The Use of a Protein Enhanced Surfactant to Increase Yield of Alfalfa

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Authors' contributions

This work was carried out in collaboration between both authors. Author BTS designed the experiment, provided data collection/processing, literature review and served as the principle author of the manuscript. Author BSG provided the editing and the statistical analysis portion of the article. Both authors read and approved the final manuscript.

Short Research Article

ABSTRACT

Increasing human populations are creating challenges in food production and water management. Improving resource management with advances in technology is needed to meet these challenges. TerraStim® is a protein enhanced surfactant created by the Advanced BioCatalytics Corporation of Irvine, California and distributed by GAM Logistics (DBA TerraStim). The liquid concentrate had been tested in various bench tests with successful results that merited a field level experiment that is presented in this article. It was shown to enhance the yield of an alfalfa crop in a sandy loam soil by 27.9% and 81.6% in test fields versus their respective control fields. Although there are several ways to improve the experiment further, the results of this study are encouraging. They support a conclusion that, in a sandy loam soil type, TerraStim® can significantly improve yields of alfalfa.

Keywords: Surfactants; agricultural additives; agro-chemicals; alfalfa; water use efficiency.

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1. INTRODUCTION

Water resource issues are a prominent concern tied to the needs of a growing global population. In particular, the conservation and improved management of water resources are of high importance when addressing these issues. Agriculture accounts for 31% of the fresh water used by the United States [1]. While we are not completely dependent upon irrigation to grow our food resources, irrigated acres accounted for half the total in cropland [2]. Innovations in management, technology and implementation of these improvements as they pertain to water resources tied to agriculture will be necessary to meet the increasing demands on food production.

TerraStim® is a new product that utilizes the technologies developed by the Advanced BioCatalytics Corporation of Irvine, California. The core technology is based on a synergistic enhancement of surfactants by their combination with certain low molecular weight proteins. While inducing a profound decrease in surface and interfacial tension, the protein component of the technology has yet another effect upon aerobic metabolism of bacteria by uncoupling the catabolic and anabolic processes [3-5].

Surfactants have been utilized in agriculture to not only increase the effectiveness of agricultural pesticides in foliar uptake [6,7], but to also improve certain soil properties. For example, they have been used as soil additives to enhance water use efficiency [8], eliminate preferential flow paths [9], and to enhance the penetration/distribution of water in soil matrixes [10].

Preferential flow paths develop in irrigated soils with sandy textures where water will "preferentially" flow in deeper horizons, causing a decrease in the water use efficiency of that cropping system [11]. On the other hand, in highly hydrophobic soils, water penetration from the surface to subsurface layers may be slow to the extent that significant fraction of irrigation water will be lost due to evaporation.

Topography of a field will impact preferential flow paths as well. The result of preferential flow paths and evaporation is troubling, as they can often lead to decreased crop yields [12]. The benefits of using agricultural surfactants to alleviate this issue by spreading the water throughout the soil matrix have been explored to a degree of success [13]. In one study, golf fairway greens that were exhibiting sub-optimal grass growth were remediated by the use a surfactant. Additionally, there was the extra benefit in that the surfactant helped to dissipate the hydrophobic characteristics of the soil that had developed [9].

Another report reviewed a wide range of studies and tests using four different surfactants and four different soil properties including infiltration rates, hydraulic conductivity, capillary rise and water holding capacity. The authors concluded that introducing surfactants could significantly improve capillary rise and unsaturated hydraulic conductivity in sandy soil, although no other improvements were observed. They went further to conclude that while some literature has shown improvements to soils with certain hydrophobic characteristics, the same will not necessarily be seen for hydrophilic soils [14].

TerraStim® is a proprietary blend shown to have enhanced surfactant characteristics in that it provides reduced surface and interfacial tensions at very low concentrations compared to other commercial surfactant products. Initial agricultural greenhouse testing that has been previously conducted prompted the tests outlined below [15]. A simple comparison measuring crop water use efficiency, yield and protein content between two fields treated with TerraStim® against two similar control fields is the objective of this study.

