The Effects of Commercial Microbial Inoculant on Morphology and Yield of Soybeans planted near Wheaton Minnesota.

David Sasseville PhD, Tony A. Hagen

ABSTRACT

Agrovive microbial inoculant product Soyfx™ was field evaluated to determine the potential for increasing yields of soybeans using traditional agricultural practices. These microbial-based products were determined to cause real changes in plant growth and morphology. By hand harvesting the crop, these changes resulted in more pods and beans, larger bean size and weights, and overall yield increases. Results utilizing conventional harvest techniques typical to soybean harvesting caused these increases to be masked by preferential loss.

With the soybean variety tested the use of microbial-based products resulted in pods forming lower in the plant architecture, and these pods were filled with heavier beans, thus resulting in the preferential beans being lower to the ground than conventionally grown untreated soybeans. Traditionally set soybean combine heads failed to harvest many of these more robust pods and the resulting heavier beans were left unharvested.

Based on these observations, soybean plants that tend to grow taller and set nodes higher (i.e. leggy) may be preferred varieties to use with these microbial treatments. Also, a comprehensive nutritional program for soybeans to stimulate rapid early vegetative growth may help overcome this problem. Because soybeans depend heavily upon nitrogen fixation for its nitrogen needs, the presence of adequate Rhizobium spp. and micronutrients such as molybdenum, iron and cobalt need to be considered in soybean nutritional programs.

The microbial-based products were applied to soybeans either as a seed treatment or foliar applied. The seed treated soybeans performed better than foliar application regardless whether hand-harvested or machine-harvested. Based on these data, seed treatment is the prefer method of application. The compatibility of the microbial-based products with other soybean seed treatments would need to be evaluated.

Based on the data of these field evaluations, the microbial-based products offer real potential for increasing soybean yields. However, in doing so, with higher yields being removed from soybean fields, this enhances the need for better nutritional programs (including both soil and tissue) to ensure long-term sustainability of these higher yields.

INTRODUCTION

Microbes have the potential for producing plant growth promoting compounds, such as indoleacetic acid, abscisic acid, and others. Over the past three years, bacteria have been collected from native soil and water sources, and screened for production of plant growth regulators that have the potential for enhancing crop growth. Those bacteria that have demonstrated such potential have been isolated, identified, and patented.

These microorganisms were then matched to specific crops based on growth and yield limiting factors of those crops. In this paper, the response of these bacteria applied to soybeans is explored under actual field conditions near Wheaton Minnesota.

MATERIAL AND METHODS

The field used only natural rainfall, with no irrigation. Overall, the crop received adequate moisture for the region Fig. 1, and temperatures were moderate, with no extremes in moisture or temperature Table 1.

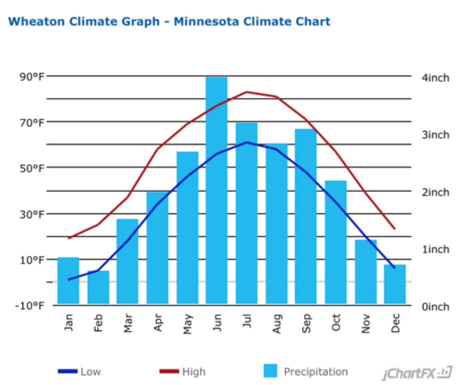


Figure 1. Mean Climatological Data For Wheaton MN.

	Jan	Feb	Mar	Apr	May	Jun
Average high in °F:	19	25	37	58	69	77
Average low in °F:	1	5	18	34	46	56
Av. precipitation in inch:	0.83	0.59	1.5	1.97	2.68	3.98
Average snowfall in inch:	9	8	8	0	0	0
	Jul	Aug	Sep	Oct	Nov	Dec
Average high in °F:	83	81	71	57	39	23
Average low in °F:	61	58	48	35	20	6
Av. precipitation in inch:	3.19	2.83	3.07	2.17	1.14	0.71
Average snowfall in inch:	0	0	0	1	5	8

Table 1. 2018 Monthly Mean Climatological Data

<u>Field soils for the study are:</u>

Doran-Mustinka silty clay loams,
0 to 2 percent slopes
76.9% , 263 acres
ANTLER-MUSTINKA complex,
0 to 2 percent slopes
22.5%, 76.8 acres
Croke very fine sandy loam,
0 to 2 percent slopes
0.6% 2.0 acres

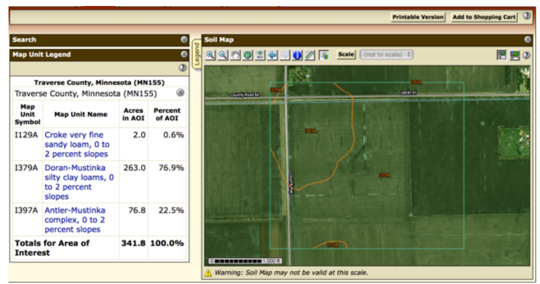


Figure 2. Soils Map for Trial Field

<u>Field Planting Data</u>

The field was planted with Soybeans from Titan Pro seed, Variety 16L86. The seeds were planted in three distinct trial types. Appendix 1. 2018 Planting Map.

