Field Evaluation of Biopesticides to Control Diseases of Green Beans

February 2013

Final Report
to:

Fraserland Organics
Lower Mainland Horticultural Improvement Association
Organic Sector Development Program

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SUMMARY

Botrytis (*Botrytis cinerea*) and Rhizoctonia (*Rhizoctonia solani*) are two fungal diseases that proliferate easily in southwestern BC. Organic growers mainly rely on cultural controls to manage these pathogens. However, because Botrytis and Rhizoctonia affect a broad range of crop and overwinters in the soil and decaying plant debris, management of these diseases is a challenge. Organic growers would largely benefit from reliable biofungicide options. This study consisted of two experiments. First a foliar trial that examined four foliar sprays 1)Serenade (*Bacillus subtilis)*, 2)Actinovate (*Streptomyces lydicus*), 3)Influence (garlic extract), 4)Regalia (*Reynoutria sachalinensis*), compared to a water Control. Second a seed treatment trial comparing the seed treatment, Heads Up (Saponins of *Chenopodium quinoa*) to an untreated Control. In both experiments, the objective was to measure the efficacy of products for control of Botrytis and Rhizoctonia on beans. Foliar treatments began one week before flowering stage and were sprayed every five to seven days for a total of seven applications. The seed treatment was applied the day of planting and seeds were planted by hand. We did not observe any significant treatment effect of any of the treatments (foliar and seed) on Botrytis incidence or Rhizoctonia incidence and severity, nor did we see any detrimental effect on plant stand or yield. However, we did observe a trend towards Actinovate, Regalia and Influence treated plots having a fewer beans with Botrytis when compared to the Control. Furthermore, we did observe fewer plants with severe Rhizoctonia infections in the Actinovate and Regalia plots, but again these trends were not significant. These treatments would be good candidates for further evaluations as the 2012 field season was unusually hot and dry, and thus environmental conditions for the development of Botrytis was not optimal.

INTRODUCTION

Botrytis (*Botrytis cinerea*) and Rhizoctonia (*Rhizoctonia solani*) are two fungal diseases that proliferate easily in southwestern BC. Organic growers mainly rely on cultural controls (e.g. rotation and wider spacing between plants) for management of these diseases. However, because Botrytis and Rhizoctonia affect a broad range of crops and overwinter in the soil in decaying plant debris, management of these pathogens is a challenge, even with cultural controls. While conventional bean growers have multiple spray options for Botrytis (Rovral, Lance, Switch , Fontelis) and seed treatments for Rhizoctonia (Thiram, Captan, Apron), organic growers are left with very limited tools, especially when the weather is favorable (cool and wet) to fungal disease development.

On beans, *Botrytis cinerea* infection starts on either immature or decaying flowers or via wounds. As the disease progresses pods become infected resulting in a visible grey-mould. The disease can lead to rejection of an entire crop by processing companies. Losses are especially significant for September harvested crops, due to the favourable environmental conditions and high disease pressure. *Rhizoctonia solani* symptoms are damping-off of seedlings and root rot which impacts plant stand and vigour. In favorable conditions up to 100% of plants can be infected by the disease in a field, resulting in reduced yield.

For organic bean production there is only one registered product for Botrytis suppression - Serenade a biofungicide formulation containing the bacterium *Bacillus subtilis* (strain QST-713). It is registered in Canada for many diseases and crops including Botrytis on beans. Experiments conducted with various strains of *B. subtilis* have shown that it can control or suppress *B. cinerea* on grape leaves (Trotel-Aziz *et al*. 2008), strawberry fruits (Essghaier *et al.* 2009) and tomato plants (Sadfi-Zouaoui *et al.* 2008). However, Bardin *et al.* (2011) have shown that the strain of *B. cinerea* can greatly influence the efficacy of Serenade - with efficacy ranging from 0 to 80% on lettuce, depending on the Botrytis strain tested. Additionally, other diseases that could potentially be managed by *B. subtilis* include *Pythium* (Walker *et al.* 1998), *Alternaria solani, Phytophthora cryptogea* and *Rhizoctonia* spp. (Todorova and Kozhuharova 2010).

