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Confidential to Heads Up® Plant Protectants Inc.
Evaluation of Heads Up® Plant Protectant Efficacy in Reducing White Mold Incidence/Severity on Dry Edible Bean in Southern Alberta

FINAL REPORT

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1. Purpose

1.1. To evaluate the efficacy of Heads Up® Plant Protectant as a seed treatment for control of white mold on dry edible bean.

1.2. To evaluate the efficacy of Heads Up® Plant Protectant tank mixed with an industry standard seed treatment fungicide (Cruiser Maxx® Beans + Streptomycin) for control of white mold on dry edible bean.

2. Background

White mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most devastating diseases of pulse and legume crops in many areas of the world. It is the main production constraint in dry bean production in many areas of western Canada. Crop rotation is of marginal effectiveness in managing the disease due to the pathogens ability to survive many years in soil as sclerotia. Fungicides are a primary method of disease management however the loss of Ronilan EG (Vinclozolin) has left the dry bean and soybean industry with no fungicide alternatives that equal its efficacy and flexibility. New fungicides, combinations and additives are currently being sought to fill the gap in white mold management on beans.

Heads Up® Plant Protectant is a "is a natural source plant defense 'activator'" that "can be beneficial in controlling several types of fungal and bacterial diseases." (http://www.sar-headsup.com/history.php). It is currently registered in the USA for use on beans and soybeans for control of white mold.

3. Summary of the Test Method

3.1. Test Materials

Table 1. Test Organisms:

#	PLANT SPECIES	MARKET CLASS	CULTIVAR
1	*Phaseolus vulgaris L.*	Pinto	Winchester
#	**PATHOGEN SPECIES**	**DISEASE**	**SOURCE**
1	*Sclerotinia sclerotiorum* (Lib.) de Bary	White mould	Sclerotia in Soil

Table 2. Treatment Products

Treatment Product	Source	Active Ingredient
CruiserMaxx® Beans	Syngenta	Thiamethoxam (22.61%) Mefenoxam (1.7%) Floudioxonil (1.12%)
HeadsUp®	HeadsUp® Plant Protectant	Chenopodium extract
Seed Dressing	Omex	Nutritional seed treatment
Exp. Mn	Omex	Manganese foliar nutritional supplement

3.2.

3.3. Plot Design

3.3.1. The plot included treatment entries from two customers. The overall, experimental plot was designed as a randomized, complete block containing 16 treatments and 4 replicates. Of the sixteen total treatments, eight are included in this report. The eight treatments and one control are listed in Table 3, below. The subplot arrangement is shown in Figure 1.

Table 3. Project Treatment List

Trt No.	Treatment name	Placement & timing	Product Rate
1	CruiserMaxx® Beans	Seed;	195mℓ/100kg;
2-8	Other customer plots		
9	Tank-mix: Omex SeedDressing & HeadsUp® & CruiserMaxx®Beans; + Foliar Mn	 Seed, Seed, Seed, Foliar;	 6mℓ/kg, 1g/ℓ, 195mℓ/100kg, 1ℓ/ac;
10	Other Customer plot		
11	HeadsUp®	Seed	1g/ℓ
12	Sequential application: 1 - CruiserMaxx®Beans 2 - HeadsUp®	 Seed Seed	 195mℓ/100kg 1g/ℓ
13	Sequential application: 1 - HeadsUp® 2 - CruiserMaxx®Beans	 Seed Seed	 1g/ℓ 195mℓ/100kg
14	Tank-mix: CruiserMaxx®Beans & HeadsUp®	 Seed Seed	 195mℓ/100kg 1g/ℓ
15	Tank-mix: Omex SeedDressing & HeadsUp® & CruiserMaxx®Beans	 Seed Seed Seed	 6mℓ/kg, 1g/ℓ, 195mℓ/100kg,
16	Untreated Check	None	None

Figure 1. Plot Diagram of Treatments

3.4. Seed Treatment

3.4.1. For exclusive or sequentially applied seed treatments, CruiserMaxx® seed treatment was added to 1kg of 'Winchester' bean seed in a rotating drum treater at a rate of 1.95mℓ /1kg for each treatment. Beans were treated for 2 minutes and laid out in a single layer to air dry.

3.4.2. For exclusive or sequentially applied seed treatments, 5.9 mℓ of a 1g/ℓ solution of HeadsUp® was added to 1 kg of 'Winchester' seed in a rotating drum treater, as in section 3.4.1.