The first treatment was a seed treatment using a MyYield™ seed treater with only the trial product. No additional binders were used. It was applied at 8oz of product per 100lb/wt. of seed.

The second treatment was a foliar application put on at second trifoliate. At a rate of 1 quart per acre tank mixed with 10 gallons of water.

The Third treatment was not treated with any additional products other than standard treatments listed.

Standard Treatments for all trials

The seeds were planted on 5-12-18. Pre emergent chemical was Valor EZ @ 2 oz per acre

Post emergent herbicide on all treatments was 32oz. per acre of Liberty and 16oz. of Generic Dual applied on 6-29-18 Appendix 2 Chemical Application Map.

Seed was planted at a variable rate with an average of 145,000 per acre. Seed was planted in 22 inch rows. Appendix 3 Seed Planting Rate Map.

Manual Harvest Methods

The manually harvested soybeans were sampled with an N of 5 randomized structure trial of 1/10,000 of an acre (28.7 inches single row). Based on the Purdue recommendations *(Shaun Casteel, 2010)*

The samples then had the pods removed and separated and counted.

The pods were then separated in each of 5 samples per treatment by :

1 Single pod single bean
2 Double pod single bean
3 Triple pod single bean
4 Double pod 2 bean
5 Triple pod 2 bean
6 Triple pod 3 bean
7 Quad pod 4 bean
8 Total number of individual plants

The pods were then ruptured and the beans were collected.

The beans were then separated by 100 bean counts and weighed.

These were then separated as a composite by size using a ¼ inch (USA Standard testing sieve, woven wire cloth test sieve, Stainless Steel test frame material, Mesh size ¼ inch, Mesh size Range Coarse, Wire Diameter 1.8mm, Opening size 6.3mm, outside diameter 8 inches, Frame inside Diameter 8 inches, overall depth 2 inches., ASTM E11 standards) sieve from a 1/8 cup measuring cup to get a consistent volume.

These were then further counted to determine number above and below ¼ inch in size.

These separated beans were then weighed to determine weight of the sample by size.

Standard Mechanized Harvest Methods

The field was harvested by trial type on October 23, 2018. The combine was operated under normal conditions at an average speed of 4mph. The head was set at a height normal to standard operations (approximately 3 inches from ground level) for soybean harvest in this geographic area.

Samples were then collected in gallon bags taken from the combine hopper at the end of a single round up and down the field in each treatment.

Random samples from the combine harvested sample bags using a 1/8 cup measuring cup to pull samples and used the sieve to separate the sample into less than and greater than ¼ inch.

The beans were then separated by size counted to determine number above and below ¼ inch in size.

These separated beans were then weighed to determine weight of the sample by size.

Sample data was then entered into excel and compared values from each treatment. This data was then entered into SASS and analyzed for statistical significance.

Each sample treatment was compared analyzing both hand harvested and machine harvested.

Equipment used:

Scale: JSR-100 Scale, 100g precision .01g Capacity

Sieve: USA Standard testing sieve, woven wire cloth test sieve, Stainless Steel test frame material, Mesh size ¼ inch, Mesh size Range Coarse, Wire Diameter 1.8mm, Opening size 6.3mm, outside diameter 8 inches, Frame inside Diameter 8 inches, overall depth 2 inches. ASTM E11 standards

Automatic Seed Counter: Goldenwall Automated seed counter machine for various seed shapes: SLY-C

Moisture Sensor: Case IH Agriculture Moisture tester 040 Grain

Combine: John Deere S680

Head: John Deere 640 FD

Average speed: 4 Mph

Results

Total Pod Counts were indicative of a difference between the seed treatment of the Soybean seeds and the foliar application. Fig 3. This was seen in the very large increase in total beans per trial sample as well. Fig 4.

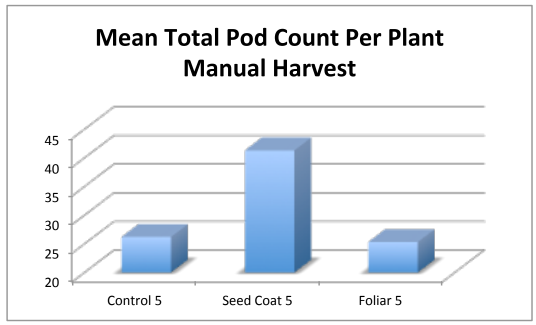


Figure 3. Total pod counts per plant manual harvest n=5.

