

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

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 (Kenny et al. 2009). While we are not completely dependent upon irrigation to grow our food resources, irrigated acres accounted for half the total in cropland (2007 USDA Census of Agriculture, 2009). 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.

Swift Wet® is a new product that utilizes the technologies developed by the Advanced BioCatalytics Corporation of Irvine, California (www.abiocat.com). The base technology is based on a synergistic enhancement of surfactants by their combination with certain low molecular weight proteins. This combination has been shown to greatly enhance the ability of the surfactants to perform in many applications such as oil remediation, cleaning, wastewater treatment and agriculture (Podella and Baldrige, 2005). While inducing a profound decrease in surface and interfacial oil/water tension as part of the application to these industries, the protein component of the technology has yet another, more subtle effect upon aerobic metabolism of bacteria (Podella et al., 2009).

An “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 bench experiments and field tests (Podella et al. 2008). The results of the experiments show a leak of protons across the cell membranes. Thus

“uncoupled” bacteria process organic nutrients at a higher rate, maintaining the proton motive force at a level necessary for the general maintenance of the bacteria and their survival. The uncoupling effect can have a number of effects upon a soil system that are currently being explored, but of most interest to this experiment are the effects that the protein enhanced surfactants have upon a test crop. In greenhouse tests previously conducted, the ability of Swift Wet to stimulate root growth of certain plants under limited water supply has been reported (Bethke et al. 2009).

Surfactants have been utilized in agriculture to not only increase the effectiveness of agricultural pesticides in foliar uptake (Tadros, 1995, Wang and Liu, 2007), but to also improve certain soil properties. For example, they have been used as soil additives to enhance water use efficiency (Karagunduz et al. 2001), to eliminate preferential flow paths (Oostindie et al. 2008), and to enhance the penetration/distribution of water in soil matrixes (Cooley et al. 2009).

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 (Ritsema and Dekker, 2000). 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. A fine balance is desirable between these two extremes. The 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 (Jordan et al, 2013). The benefits of using agricultural surfactants to alleviate this issue by spreading the water throughout the soil matrix has been explored to a degree of success (Arriaga et al, 2009). In one study, golf fairway greens that were exhibiting sub-optimal grass growth were remediated by the use of a surfactant. Additionally, there was the extra benefit in that the surfactant helped to dissipate the hydrophobic characteristics of the soil that had developed (Oostindie et al. 2008).

Another report reviewed a wide range of studies and tests using four different surfactants and four different properties in a silt loam and sandy soil using laboratory column tests. The four properties tested were infiltration rates, hydraulic conductivity, capillary rise and water holding capacity. They 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 (Mobbs et al. 2012).

Results for crop yield increases are limited and not strongly reinforced. 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 (Wolkowski et al. 1985). The type of surfactant used was non-ionic. 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 (O'Neil et al. 2005).

Swift Wet® 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. The initial agricultural greenhouse testing that has been previously conducted prompted the tests outlined below with a limited scope of measuring crop water use efficiency, yield and protein content. Not included in this study is the potential positive or negative effect the microbial aspect could have upon the soil biota. Given that many of the nitrogen cycling processes are based upon microbial activity (Sylvia, 2005, Emerich, 2009), further study upon the positive result of this experiment would be highly merited.

Experiment Design

Site Description and Testing Methods

The experiment has been run from August 9th 2013 until harvest time around September 10th 2013. The aim was to determine the effect that the Swift Wet[®] product has upon yield as it relates to water applied in the standard described by the University of Nebraska as crop water use efficiency (Odhiambo et al. 2011). 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 Hammer, Idaho in Jefferson County. A simple test was conducted on two sites watered with Swift Wet[®] against two controls.

The soil of the test site is a mixed area between a sandy loam and a loamy sand having a slightly alkaline pH, averaging 8.4 with an average bulk density of 1.53 g/cc. The soils were formed from wind deposits and are excessively drained. The infiltration rate is found to vary between 6.0-20 inches/hour (USDA Soil Survey, Jefferson County, 1979)

The experiment proceeded with yield as the primary test variable measured and recorded as dry plant biomass in grams/square meter and converted to tons/acre. The second test variable to evaluate the water use efficiency within alfalfa was the total volume of water used to produce the recorded yield. This is recorded as the volume of irrigation water used, accounting for irrigation system efficiency, precipitation, and changes in soil water storage. Protein content is 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 test sites are referred to as West and East sites, both with a corresponding control site in the two fields. The two test sites had Swift Wet® applied to them and were compared to two control sites that have only water applied to them. Both test and control sites were watered for the same amount of time (24 hrs). Test sites were randomly selected in the fields by dividing the field sections where the irrigation sprinkler system stops for watering cycles.