3.4.3. For the tank-mix treatments:

3.4.3.1. In treatment #14, 6mℓ of a 1 g/ℓ aqueous solution of HeadsUp® was added to 1.95mℓ of CruiserMaxx® Beans and applied to 1 kg of 'Winchester' seed as in section 3.4.1.

3.4.3.2. It was desirable to apply less than 8mℓ of solution per kg of seed in any single application in order to avoid damaging the seed. Thus, for treatments #9 and #15, HeadsUp® was first dissolved in Omex seed dressing, rather than water, at the stated rate of 1 g/ℓ and 6mℓ was tank-mixed with 1.95mℓ of CruiserMaxx® Beans. This tank-mix was then added to 1 kg of 'Winchester' seed as in section 3.4.1.

3.4.4. Dried seed was collected and packaged into 16 packets, each containing130 seeds for planting in four-row subplots at a rate of 1 packet per row. Packaged seed was kept at 5ºC until use.

3.5. Preparation of the field, seeding and agronomy

3.5.1. Field 75 of Lendrum farm, located at Alberta Agriculture's Crop Diversification Centre South facility in Brooks, AB was used to house the plot.

3.5.2. The field was opened with a vibrashank-style cultivator. The top 6" of topsoil was adjusted to 50lb N/acre and Rival® herbicide was applied for weed control and worked into the soil twice with cultivator and harrows.

3.5.3. Beans were seeded in four-row subplots using a tractor-mounted, four row cone seeder using the individual seed packets prepared in section 2.3. Seed was sown at a depth of 3-5 cm into 8m rows with 70 cm separation between rows.

3.5.4. Four replicates of each variety were planted using a randomized, complete block design, according to the trial map in figure 1.

3.6. Plot disease inoculation and plot maintenance

3.6.1. Following stand establishment, sclerotia of *Sclerotinia sclerotiorum* were hand-scattered over the subplots, between the rows. The bean subplots were hilled using a cultivator with narrow shovels. This process also had the benefit of incorporating the sclerotia.

3.6.2. Irrigation was performed as needed for crop growth and establishment, as well as to encourage disease pressure in the plots. Weeding was performed manually throughout the growing season.

3.7. Foliar Treatments:

3.7.1. The foliar Manganese application in treatment #9 was prepared at a rate of 1 ℓ/ac. The Manganese spray solution was mixed with lab-grade water to generate a 2ℓ, aqueous solutions. The foliar spray solution was used the day of preparation.

3.7.2. Foliar sprays were applied using a CO_2 pressurized, 3m boom sprayer, set at 37PSI with four, active XR TEEJET 8003VS nozzles. Applications were manually calibrated to deliver 250 mℓ of solution per subplot. Water was used to rinse the sprayer between treatments.

3.7.3. The first spray application was performed at 20% bloom. Refer to Figure 2 for a bloom staging guide for dry beans. As no disease pressure was observed at this point, two further applications were performed 10 days and 20 days after the first application.