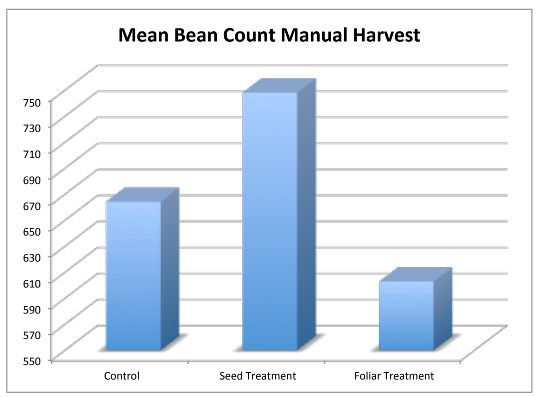


Figure 4. Mean bean count per trial sample n=5.

These increases in pods and beans led to an increase in yield in the manual harvest data of 15.65 bushels per acre between the seed treatment and the control. Fig. 5

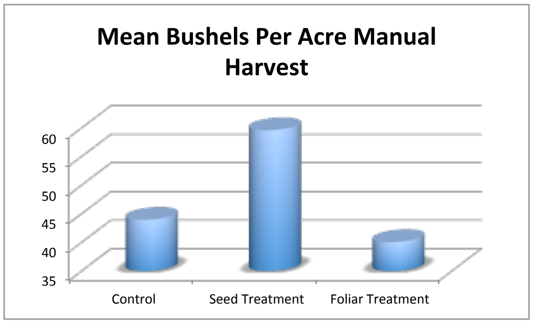


Figure 5. Mean Bushels Per Acre Manual Harvest.

The data was reviewed as it pertained to pod type differences and found that three potential location pods with one bean in them were significantly higher in the seed coated trial. This data revealed an increase of 14 extra 3 locus one bean pods. Fig. 6 Mean Pod Count Per Plant.

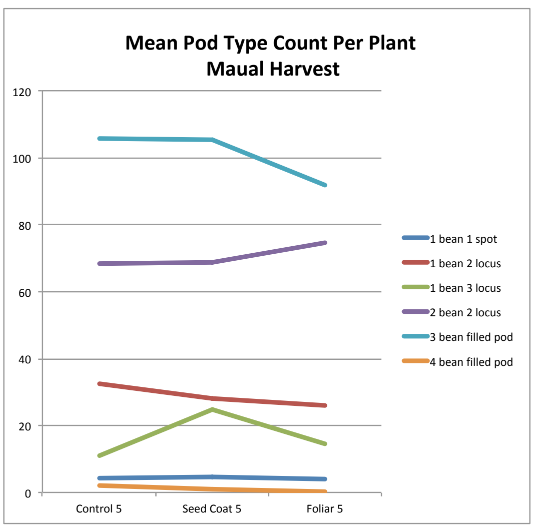


Figure 6. Mean Pod Count Per Plant.

The data shows a significant unfulfilled potential as bean locus in existing bean pods that had a difference of 63.2 beans per plant. Fig 7

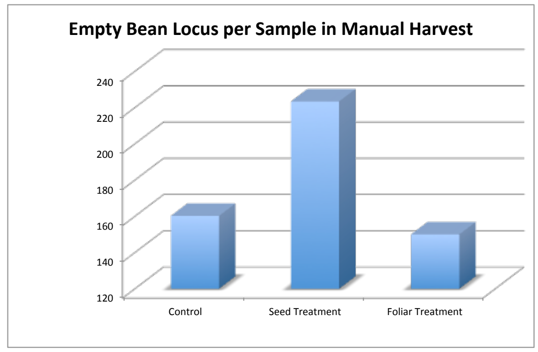


Figure 7. Empty Beans per Sample.

These unfulfilled bean sites represent a large potential for increased yield. The estimated potential of 17.94 bushels per acre from seed coat treatment could be realized if the limitations of translocation and fill could be overcome. Fig. 8

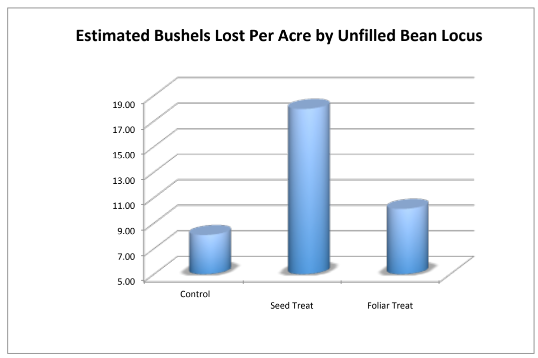


Fig 8. Unfulfilled bean locus potentials in bushels per acre

The difference in bean weight became obvious as 100 count bean weights between the trial types were analyzed. The seed coat showed an increase of 3 grams per 100 beans versus the other two treatments . Fig. 9

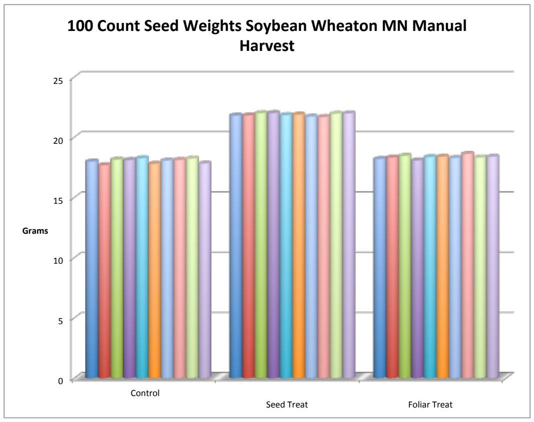


Figure 9. 100 count bean weights by treatment.