Four additional options that should be evaluated against bean diseases are Actinovate (*Streptomyces lydicus*), Influence (garlic extract), Regalia (*Reynoutria sachalinensis*) and Heads Up (Saponins of *Chenopodium quinoa*). Actinovate is a biofungicide that contains the bacterium *Streptomyces lydicus*. Antimicrobial compounds produced by *S. lydicus* have been shown to have negative effects on *B. cinerea* (Lu *et al*. 2008) and other common plant pathogens such as *Rhizoctonia solani* (Sabaratnam and Traquair 2002; Farrag 2011), *Pythium ultimum* (Crawford 1993; Trejo-Estrada *et al.* 1998; Yuan and Crawford 1995) and *Fusarium oxysporum* (Lu *et al.* 2008). Actinovate is currently registered to control powdery mildew and Botrytis fruit rot in field and greenhouse strawberry. Extracts from garlic, the sole active ingredient in Influence, have been studied for their antifungal activity against a number of fungal diseases including Botrytis (Wilson *et al.* 1997). However, it is only recently that reliable formulations are available to growers. Influence is registered for the control of Powdery mildew in greenhouse pepper and tomato. In Petri dish assays, Tedeschi *et al*. (2011) showed that garlic extracts have fungicidal activity against *B. cincerea* and *Sclerotium rolfsii*. Regalia contains extracts from giant knotweed (*Reynoutria sachalinensis*) which induce plant resistance reactions to certain diseases when applied to growing plant tissues, known as systemic acquired resistance (SAR). Regalia is registered in Canada for the suppression of *Botrytis cinerea* on grapes and tomatoes and Powdery mildew on cucurbits, grapes and strawberries. Heads Up is made of saponins of *Chenopodium quinoa* and is registered in Canada as a seed piece treatment for potatoes and soybeans for the suppression of *Rhizoctonia solani*. Like Regalia, the mode of action of Heads Up is to trigger SAR to subsequent pathogens attack.

The objective of this study was to test potential biofungicide solutions to Botrytis and Rhizoctonia for organic green bean production. Products were tested in two separate trials - one for foliar-applied products and the other for the seed treatment.

MATERIAL AND METHODS

Trial A- Foliar trial:

Study site: The trial was conducted in three organic (processing) bean fields in Delta, BC during the 2012 field season. All three fields were planted with the Botrytis-susceptible variety Cadillac. Planting and weed control were done by the grower. There were no insect or disease control activities in any of the fields and none of the fields were irrigated. Field 1 and Field 2 was planted on July 12 and Field 3 on July 13. The fields were harvested on September 24 (Field 2 and 3) and on September 25 (Field 1).

Treatment Description and Plot Layout: In order to evaluate the efficacy of biofungicides for Botrytis and Rhizoctonia control in beans, the trial examined four possible solutions 1) Actinovate 2) Serenade 3) Influence 4) Regalia compared to a water Control (Table 1). Each of the five treatments were replicated eight times, for a total of 40 plots/field and treatments were randomly assigned to plots. Plots were 2 m long and 1 m wide and were separated by 0.5 m buffer. Plots were laid out in a 4 X 10 grid in all fields (Appendix I).

Treatments began one week before flowering stage as this is when primary Botrytis infection occurs (Agrios 2005). Plots were sprayed weekly, from August 10 to September 7; spray frequency increased to every five days from September 7 to September 17, as environmental conditions (period of morning dew) were more favourable for disease development. (NB: overall the environmental conditions were not favourable for Botrytis development in 2012, see below). Plots received a total of seven applications (Table 2). Treatments were applied with a backpack sprayer hand-pumped to maintain full pressure. As per the label, plants were sprayed to allow good coverage but to avoid run-off (900L/ha or 0.18 L of spray solution/plot).