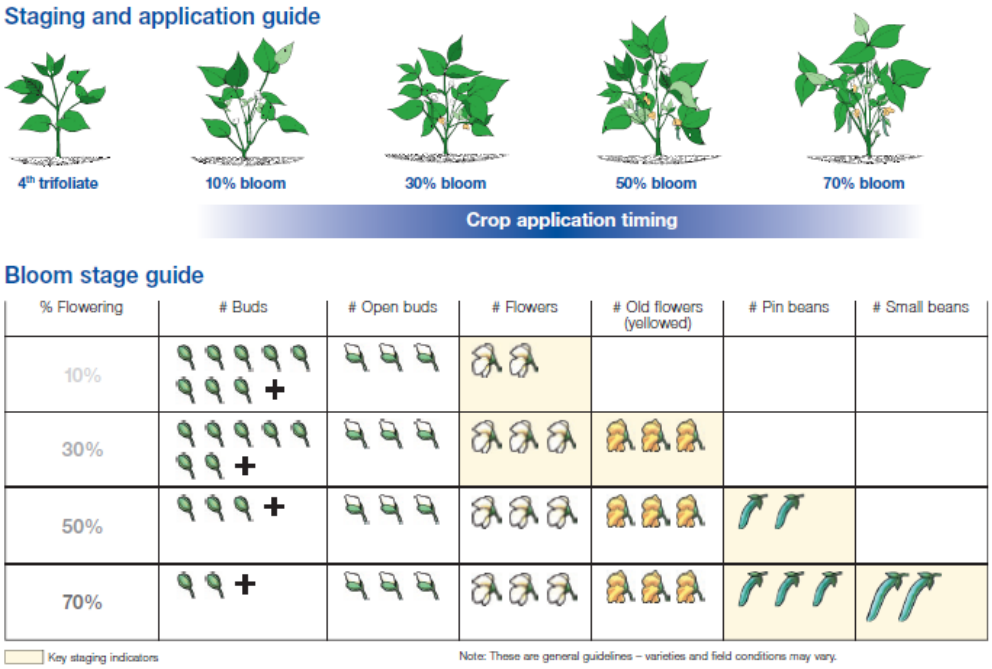


Figure 2. Bloom stage guide
(Source: http://hoegys.ca/cms/files/file/lance_beans.pdf)

3.8. Data Collection

3.8.1. Beginning two weeks after planting, emergence counts were taken on a daily basis, until counts began to plateau, with a final stand count taken 30 days after planting (DAP). A 6m portion of the central rows was marked to ensure that the length of the enumeration area was constant. The two centre rows of each subplot were enumerated and the sum of all plants was reported.

3.8.2. White mould ratings were performed weekly following the onset of the disease. Twenty five plants were chosen at random from the center two rows of each subplot and rated for disease incidence and severity.

3.8.2.1. Disease incidence was determined as the percentage of plants showing disease symptoms of any stage.

3.8.2.2. Disease severity was determined on the Kutcher 0-5 rating scale based on the portion of the plant affected by the disease. The scale is based on the following severity estimates:

Severity Rating:
0 = no disease observed
1 = infection limited to pods only
2 = ¼ of plant affected, 1-2 main branches
3 = ½ of plant affected, 2-3 main branches
4 = ¾ of plant affected, 3 or more branches
5 = main stem lesion near the base affecting the whole plant

3.8.2.3. The overall disease index was calculated for each subplot by taking the sum of the values of all disease ratings as a percentage of the maximum possible sum of disease ratings (the number of plants x a rating of 5).

3.8.3. Beans were undercut when pods reached approximately 75% buckskin appearance, with the intention of drying the plants for harvest and calculation of yield. Unfortunately, five days after cutting, a severe windstorm struck the site and 15 subplots were damaged sufficiently to prevent collection of their yield data.

3.8.4. The remaining plots were machine-harvested using a Wintersteiger plot combine.

3.8.5. Harvested seed from each subplot was weighed, cleaned using a forced-air seed blower and re-weighed.

3.8.6. A 50g sub-sample was collected from each subplot and analyzed for moisture content. Final yield values were standardized to 15% moisture.

3.9. Data analysis

3.9.1. ARM8 software (Gylling Data Management Inc.) was used to perform an Analysis of Variance (ANOVA) on all plant emergence counts, as well as on all disease incidence, severity and index ratings, and also on the standardized yield and dockage measurements. When significant F values were obtained ($p \leq 0.05$), mean separations were performed using LSD at a significance level of $p \leq 0.05$.