2. EXPERIMENTAL DETAILS AND METHODS

The experiment ran from August 9 th2013 until harvest on September 10 th2013. The aim was to determine the effect that the TerraStim® product has upon yield as it relates to the standard water application described by the University of Nebraska as crop water use efficiency [16]. Protein content had also been measured and analyzed. The test crop was alfalfa, which has been grown upon the test site for the previous eight years. The test site is located 2 miles north east of Hamer, Idaho in Jefferson County. A simple test was conducted on two sites watered with TerraStim® against two controls.

Soil of the test site has a texture mix between a sandy loam and a loamy sand. The pH is alkaline averaging 8.4 with a mean bulk density of 1.53 g/cc. Soils of the area were formed from wind deposits and are excessively drained. The infiltration rate varies between 6.0-20 inches/hour [17]. The east test and control fields have minor observable topographical differences, as the slope is 4 to 5 degrees steeper in the east fields than that of the west fields.

Yield was the primary test variable measured and recorded as dry plant biomass in grams/square meter and converted to tons/acre. The secondary test variable to be evaluated was water use efficiency as the total volume of water used to produce the recorded yield. This was recorded as the volume of irrigation water used, accounting for irrigation system efficiency, precipitation, and changes in soil water storage. Protein content was measured as well from the harvested plots as a third test variable.

The test occurred on two different fields that share a water source. The fields are referred to as West and East sites. Each of the two sites had a designated control section and a treatment section. Test sections were randomly selected in the fields by dividing the fields into sections where the irrigation sprinkler system stops for watering cycles and has a width defined by sprinkler application distance. The control sections for each site where chosen first and a buffer zone of at least two sections was given on either side for the selection of the treatment sections. Two treatment sections had TerraStim® applied to them and were compared to two control sections that had only water applied to them. Both test and control sites were watered for the same amount of time (24 hrs).

Three harvests occur each season for the test sites. The test period took place during the third and final harvest of the year. It begins in early August every year and ends with harvests in mid-September. There are three to four watering cycles that occur during each harvest session.

Both of the west field sections occupied approximately 3.6 acres, and both East field sections are approximately 1.38 acres each in size. Fields where mapped and drawn to scale with a grid system assigning numbers to square meter plots. Plots where assigned numbers, and a random number generator was used to select

50 points from the East sections and 30 points from the West field sections for sampling. The east site had a greater degree of topographical difference with several steep ($\geq$10°) sections that funneled into the middle of the plot. It also had a west to east aspect. The west section ran south to north and had a maximum slope of 4°. While this did not explain the differences in experimental findings in any significant way, it should be noted as the primary observable difference between the two sites.

TerraStim® was applied directly into the main line using an Agri-Inject Chemigation pump that is calibrated to deliver the product to the water line at a 0.6 gallons/hour using the factory calibration method incorporated into the pump system. The watering cycle prescribed to the site is twenty-four hours long at a rate of 450 gallons per minute. This yields a total application per cycle at 648,000 gallons or 23.85 acre-inches. The recommended dosage of TerraStim® is 10 ppm when mixed with water. Therefore, approximately 6.48 gallons of TerraStim® were applied per cycle by running the chemigation pump for 10.8 hours per cycle during the 24 hour watering cycle for each field section.

Precipitation was measured using a field tipping bucket and data logger. The existing irrigation system was a wheel line sprinkler system, and has a measured irrigation efficiency of 0.68. This value is similar to other sprinkler systems of the same type. Irrigation efficiency was calculated before the experiment began. Fields were mapped and drawn to scale with a grid system assigning numbers to 10 square meter plots. Plots where assigned numbers, and a random number generator were used to select 30 points from both the East and West fields for sampling. Amount of water delivered to those points was measured and the efficiency was calculated for the system.

Soil cores were taken the day watering began and the day of the test harvest. Fifteen were taken from each of the four test sites. Cores were sampled to a depth of twelve inches as based upon the average A horizon depth of the sites and the maximum depth that the hand core device can achieve while ensuring that the cores are consistently intact. Soil cores were transported in a cooler, having been stored in small paper bags to Montana State University. The Cropping Systems Laboratory oven and scale where used to measure change in weight before and after drying. Gravimetric water

content was measured and converted to percent volumetric water utilizing the found average value of bulk density of the soil.