When the data is analyzed the 100 count weights comparing manual harvest and the mechanical harvest the data shows a difference that has to be related to a loss of beans due to mechanical harvest methodology. A portion of the extra weight in the beans is lost in the mechanical harvest in the seed coat trial plots. This represents a significant data point that is explained from a bottom up filling of soybeans. (literature review. Article.) Fig 10.

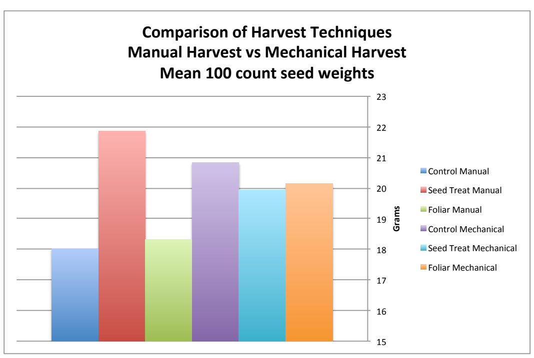


Figure 10. Harvest Technique Comparison of Bean Weights.

The data does however shows the large increase in yield not reflected in the combine from the harvest of the field due to all pods being collected in the manual sampling methodology.

Results from the differential testing of size versus weight were done to determine the correlation of treatment and bean development. The result of using the seed coat method showed a mean increase of 286 beans greater than ¼ inch per sample. There was a corresponding decrease of 199.4 beans smaller than ¼ inch. Fig 11

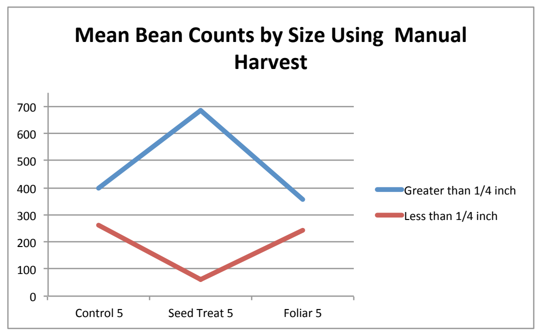


Figure 11. Mean Bean Count by Size.

This differentiation was also shown in the weight of beans greater than ¼ inch in size by 74 grams per sample using seed treatment. The decrease in weight of less than ¼ inch was 32.8 grams per sample. Fig 12

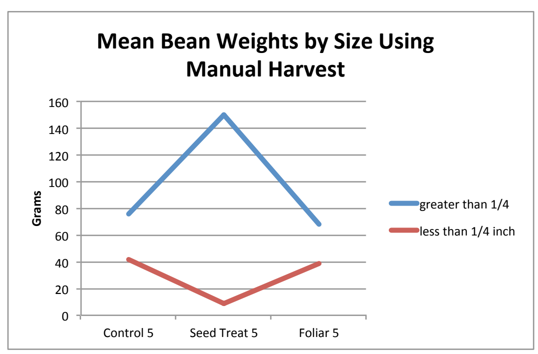


Figure 12. Mean Bean Weights by Size.

This bean count ration differential is smoothed by mechanical harvest which is shown in Figure 13.

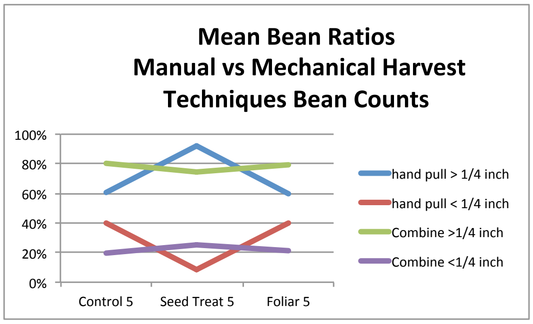


Figure 13. Mean Bean Count Ratios by Harvest Technique.

The mean bean weight was also modulated to remove benefit of the increase in bean weights by size using mechanical harvest. Fig 14.

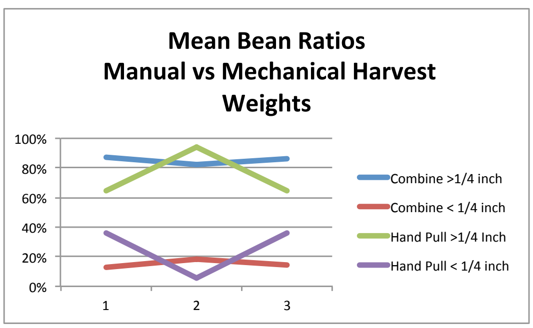


Figure 14. Bean Weight Ratios by Harvest Technique.