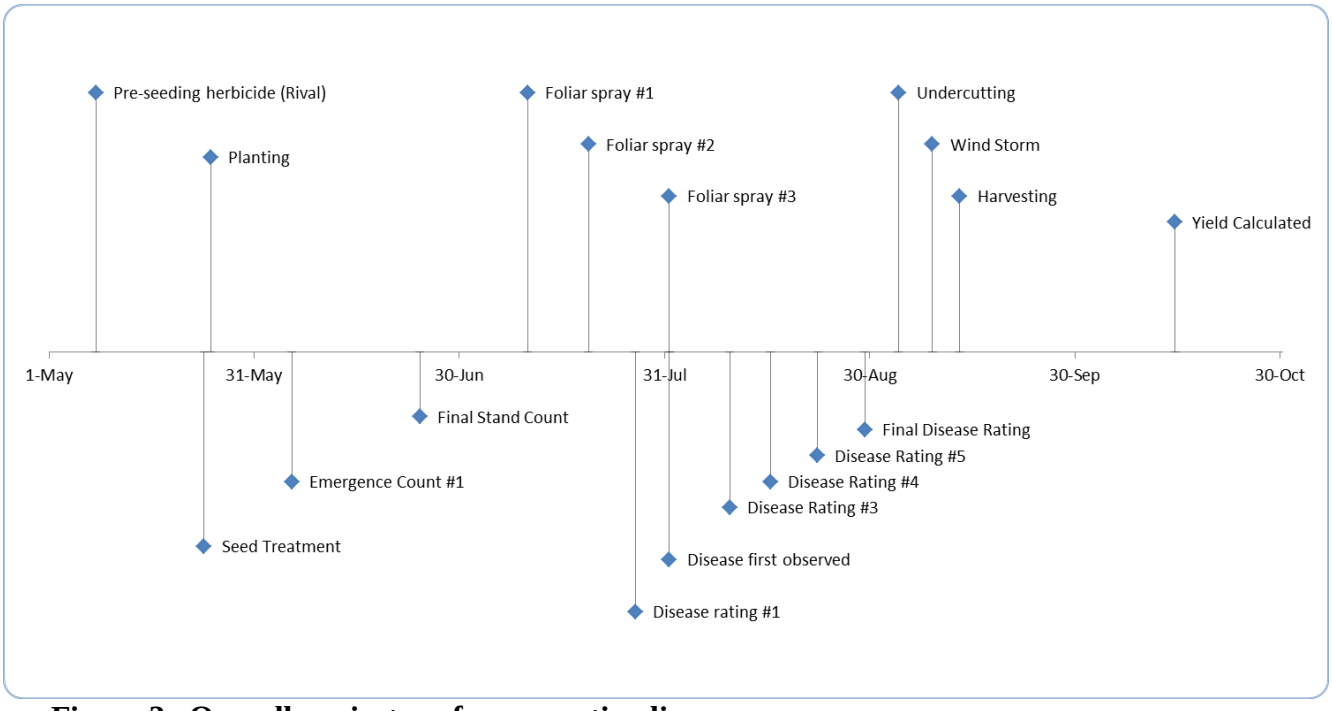


Figure 3. Overall project performance timeline

4. Results

4.1. Emergence

4.1.1. A graph of the average stand count for treatment s at 30 DAP is in Figure 4.

4.1.2. When an ANOVA was performed on seedling emergence data, no significant differences were observed on single date or on the final stand count. Raw data and ANOVA results may be found in Section 9.2.

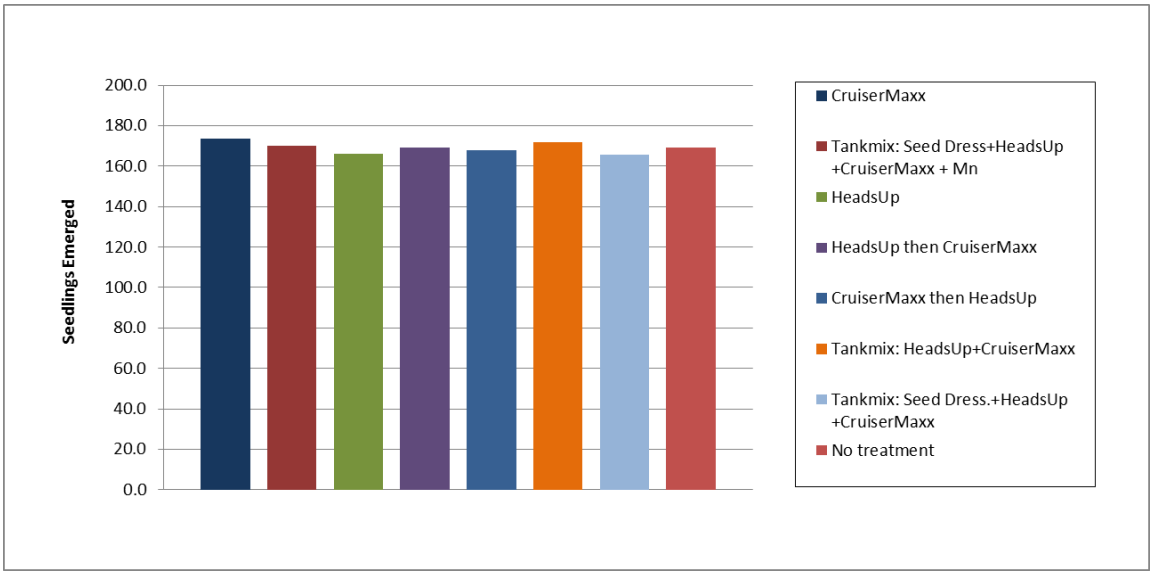


Figure 4. Average Total Emergence 30 DAP

4.2. Disease incidence, severity and index

4.2.1. Six disease ratings were performed on the plot, starting on July 27th, however disease symptoms were not observed until August 1st.