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 or four watering cycles that occur during each harvest session.

Each of the West fields occupies approximately 3.6 acres, and both East fields are approximately 1.38 acres each in size. Fifty samples were planned in each of the West fields, and the East fields had thirty samples apiece. The East site had a greater degree of topographical difference with several steep ($\geq 10^\circ$) 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 does not explain the differences in any significant way, it should be noted as the primary observable differences between the two sites.

Swift Wet® 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 gallon application per cycle at 648,000 gallons or 23.85 acre-inches. The recommended dosage of Swift Wet® is 10 ppm when mixed with water. Therefore, approximately 6.48 gallons of Swift Wet® 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 is 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. The irrigation efficiency was calculated before the experiment began. The fields were mapped and drawn to scale with a grid system assigning numbers to 10 square meter plots. The plots were assigned numbers, and a random number generator was used to select 30 points from both the East and West fields for sampling. The 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. The soil cores were 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. The gravimetric water content has been 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 is 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 $\frac{1}{2}$ inch of the surface and placing them into bags to be dried.

Irrigation stopped roughly one week before harvest. 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 then converted to tons/acre. Fifteen sample points from each of the test plots and each of the control plots will be randomly selected to be ground down for crude protein measurements that will be submitted for laboratory calculation.

Statistical Evaluation of Results

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

$$Z = \frac{(\bar{X}_1 - \bar{X}_2) - (\mu_1 - \mu_2)}{\sqrt{\left(\frac{\sigma_1^2}{n_1}\right) + \left(\frac{\sigma_2^2}{n_2}\right)}}$$

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

$\bar{X}_2$: 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.

Results

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.

$$\text{WUE} = \text{Yield (Tons/Acre)} / (\text{Precipitation} + \text{Irrigation Inches} + \text{Change in soil water content (inches)})$$

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

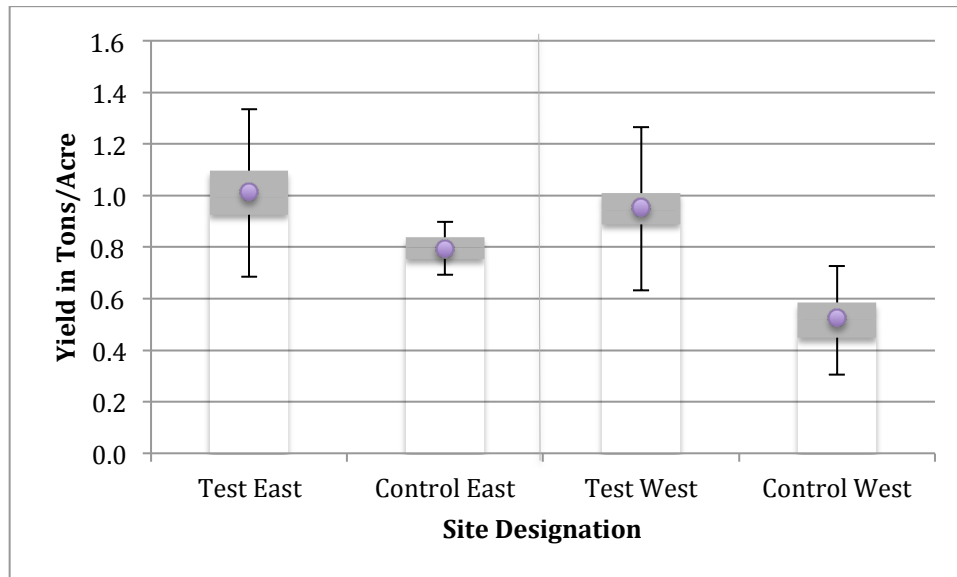
Table 2 summarizes the values found and calculated throughout the harvest session. The irrigation value was calculated based upon the irrigation efficiency and the three watering cycles that each of the sites was given throughout the course of the harvest session.