Crop sampling took place approximately two days before harvest, which was subject to weather conditions and field saturation levels. Sampling plots were randomly assigned by dividing the plots into four quadrants. Points were selected from within each of those quadrants by dividing them into square meter sections and using a random number generator to select random points. Points were marked with tall flags that served as the center for square meter transects. Harvest occurred by collecting all plant material within the square meter transect plot down to within ½ inch of the surface and placing them into bags to be dried.

Irrigation stopped roughly one week before harvest on September 3rd. Upon harvesting, samples were collected from both test fields and both control fields. Then, the samples were dried and weighed to determine dried plant biomass in grams per square meter and than converted to tons/acre. Fifteen sample points from each of the test plots and each of the control plots were randomly selected to be ground down for crude protein measurements that were submitted for laboratory calculation.

3. STATISTICAL EVALUATION OF RESULTS

To calculate the statistical validity of our results, we utilized a Z-test designed for two samples. This equation (1) is as follows:

(1)

$$z = \frac{(\overline{X_1} - \overline{X_2}) - (\mu_1 - \mu_2)}{\sqrt{\left(\frac{\sigma_1^2}{n_1}\right) + \left(\frac{\sigma_2^2}{n_2}\right)}}$$

where

$\overline{X}_1$: the mean of sample 1 (test)

$\overline{X}_2$: the mean of sample 2 (control)

σ_1 : the standard deviation of sample 1 (test)

σ_2 : the standard deviation of sample 2 (control)

n_1: the sample size of sample 1 (test)

n_2: the sample size of sample 2 (control)

$(\mu_1 - \mu_2)$: the expected difference between the mean of sample 1 and the mean of sample 2

The Z-test is one-tailed, since we are looking to evaluate whether the test samples produced a higher yield than the control samples. The research hypothesis is:

H_0: $\mu_1 - \mu_2 = 0$
H_1: $\mu_1 - \mu_2 > 0$

If we reject the null hypothesis, we reject that the two means are the same, accepting the alternative hypothesis stating that the test mean is greater than the control mean. The significance level of a test is the probability of wrongly rejecting null hypothesis. The power level of a test is the probability of correctly rejecting the null hypothesis.

The first Z-test will see if, in fact, the two samples are statistically different from one another, with no expected difference. If the test sample proves to be greater than the control sample, subsequent Z-tests will evaluate if the test sample is statistically greater than 105%, 110%, 115%, 125%, 150%, and 175% of the control sample.

4. RESULTS AND DISCUSSION

The experiment ended with only three watering cycles occurring throughout the course of the experiment. The total growing time was approximately five weeks long. The third harvest cycle usually occurs in this fashion. Statistically significant differences in the treatments were still measureable and observable.

Crop water use efficiency (WUE) is a benchmark value used to measure the differences in treatments tested through irrigation systems. It does not account for losses in deep horizon percolation, evapotranspiration or soil respiration. It does provide a simple method for measuring differences observed in treatments that have similar site characteristics and therefore suits the conditions of the test well, given the remote location and subsequent equipment limitations. The equation is written below.

WUE = Yield (Tons/Acre)/(Precipitation + Irrigation Inches + Change in soil water content (inches))

Basic statistical values for yield are listed in Table 1.

Table 1. Test plot yields (tons/acre)

	Test East	Control East	Test West	Control West
Mean	1.016	0.794	0.955	0.526
Std. Deviation	0.121	0.055	0.102	0.082
Minimum	0.835	0.685	0.769	0.413
Q1	0.925	0.753	0.889	0.448
Median	0.993	0.799	0.942	0.518
Q3	1.095	0.837	1.009	0.584
Maximum	1.336	0.898	1.266	0.727
n	30	30	50	50

Table 2 summarizes the values found and calculated throughout the harvest session. The irrigation value was calculated based upon the irrigation efficiency and the three watering cycles that each of the sites was given throughout the course of the harvest session.