4.2.2. Disease incidence and severity peaked with the August 23rd rating set and declined with the last rating on August 30th. This was likely due to the turning of the colour in the plants making observation of symptoms more difficult and the decomposition state of dead plants with previous, high ratings.

4.2.3. Although disease pressure peaked on August 23rd, when subplots were averaged across the entire plot, the average plot incidence was only 31% on that date and the average plot severity was 1.1 out of 5.

4.2.4. Despite the relatively low disease pressure, statistically significant differences between treatments were observed in incidence, severity and overall disease index. Mean separation test results from this date are shown in Table 4

4.2.4.1. CruiserMaxx®Beans did not significantly differ from the untreated check for any of disease incidence, disease severity or index of disease.

4.2.4.2. In all, three of the six treatments utilizing HeadsUp® were significantly lower in disease incidence than both the CruiserMaxx® and untreated checks, namely 1)HeadsUp® alone, 2)the Tankmix of HeadsUp® and CruiserMaxx® Beans and 3) the tankmix of Omex seed dressing, HeadsUp® and CruiserMaxx®Beans with foliar Manganese applied.

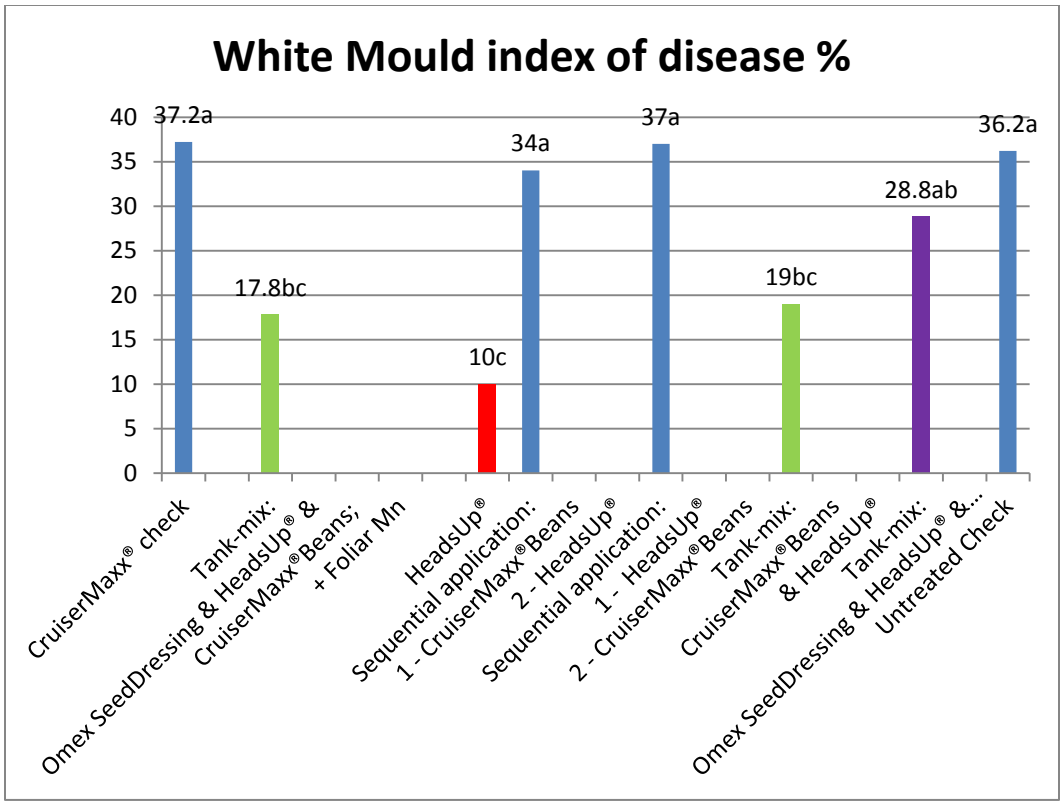
4.2.4.3. Sequentially applied treatments of HeadsUp® and CruiserMaxx®Beans did not significantly differ from control checks, regardless of the order of product application.

4.2.4.4. HeadsUp® alone had the lowest disease incidence, severity and index, however it was not statistically superior to the other two successful treatments.