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

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

Chart 1: Yield Box and Whisker Plot



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

Table 3: Differences and Statistical Validity for East Field

(all units in grams/square meter)

$$\bar{X}_1 = 1.016$$

$$\bar{X}_2 = 0.794$$

$$\bar{X}_1 - \bar{X}_2 = 0.221$$

$$n = 30$$

	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%

Table 4: Differences and Statistical Validity for West Field
(all units in tons/acre)

$$\begin{aligned}\bar{X}_1 &= 0.955 \\ \bar{X}_2 &= 0.526 \\ \bar{X}_1 - \bar{X}_2 &= 0.429 \\ n &= 50\end{aligned}$$

	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)

$$\begin{aligned}\bar{X}_1 &= 0.978 \\ \bar{X}_2 &= 0.627 \\ \bar{X}_1 - \bar{X}_2 &= 0.351 \\ n &= 80\end{aligned}$$

	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%

Discussion & Conclusions

The results of the experiment show that the plots that had Swift Wet® applied to them produced significantly greater yields than that of the plots that received only water. The 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. The water use efficiency values are therefore significantly higher as well.

The test yields from the East Field were greater than 10% larger than those from the control group, with over 99% statistical significance and a strong power level. 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. Aggregated, the test yields from both the East and West Fields were more than 50% greater than the control yields from both fields. The statistical significance of this result is greater than 95% with a 95% power level.

These findings show that given the water added to the system, the alfalfa crops in the sites with sandy soil produce greater yield when applied with the treated water, than in control. The reduction in surface tension and interfacial tension as a result of the surfactant effects upon the soil system correlates with the increased yields as it did in several other published studies (McCauley, 1993, Hagenhoff et al. 2010).

Crude protein content was measured at the Montana State University McCall Lab. Values for the tests and controls were not significantly different being 19.7 % for the tests and 19.6% for the controls. This corresponded with the literature as no reports have as yet found any other results that contradict this.

Taking the reviewed literature into account, the results of the yield can likely be attributed to a couple of different factors. Mobbs et al. 2012 concluded that surfactants in hydrophobic and sandy soils perform better in terms of yield and several other factors like permeability, hydraulic conductivity and permeability with the addition of surfactants. This correlates well with the results given the high sand content of the soil, the dry environment of the site, and the slightly calcareous nature of the soil, which contributes to top and sub soil deposits of salt that could have limited harvests in the controls. The yield observed in the eastern test site was 27.9% greater than that of the control site while the west test site was 81.6% greater.

Future experimentation should also be exercised on a more challenging site that does not exhibit hydrophobic characteristics and that does not possess such a well-drained, sandy soil type. 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 the strong microbial effect the protein component of Ag-Star has, this would be of strong interest. Furthermore, testing the application of Ag-Star on other crops would be of interest.

In addition, it would be worthwhile to perform more in-depth statistical analysis of crops treated with Swift Wet®. In particular, performing Ordinary Least Squares regression analysis of yield as impacted by the volume of Swift Wet® product used, water used, change in soil water content, hours of sunlight, nitrogen content, carbon content, microbial population and other potential independent variables would be extremely useful in determining how application of the Swift Wet® product relates to crop yields while controlling for other factors. Furthermore, testing these applications on a variety of crops and in different soil types would provide additional information.

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, Swift Wet® can significantly improve yields of alfalfa.