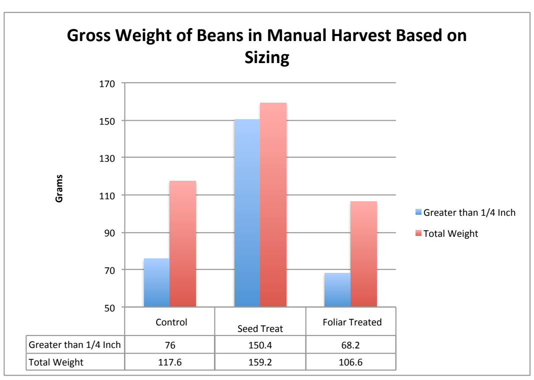


Figure 15. Gross Weight of Beans by Sample by Size and Weight.

Yield of beans were shown to be greater in both sizes of beans by weight by utilizing seed coat method of application. The data showed a substantial increase in the greater than ¼ inch weights in the seed treatment versus control.

Discussion

Yields were increased in the seed coat application of the trial product exclusively in the semi-bush soybean used in the trial. This yield increase was seen in overall size distribution, total bean counts, total bean weights and pod counts. The bean plants were shown to fill from the bottom up and the manual harvest was able to capture these increases while mechanical harvest was unable or incapable of capturing these larger and heavier beans due to placement on the plant architecture. The increase in pods were seen primarily in the lower portions of the plant at growth due to the lowest branches promoting pod set early and allowing the pods to get advantageous access to photosynthetic carbohydrates early. The beans were noted to close canopy up to two weeks prior to untreated plants across the treatment area, which allowed the plants to maintain additional water in the field areas around the seed treated plants preventing loss due to evaporation. The beans showed an increase in size in leaf surface area, and an increase in stem girth, which was advantageous to reduced lodging. The beans showed a dramatic reduction in leaf wilt Fig 16 due to stomatal daily heat response from the treatments. The plants also showed an increase in plant height and overall mass. Plant height began to express itself very early in the plant growth cycle. Fig 18. (Early season pictures on the seed treated plants and untreated control plants). Late in the season while the pods were ripening the plants demonstrated a substantial increase in growth. Fig. 19 which correlates to an increase in photosynthate that could translocate to upper bean pods.

Conclusion

A yield increase of 15.65 bushels per acre was seen in the seed coat versus control in the manual harvest. This was a significant increase in yield and would lead to a dramatic increase in profitability in the field. The capture of these increases in a mechanical harvest needs examination.

Effect of inoculation of seed coat

The difference between seed coat and control yield can be broken down in several data points.

The overall bushels by weight was a 15.65 bushel per acre increase. This is broken down further by total pod count difference of 15.1 more pods per plant. These pods were represented by 14 more 3 bean pods per plant in the seed treated than the control. Total bean counts were increased in the seed treatment sample mean by 83.8 beans per 1/10,000 of an acre which was a 12.6% increase in beans in comparison to control. These beans had a test weight difference of 3.86g per 100 count weight. This represents a 21.3% increase in weight of the beans. This increase in weight was noted by the grower in a discussion with the elevator that was relayed to us. The elevator informed the grower that "they had the highest test weights in the county." The size of the beans was also altered by the seed treatment. The beans were larger in the seed treatment than the control. The number of bean seeds greater than ¼ inch was 92% for seed coat treatment and only 60% for control in manual harvest. This was not seen in the mechanical harvest data. The mechanical harvest data represented the number of beans greater than ¼ inch were 74% for the seed coat treatment and 80% for the control. This shows a modulating of the effect by the harvest techniques. This is supported by the field observation of a large number of three bean pods left behind on the remaining stalks after mechanical harvest in the seed treated plots versus the control plots. Additional research is recommended to determine impact on yield from loss in the field by pods left on the stalk due to plant pod heigh.

The yields realized in the trial plots will need to be examined in depth to find a solution to the lost yield by mechanical harvesting.

Realized Yield Potential

Potential causes for mechanical harvest loss of in field yield are initially proposed to be related to plant architecture differences and overall plant morphology.

The seed treatment of the soybean plants were noted to have caused the initial two nodal leaves to form large pod bearing locations that were populated by full pods of primarily 3 bean size. These branches were monitored throughout the season and were noted at all stages during reproduction. These primary branches were the main pods left behind after harvest of the treatment plots.

Further research is required to determine if a different genetics that could place these nodes higher in the plants architecture could place then in the zone of inclusion of current technology for mechanical harvest.

The standard header height of 3 inches and a lowest nodal height of 1 inch is used to determine height of soybean plant needed to gain yield in each of the following scenarios. Yield recovery of 10,20,50,75,90, and 100 percent of lost yield to header height mechanical harvest loss. See Table 2.

Percentage Recovered	Increased Height of first node In Inches	Bushels per Acre Recovered
10%	0.2	1.57
20%	0.4	3.13
50%	1.0	7.82
75%	1.5	11.73
90%	1.8	14.09
100%	2.0	15.65

Table 2. Plant first nodal height recovery data.