Table 4. Mean separations summary for August 23rd disease rating

Trt	Treatment Description	Disease Incidence (%)	Disease Severity (0-3)	Index of Disease (%)
1	**CruiserMaxx® check**	49.0 a	1.860 a	37.20 a
2-8	Other customer			
9	**Tank-mix**: Omex SeedDressing & HeadsUp® & CruiserMaxx®Beans; + Foliar Mn	28.0 bc	0.890 bc	17.80 bc
10	Other customer			
11	HeadsUp®	18.0 c	0.500 c	10.0 c
12	**Sequential application**: 1 - CruiserMaxx®Beans 2 - HeadsUp®	41.0 ab	1.700 a	34.00 a
13	**Sequential application**: 1 - HeadsUp® 2 - CruiserMaxx®Beans	47.0 a	1.850 a	37.00 a
14	**Tank-mix**: CruiserMaxx®Beans & HeadsUp®	31.0 bc	0.950 bc	19.00 bc
15	**Tank-mix**: Omex SeedDressing & HeadsUp® & CruiserMaxx®Beans	40.0 ab	1.440 ab	28.80 ab
16	**Untreated Check**	48.0 a	1.810 a	36.20 a
CV%		28.31	31.38	31.38
Replicate F		5.797	5.828	5.828
Replicate Prob/F		0.0047	0.0046	0.0046
Treatment F		4.321	5.959	5.959
Treatment Prob/F		0.0042	0.0007	0.0007

*- numbers followed by the same letter rating are not significantly different by *LSD* ($p<0.05$)