References

- Arriaga, F.J., Lowery, B., Kelling, K.A. (2009) Surfactant Impact on Nitrogen Utilization and Leaching in Potatoes. *American Journal of Potato Research*, vol. 86 (5), pages 383-390.
- Bethke, J.A., Michalaw, A.H., Goldfeld, M., Podella, C., Baldridge, Villavicencio, J. (2009) Root mass promotion by a yeast stress-induced protein - surfactant complex in greenhouse studies. *Proceedings of the ASA-CSSA-SSSA 2009 International Annual Meetings*. AnMtgsAbst2009.55307.
<https://scisoc.confex.com/crops/2009am/webprogram/Paper55307.html>
- Cooley, E.T., Lowery, B., Kelling, K.A., Speth, P.E., Madison, F.W., Bland, W.L., Tapsieva, A. (2009) Surfactant Use to Improve Soil Water Distribution and Reduce Nitrate Leaching in Potatoes. *Soil Science*, vol. 174 (6), pages 321-329.
- Emerich, D.W., Krishnan, H.B. editors, (2009) *Nitrogen Fixation in Crop Production*. American Society of Agronomy, Madison, WI.
- Hagenhoff, K., Dong, J., Chowdhry, B.Z., Leharne, S.A. (2010) Aqueous Solution of Anionic Surfactants Mixed with Soils Show a Synergistic Reduction in Surface Tension. *Water, Air, Soil Pollution*, vol. 209 (1), pages 3-13.
- Jordan, M.O., Kelling, K.A, Lowery, B., Arriaga, F.J., Speth, P.E. (2013) Hill Shape Influences on Potato Yield, Quality, and Nitrogen Use Efficiency. *American Journal of Potato Research*, vol. 90 (3), pages 217-228.
- Karagunduz, A., Pennell, K.D., Young, M.H. (2001) Influence of a Nonionic Surfactant on the Water Retention Properties of Unsaturated Soils. *Soil Science Society of America Journal*, vol. 65 (5), pages 1392-1399.
- Kenny, J.F., Barber, N.L., Hutson, S.S., Linsey, K.S., Lovelace, J.K., and Maupin, M.A. (2009). *Estimated use of water in the United States in 2005*: U.S. Geological Survey Circular 1344, 52 p.
- McCauley, G.N. (1993) Nonionic surfactant and supplemental irrigation of soybean on crusting soils. *Agronomy Journal*, vol. 85 (1), pages 17-21.
- Mobbs, T.L., Peters, R.T., Javenport, J.R., Evans, M.J., and Wu, J.Q. (2012) Effects of four soil surfactants on four soil-water properties in sand and silt loam. *Journal of Soil and Water Conservation*, vol. 67 (4), pages 275-283.
- Odhiambo, L.O., Kranz, W.L., Dean, E.E. (2011) *Irrigation Efficiency and Uniformity, and Crop Water Use Efficiency*. University of Nebraska Extension, Lincoln, Nebraska.

O'Neill, M., Heyduck, R., and Prior N. (2005) Soil surfactants for improved soil moisture and nutrient use efficiency in pinto beans. New Mexico State University Agricultural Science Center Annual Progress Report: 133–137. Farmington, NM: New Mexico State University, <http://cahe.nmsu.edu/aes/farm/projects--results.html>.

Oostindie, K., Dekker, L.W., Wesseling, J.G. and Ritsema, C.J. (2008) Soil Surfactant Stops Water Repellency and Preferential Flow Paths. *Soil Use and Management*, 24, pages 409-415.

Podella, C.W. and Baldrige, J. (2005) Changing the Nature of Surfactants. *Industrial Biotechnology*, vol. 1 (4), pages 288-291.

Podella, C.W., Hooshnam, N., Krassner, S.M. and Goldfeld, M.G. (2009) Yeast protein-surfactant complexes uncouple microbial electron transfer and increase transmembrane leak of protons. *Journal of Applied Microbiology*, vol. 106 (1), pages 140–148.

Ritsema, C.J, Dekker,L.W. (2000) Preferential flow in water repellent sandy soils: principles and modeling implications. *Journal of Hydrology*, volumes 231-232, pages 308-319.

Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A. (2005) *Principles and Applications of Soil Microbiology*, 2nd edition, Prentice Hall, New Jersey.

Tadros, T.F. (1995) *Applied Surfactants: Principles and Applications*. Marcel Dekker, New York. Ch. 14, Applications of Surfactants in Agrochemicals.

United States Department of Agriculture, National Agricultural Statistics Service, 2009. 2007 United States Census of Agriculture. U. S. Department of Agriculture, National Agricultural Statistics Service, Fairfax, Virginia.

United States Department of Agriculture, Jorgenson, Wendel, United States Soil Conservation Service, 1979. *Soil Survey of Jefferson County*. USDA, Fairfax, Virginia.

Wang, C.J., and Lui, Z.Q. (2007) Foliar uptake of pesticides-Present status and future challenge. *Pesticide Biochemistry and Physiology* vol. 87 (1), pages 1-8.

Wolkowski, R.P., Kelling, K.A., Oplinger, E.S. (1985) Evaluation of three wetting agents as soil additives for improving crop yield and nutrient availability. *Agronomy Journal*, vol. 77, pages 695–698.