Table 2. Yield and test conditions

	Test East	Control East	Test West	Control West
Yield (Tons/Acre)	1.016	0.794	0.955	0.526
Precipitation (Inches)	0.18	0.18	0.18	0.18
Irrigation (Inches)	47.63	47.63	47.63	47.63
Δ Soil Water (Inches)	0.198	0.401	0.0364	-0.344
WUE (Tons/acre inch)	0.021	0.0165	0.020	0.00111

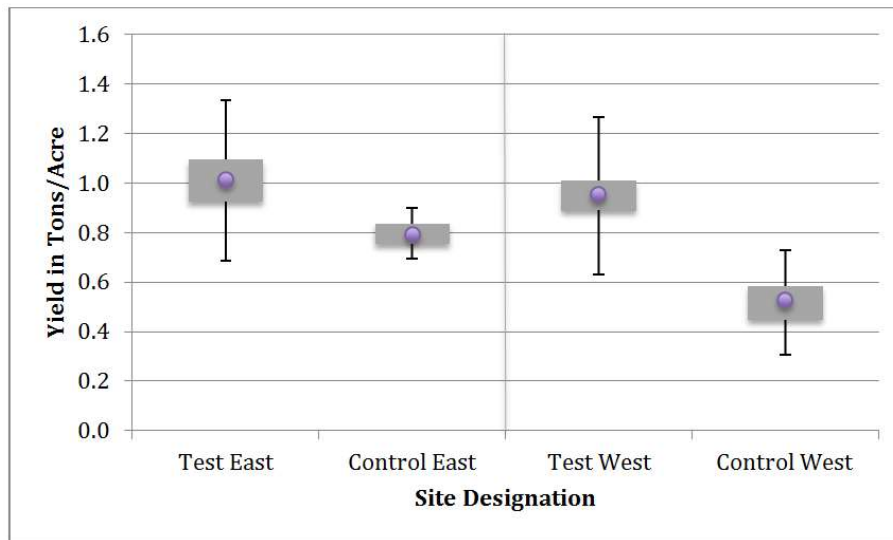Fig. 1 shows the calculated graphical summary of the results of the yield component of the experiment.

Fig. 1. Yield box and whisker plot

Table 3. Differences and statistical validity for east field

(all units in grams/square meter)

$\overline{X}_1 = 1.016$
$\overline{X}_2 = 0.794$
$\overline{X}_1 - \overline{X}_2 = 0.221$
$n = 30$

	0% difference	5% difference	10% difference	25% difference
$\mu_1 - \mu_2$	0.0	0.038	0.076	0.191
z statistic	9.13	7.55	5.98	1.25
Significance level	$P<0.001$	$P<0.001$	$P<0.001$	$P=0.15$
Power level	99%	99%	77.38%	0.00%

The test and control yields from each of the four plots in this experiment were then analyzed. Table 3 presents the differences between the test and control yields, and the statistical significance of the difference. Table 4 does the same for the West Field, and Table 5 presents these findings for the East and West fields aggregated for both the test and control treatments.

Results of the experiment show that the plots that had TerraStim® applied to them produced significantly greater yields than that of the plots that received only water as illustrated in Fig. 1 and Table 1. Water use efficiency values provide a way to compare yields with one another. Both of the tests produced higher tons/acre than the controls and given the short growing period the test occurred in, the difference is quite notable as shown in Table 2. Water use efficiency values are therefore significantly higher as well (Table 2).

Test yields from the East field were 10% greater than those from the control group, with over 99% statistical significance and a strong power level (Table 2 and Table 3). The West field showed even greater improvement, with yields that were over 75% larger than those of the control group, with over 95% statistical significance and a 95% power level (Table 2 and Table 4). Aggregated, the test yields from both the East and West fields were more than 50% greater than the control yields from both fields as shown in Table 6. The statistical significance of this result is greater than 95% with a 95% power level.