Table 1. Description of the three treatments evaluated against the water Control for Botrytis control in organic beans.

Trade Name and Manufacturer	Active Ingredient	Rate	Amount of product/plot
Actinovate Natural Industries, Inc.	*Streptomyces lydicus*	425g/ha	0.085 g
Serenade Max AgraQuest, Inc	*Bacillus subtilis*	3.36kg/ha	0.67 g
Influence AEF Global Inc.	Garlic extract	3.6%	6.48 mL
Regalia Max Marrone Bio Innovations, Inc.	Extract of *Reynoutria sachalinensis*	0.25%	0.45 mL

Water			0.18 L

Inoculation of Plots: Plots were inoculated twice with Botrytis in order to ensure even Botrytis pressure throughout the trial area in all three fields. Natural infection of Botrytis usually occurs during the month of September providing that the weather is cool and wet. However, due to dry weather throughout August and September, natural infection of our plot did not occur.

For the first inoculation, Botrytis-infected plant material was introduced into the plots after the fifth spray (September 7). Inoculation consisted of placing a 1-cm section of a Botrytis infected bean pod in the middle of each plot. In order to infect those beans with Botrytis, incisions along bean pods was made with a scalpel and beans were placed on a damp paper tower in a plastic container (one layer of beans). Then Botrytis infected potato flowers were placed on the beans. The container was placed in a cool (18°C) room for five days to allow the Botrytis to infect the beans and grow mycelium and spores (Fig. 1). This material was also used for the second inoculation which occurred after the sixth spray (September 13). For this inoculation, 10 infected beans (with mycelium and spores) were placed in a spray bottle containing room temperature water to create a spore suspension. This suspension was used to inoculate the plots by shaking well while spraying the solution on the plots (Siva Sabaratnam, BC Ministry of Agriculture, personal communication). These inoculations appear to have resulted in infection of the immature pods (the ones with flower petals at the time of inoculation) but did not result in secondary infection to the mature pods due to the lack of humidity.

Table 2. Summary of sprays and inoculations dates.

Spray 1	Spray 2	Spray 3	Spray 4	Spray 5 1st Inoculation	Spray 6 2nd Inoculation	Spray 7
August 10	August 17	August 24	August 31	Sept. 7	Sept. 12	Sept. 17

Figure 1. Botrytis infected green bean pods used for inoculating field plots.

Assessment and Analysis: One week after the last spray (September 24 (Field 2) and September 25 (Field 1 and 3)), beans along a 30-cm transect in the middle of each plot were assessed. Total number of plants, number of plants with *Rhizoctonia solani* (including superficial and severe symptoms), number of plants with severe Rhizoctonia (lesions throughout the stem with limited root system (Fig. 2a) as supposed to only superficial lesions (Fig. 2b)), bean yield (g), total number of beans (healthy and diseased, immature and mature), and the weight and number of beans with Botrytis (only immature beans were affected by Botrytis in our plots) were recorded for each plot. Effect of foliar treatments on disease incidence, severity, and yield parameters was analyzed using two-way (Field X Treatment) ANOVA. All proportion data were arc-sine transformed prior to analyses and all analyses done using JMP-In Version 5.1 (SAS Institute, Chicago, IL).

Figure 2a-b. Plant with severe Rhizoctonia (left) and superficial Rhizoctonia lesions (right)

Trial B-Seed treatment trial

Study site: The trial was conducted in the same three organic bean fields as the foliar trial. Plots were planted by hand on July 13 in an area of the field left unplanted by the growers.

Treatment Description and Plot Layout: In order to evaluate the efficacy of Heads Up seed treatment for disease control in beans, we compared Heads Up treated plots with Untreated Control plots. Each treatment was replicated ten times, for a total of 20 plots/field. Plots were 2 m long and 1 m wide and were separated by 0.5 m buffer. Cadillac seeds were planted following the grower's seed spacing of 2.54 cm apart and 3.81 cm deep; with this spacing we planted a total of 79 seeds in the 2 m long plots. Treatments were randomly assigned to plots. Plots were laid out in a 4 X 5 grid in all fields (Appendix II).