5. Yield and Dockage

5.1. Yield data was collected on subplots not damaged by wind.

5.2. Yield and dockage were not significantly different between treatments

5.3. Average standardized yield and dockage are shown in Figures 5 and 6.

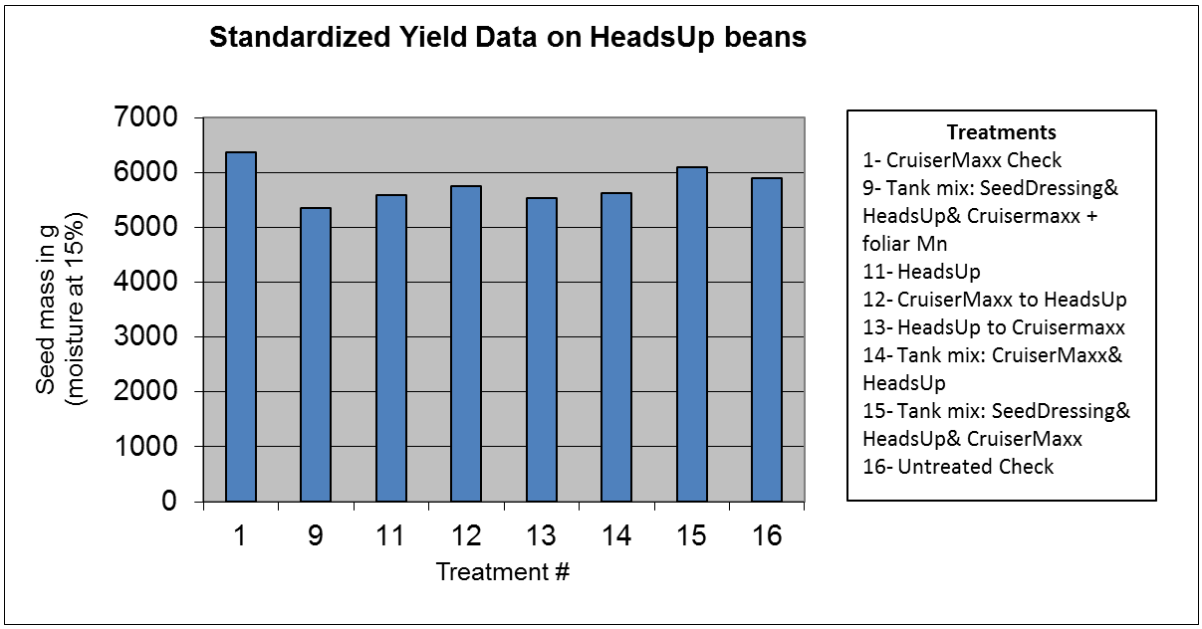


Figure 5. Yield data for Omex treatments

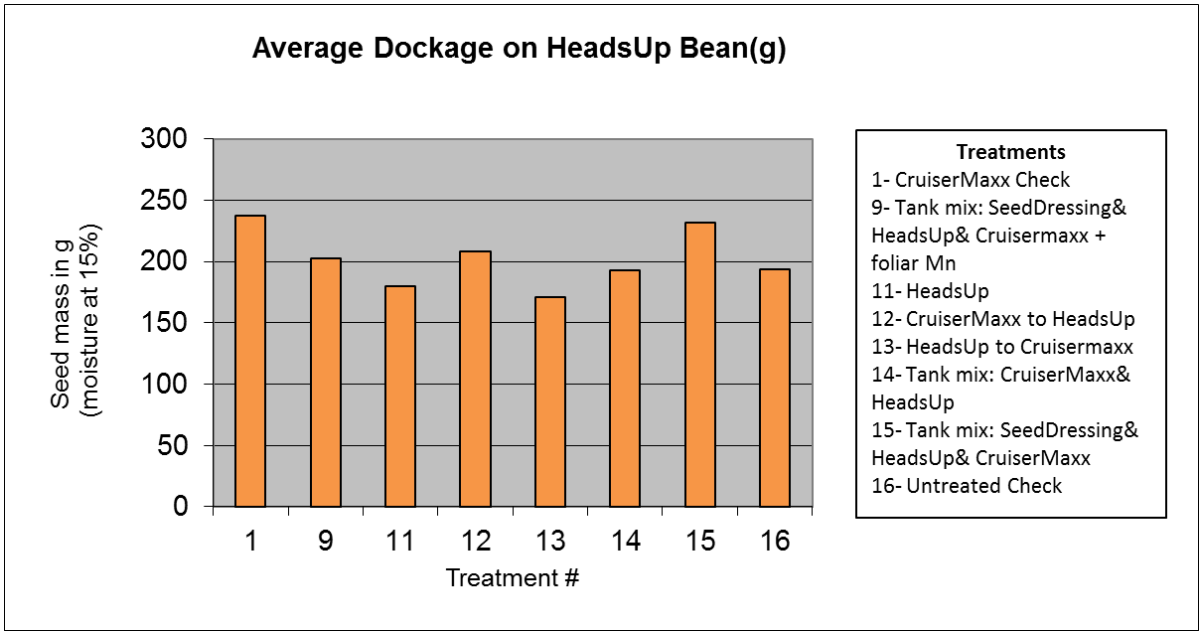


Figure 6. Dockage on Omex dry bean treatments

6. Discussion and Conclusions

6.1. No statistically significant differences were observed in emergence between seed treatments.

6.2. Disease pressure was lower than expected in 2012, with only a 31% disease incidence and a 1.1 disease severity rate in the plot, as a whole, despite artificial inoculation of the site. Discussion with representatives of Viterra in Bow Island and Vauxhall has lead us to the conclusion that weather conditions this summer were not conducive to the development of the disease in dry beans. The wet spring, in conjunction with the long dry periods in July and August (Section 8.3) seems to have allowed the beans to develop ahead of the disease, reaching plant maturity much faster than usual. This is confirmed by the lack of disease in the plot until well after the plants reached 20% bloom.

6.3. Despite the low disease pressure, three treatments performed significantly better than both the untreated check and the CruiserMaxx® check. HeadsUp® alone, HeadsUp® in tankmix with CruiserMaxx®beans, and HeadsUp® in tankmix with Omex Seed Dressing and CruiserMaxx®beans with supplemental Manganese all had statistically lower disease incidence, severity and index of disease when compared to the treated and untreated checks.

6.4. No statistically significant differences were seen in yield or dockage. Due to the low disease incidence and severity of the overall plot and the later-than-normal onset of the disease, it is likely that yield was not affected enough to give statistical separation between treatments.

6.5. Repetition of this project under stronger disease pressure is recommended.

7. Recommendations

7.1. Use of HeadsUp® alone and in tank mixes with CruiserMaxx®Beans resulted in lower disease incidence and severity than in either the untreated check or CruiserMaxx®beans alone, at low levels of disease pressure. Repetition of these treatments under stronger disease pressure is recommended.

8. Innovotech Personnel

Position	Staff Member
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Lead Field Technician	Ms. Margo Unruh
Field Technologists	Ms. Megan McCaig Mr. Jordan Bain Ms. Erin Tateson