These results indicate that the water added to the alfalfa crops in sites with sandy soil produce greater yield when applied with the treated water, than in the control. Reductions in surface and interfacial tensions as a result of documented surfactant effects upon a soil system correlates with the increased yields as it did in several other published studies [18,19]. Furthermore, given the site's sandy soil and historical irrigation regimen, channelization would likely have been observed and contribute to a decreased yield over time which has been documented in other similar soils [9,11]. If such circumstances were present in the fields treated, than the increase in yield could partially have been attributed to a remediation of the channelized conditions. The USDA Soil Survey classification as a well-drained soil would also support this hypothesis since the water could more easily move through the profile into deeper horizons [17]. While the soil type and past watering cycles may have attributed to the increased yield, greater harvests are not universal with the application of surfactants to all crops and soil types.

A few studies should be mentioned since they refute any universal effect of surfactants upon increased yield. Corn, soybean and potato yields did not increase over the course of a three-year study in both a silt loam and loamy sand soil that had some hydrophobic characteristics [20]. The type of surfactant used was non-ionic which is different than the surfactant utilized in the treatment of this study, which has a zwitterionic charge. More recently, a study done in New Mexico showed no significant increases in yields after testing two different types of surfactants on pinto beans in a sandy loam [21]. However, the results indicate a clear increase in yield as a result of the treatment. Other articles reviewed can aid in understanding the results when taken into account.

Table 4. Differences and statistical validity for west field

(all units in tons/acre)

$\overline{X}_1 = 0.955$
$\overline{X}_2 = 0.526$
$\overline{X}_1 - \overline{X}_2 = 0.429$
$n = 50$

	0% difference	25% difference	50% difference	75% difference
$\mu_1 - \mu_2$	0.0	0.131	0.263	0.395
z statistic	23.09	16.01	8.93	1.86
Significance level	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.05$
Power level	99%	99%	99%	95%

Table 5. Differences and statistical validity for both fields

(all units in tons/acre)

$\overline{X}_1 = 0.978$
$\overline{X}_2 = 0.627$
$\overline{X}_1 - \overline{X}_2 = 0.351$
$n = 80$

	0% difference	10% difference	25% difference	50% difference
$\mu_1 - \mu_2$	0.0	0.063	0.157	0.313
z statistic	16.76	13.77	9.28	1.81
Significance level	$P<0.001$	$P<0.001$	$P<0.001$	$P=0.05$
Power Level	99%	99%	99%	95%

Mobbs et al. [14] concluded that surfactants in hydrophobic and sandy soils perform better in terms of yield as well as several other variables like permeability and hydraulic conductivity. This correlates well with the results given the high sand content of the soil, the dry environment of the site and a calcareous soil, which contributes to top and sub soil profile deposits of salt that could have limited harvests in the controls. The yield observed in the East test site was 27.9% greater than that of the control site while the West test site was 81.6% greater (Table 2).

A review of bio-surfactants applied in agriculture expounded upon the correlation between surfactants and increased bioavailability of nutrients [22]. Increased nitrogen, phosphorus, potassium and micronutrients would have likely contributed slightly to the observed yield increases as the site typically had low organic matter content. In greenhouse tests previously conducted, the ability of TerraStim® to stimulate root growth of certain plants under limited water supply has been reported [15]. A combination of an increase in the bio-availability of nutrients coupled with enhanced root development could also be attributed to the increase in yield.

5. CONCLUSIONS

Although there are several ways to improve the experiment further, the results of this study are encouraging. They support a conclusion that, in a sandy loam soil type, TerraStim® can significantly improve yields of alfalfa.

Further study relating the increased nutrient cycling as a result of both the surfactants suggested effect upon nutrient availability and microbial contributions as a result of the uncoupling effect merits further exploration. It would also be of interest to observe the changes, if any, in nitrogen content/form and carbon content/form as well as the changes in microbial populations from the beginning of the test to the end. Given that many of the nitrogen cycling processes are based upon microbial activity [23] and that the "uncoupling" in aerobic bacteria between their catabolic and anabolic processes has been observed as a result of the introduction of the protein-surfactant complexes in various experiments [4], future experimentation exploring this phenomenon would be highly merited.

Testing the application of TerraStim® on other crops would also be of prudent. Future experimentation would be beneficial if performed on a more challenging site that does not exhibit hydrophobic characteristics and that does not posses such a well-drained, sandy soil type as well.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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