Heads Up seed treatment was applied on the day of planting. All seeds (i.e. 790 seeds) for the Heads Up plots were dipped together in 1L solution (1g of Heads Up/1liter of water as per label) for one minute and drained using a strainer. Heads Up treated seeds had air dried at the time of planting, Field 1 was planted about two hours after treatment, Field 2 about four hours and Field 3, six hours. The treated seeds were taken at random for planting in plots. Seeds to be planted in the Control plots were untreated.

Inoculation of Plots: Plots were inoculated with Botrytis the same way and same dates as foliar trial (see above).

Assessment and Analysis: Plots were assessed the same way as the foliar component of this study. Plots from Field 2 and 3 were harvested on September 24 and plots from Field 1 were harvested on September 25. Effect of seed treatments on disease incidence was analyzed using two-way (Field X Treatment) ANOVA. All proportion data were arc-sine transformed prior to analyses and all analyses done using JMP-In Version 5.1 (SAS Institute, Chicago, IL).

RESULTS

General Observations - August and September 2012, were characterized by unusually low levels of rainfall, i.e. well below 30 year daily averages (Fig. 3). As a consequence Botrytis pressure was relatively low in our plots, with the disease only observed on immature pods (Fig. 4a) and not on mature pods (Fig. 4b). Although all three fields were planted with the same Botrytis-susceptible variety disease pressure varied significantly among the three fields. Disease incidence was the highest in Field 3 at 9.8% in the Control plots (Fig. 5). In contrast levels of Botrytis in Control plots in Fields 1 and 2 were 4 and 1%, respectively (see Appendix III for raw data and summary tables).While almost all plants had evidence of Rhizoctonia, observation of severe infection (i.e. Rhizoctonia lesions throughout the stem and plants with limited root system) was also higher in Field 3, than in Fields 1 or 2 (Fig. 5). Interestingly, yield was significantly lower in Field 3 than in the other two fields (Appendix III).