The oversimplified height recovery data in Table 2 shows that even slight increases in height can lead to dramatic yield increased in mechanical harvest.

Unrealized Yield Potential Recovery

The loss of potential in the yield as demonstrated by the lack of complete fill of bean pods versus simple pod abortion can be attributed to many factors, which can effect translocation of resources and overall plant resource development. The increase in overall plant mass is suspected to be the cause of additional limitations of resources.

Further research is necessary for this unfulfilled yield potential. Recommendations for further trials include the use of micronutrient and co-enzyme products to allow for full realization of translocation potentials and the ability of the plant to maintain an increased level of hormone production.

Literature Cited

Early season photo showing seed treated plants on left and control on right

Late season seed treated on left control on right

Late season seed treated samples on left and control on right

Beans after being left in bed of truck for one hour showing difference in leaf wilt. Plant on left is seed treated and plant on right is control.

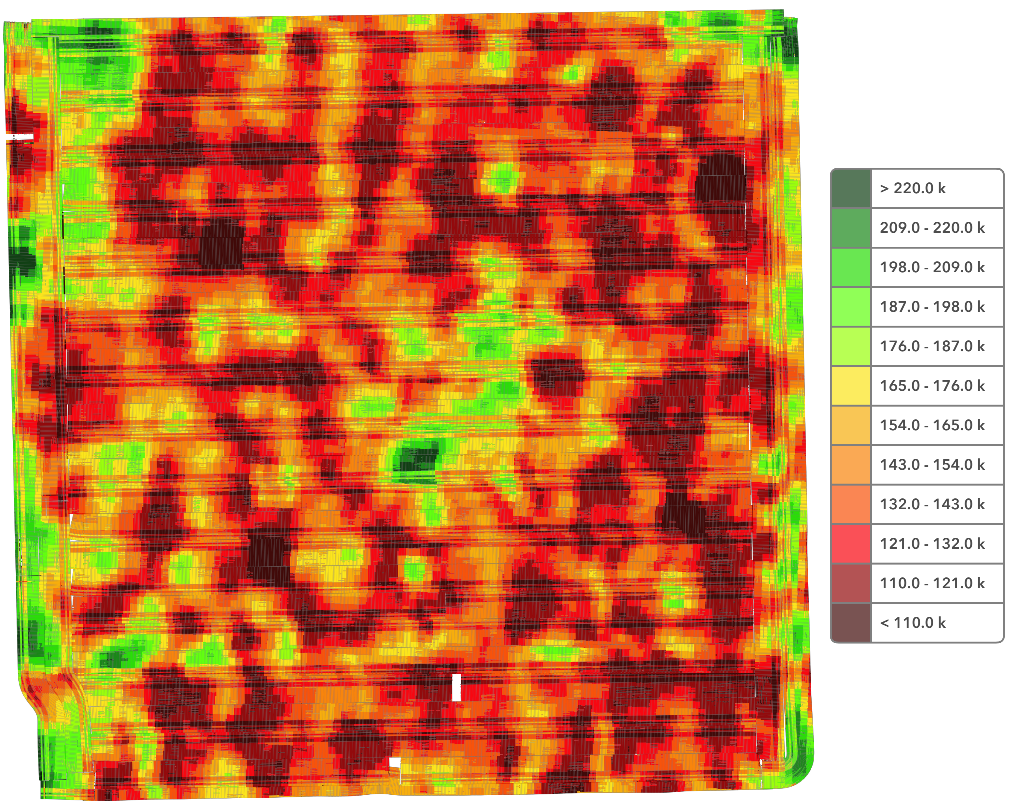
Harvest Information
2018 Plant Population Mapping

