water have shown improved human health and increased longevity [75-81].

The effectiveness of magnetized irrigation water depends on the polarity of the magnetic fields, strength of the fields, and length of exposure time to the magnetic fields. Also, research is needed to evaluate the effects of combining magnetic fields with other energy treatments, i.e., static magnetic fields could be combined with vortexed water, ceramic treated water, or water exposed to geocentric frequencies, to evaluate combined water treatments on plant responses. Further studies are needed to evaluate the combined effects of magnetized seed and irrigation treatments on plant responses. In addition, magnetized water treatments should be evaluated for improving drought tolerance and/or improved disease resistance.

The effects of magnetic fields on physicochemical water properties should be included in all magnetized irrigation water studies. The water properties would provide useful water property information that can be related to the plant or crop responses to the irrigation treatments. Quantification of water properties may provide further insights into the effects of magnetized water for improving drought tolerance [16-18] or increasing disease resistance in crops [82-84].

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