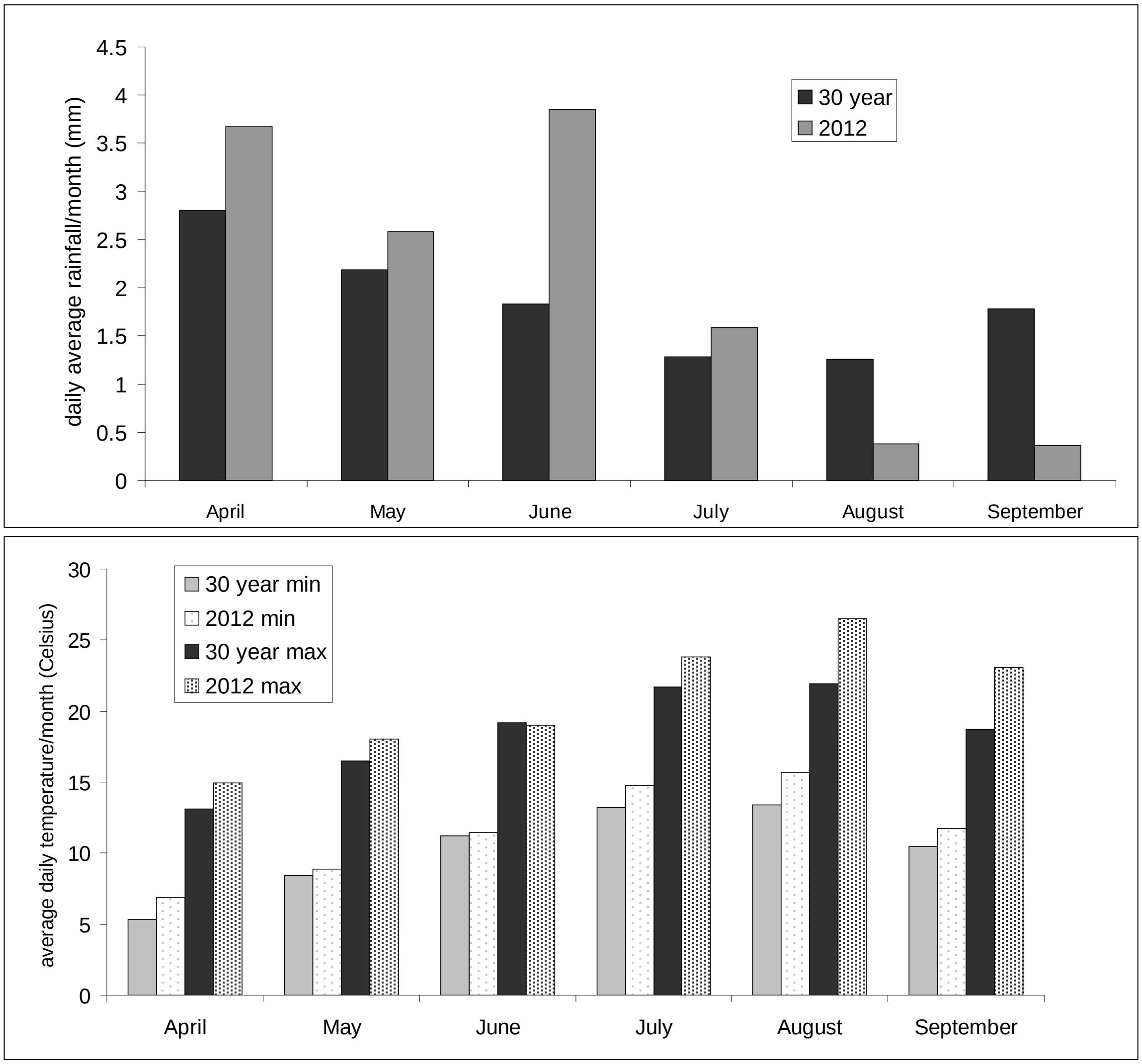

Figure 3. Comparison 30-year average and 2012 environmental conditions, (top) average daily rainfall (mm) and (bottom) average daily minimum and maximum temperature. 30 year average data based on Vancouver airport (YVR) weather record and 2012 data collected from Burnaby B.C.

Figure 4 a-b. Botrytis on immature beans (left) and on mature beans (right). We did not observed Botrytis infection on mature beans in our plots.