CLIMATE FIELDVIEW

Population

Elroys south

Raguse Farms | Raguse Farms



2018 Plant Trial Mapping

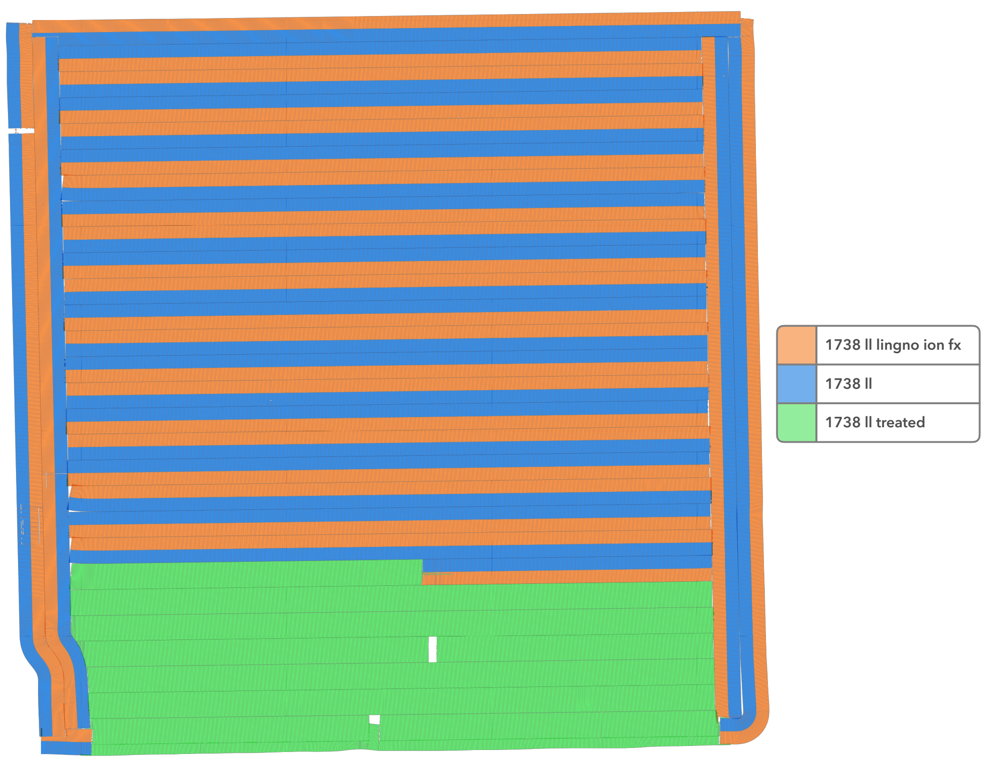
Orange is seed treatment with IONfx
Blue is treated with Lignojoule only.
Green is Seed Treated with MY Yield standard F3 treatment
North side of green is foliar treated with IONfx at second trifoliate

CLIMATE FIELDVIEW

Variety

Elroys south

Raguse Farms | Raguse Farms



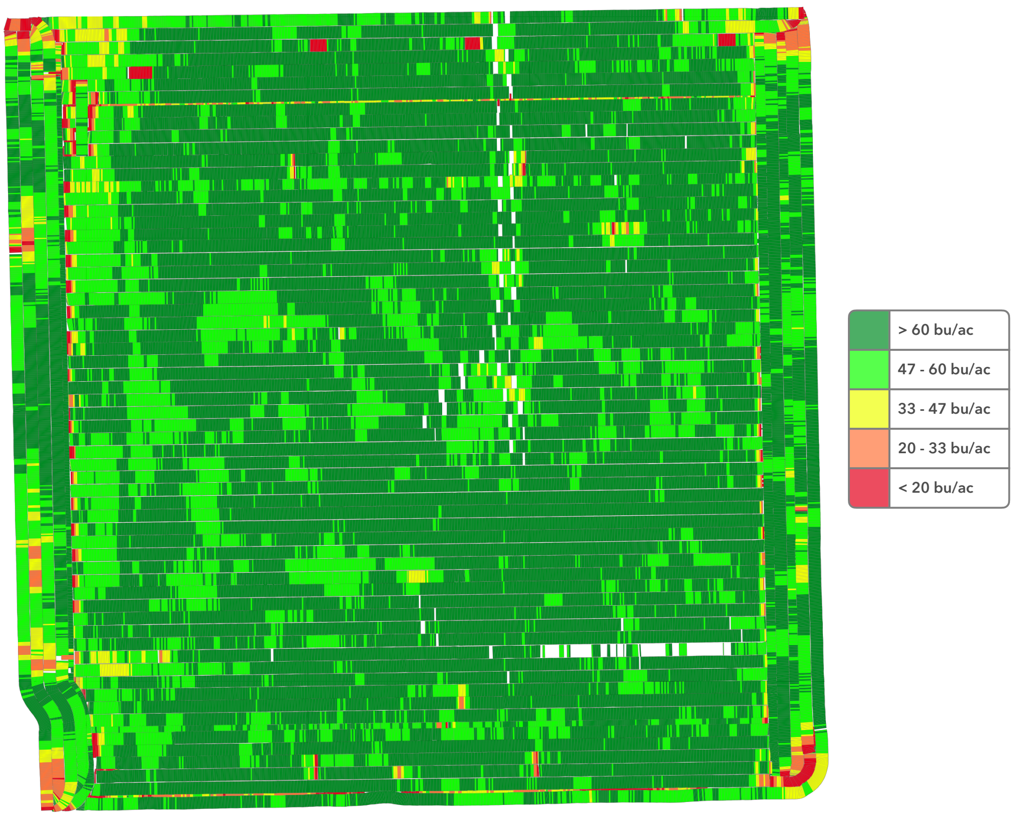
2018 Yield Mapping

CLIMATE FIELDVIEW

Yield

Elroys south

Raguse Farms | Raguse Farms



2017 Fall Soils Testing

REPORT NUMBER
17-307-1203
COMPLETED DATE **Nov 7, 2017** ACCOUNT **37230**
RECEIVED DATE **Nov 3, 2017**

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PAGE 1/1

TODAY'S DATE **Nov 07, 2017**

WARREN AG AVIATION
WARREN RAGUSE
301 12TH ST N
WHEATON MN 59296

IDENTIFICATION
RAGUSE FARMS
CLIFTON 4 NW

SOIL ANALYSIS REPORT

INFO SHEET: 956419

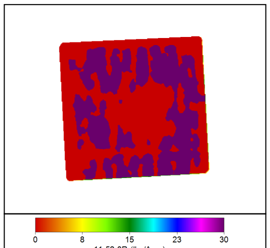
LAB NUMBER *320*	SAMPLE IDENTIFICATION	ORGANIC MATTER L.O.I.		PHOSPHORUS						POTASSIUM (NEUTRAL AMMONIUM ACETATE (EXCHANGEABLE))		MAGNESIUM		CALCIUM		SODIUM		pH		CATION EXCHANGE CAPACITY C.E.C.	PERCENT BASE SATURATION (COMPUTED)				
				P_1 (WEAK BRAY) 1:7		P_2 (STRONG BRAY) 1:7		OLSEN BICARBONATE P		K		Mg		Ca		Na		SOIL pH 1:1	BUFFER INDEX		% K	% Mg	% Ca	% H	% Na
		percent	RATE	ppm	RATE	ppm	RATE	ppm	RATE	ppm	RATE	ppm	RATE	ppm	RATE	ppm	RATE			meq/100g					
61739	LOL 3	3.1	M	56	VH	106	VH	43	VH	172	M	1144	VH	3129	M	148	H	7.4		26.3	1.7	36.2	59.7	0.0	2.4
61740	LG 5	3.3	M	35	VH	101	VH	23	VH	171	M	879	VH	2864	M	35		7.3		22.2	2.0	33.0	64.3	0.0	0.7
61741	DG 7	3.8	H	23	H	67	VH			167	M	871	VH	3230	M	45		6.9		24.0	1.8	30.2	67.2	0.0	0.8

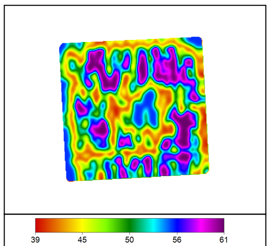
LAB NUMBER *320*	NITRATE-N (FIA)										SULFUR S ICAP		ZINC Zn DTPA		MANGANESE Mn DTPA		IRON Fe DTPA		COPPER Cu DTPA		BORON B SORB. DTPA		EXCESS LIME RATE	SOLUBLE SALTS 1:1	
	SURFACE			SUBSOIL 1			SUBSOIL 2			Total lbs/A															
	ppm	lbs/A	depth (in)	ppm	lbs/A	depth (in)	ppm	lbs/A	depth (in)		ppm	RATE	ppm	RATE	ppm	RATE	ppm	RATE	ppm	RATE	ppm	RATE		mmhos/cm	RATE
61739	13	23	0-6							23	588	VH	2.4	M	3	VL	26	VH	1.1	M	1.3	H	L	2.0	M
61740	12	22	0-6							22	34	VH	2.6	M	2	VL	25	VH	1.1	M	1.0	M	L	0.6	L
61741	20	36	0-6							36	81	VH	2.7	M	5	L	22	H	1.2	M	1.1	M	L	0.8	L

REV. 12/03

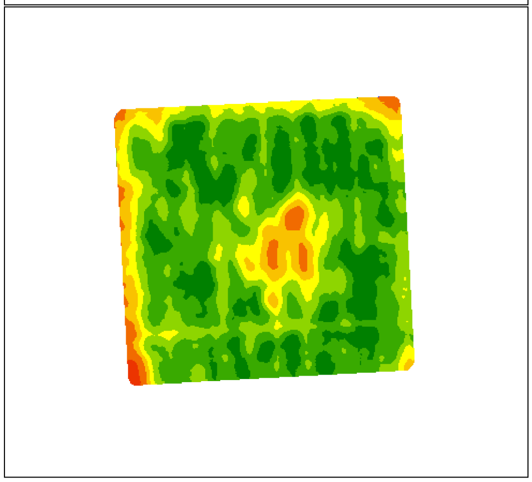
The above analytical results apply only to the sample(s) submitted. Samples are retained a maximum of 30 days.

2017 Fall Fertilization Mapping





RF FALL 17 BCAST EST



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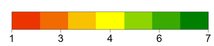

Average: 5.5 zone ID Total: 844 lbs

Client: Raguse Farms
Farm: Raguse Farms
Field: Clifton 4 NW
154.4 Ac

Grower: Raguse Farms Farm: Raguse Farms Field: Clifton 4 NW	Notes:	Layer Summary Zone Print Date 11/2/2017

Product	Rate Range	Average Rate	Investment	Required
ZONE NUM	1 3 4 6 7 ZONE NUM-(zone ID)	5	$	
11-52-0R	0 8 15 23 30 11-52-0R-(lbs/Acre)	13	$	2,028
0-0-60R	39 45 50 56 61 0-0-60R-(lbs/Acre)	50	$	7,737

Acres: 153.7 Total Investment: $ Investment Per Acre: $

2018 Seed Population Mapping