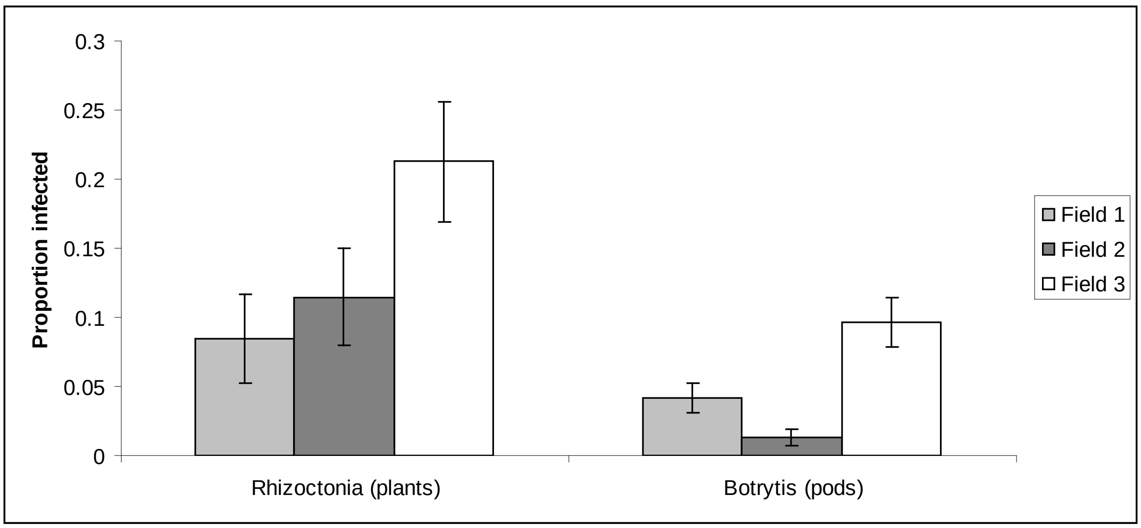

Figure 5. Rhizoctonia (at severe levels only) and Botrytis infection levels in water-only Control plots in three bean (var. Cadillac) fields, assessed September 24 or 25, 2012. Each bar is the mean ± s.e. of eight replicates/treatment.

Efficacy of Foliar Applications (Trial A)-We did not observe a significant reduction in the proportion of Botrytis infected pods following a program of seven to five day application intervals with the test products (Table 3). In Field 3, we did observe a trend towards lower incidence of Botrytis infected pods in the Actinovate, Influence and Regalia treatments, but these differences were not statistically significant (Fig. 6). The incidence of Rhizoctonia was very high in all fields and all treatments (98 to 100% of plants with symptoms of Rhizoctonia), but there were no significant treatment effects (Table 3). There were also no significant treatment effects on the proportion of plants with severe (Fig. 4) Rhizoctonia symptoms (Table 3). As with Botrytis, we did observe fewer plants with severe Rhizoctonia infections in the Actinovate and Regalia plots, in Field 3, but again this trend was not significant (Fig. 7). There were no negative effects of treatments on plant stand or yield (Table 3). We observed possible phytotoxicity symptoms in Regalia plots (Fig. 8), these symptoms were not observed in any of the other treatment plots or in the surrounding field.

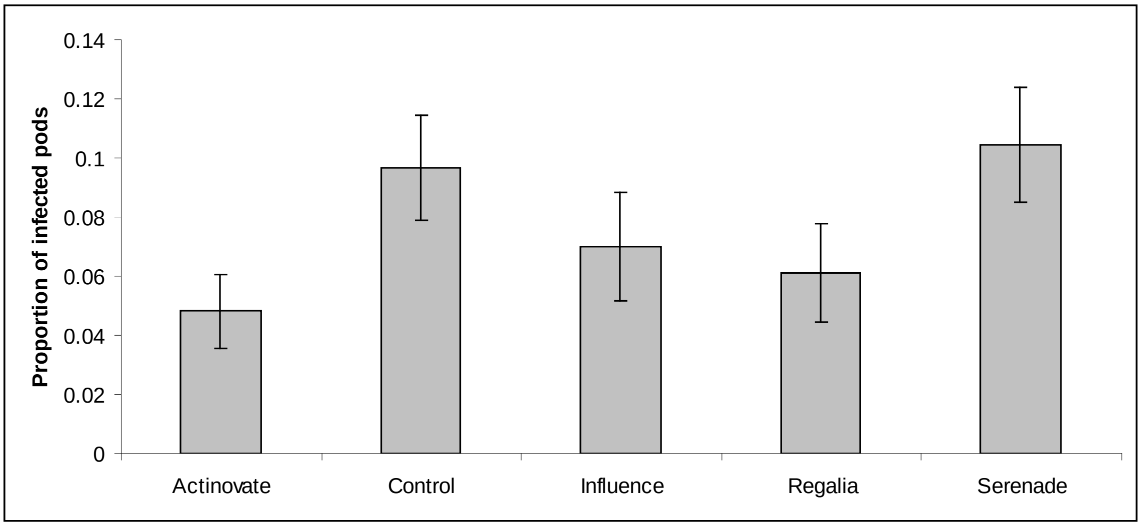

Figure 6. Effect of foliar fungicides on the proportion of bean pods infected with *Botrytis cinerea* at harvest in Field 3. Each bar is the mean ± s.e. of eight replicates/treatment.

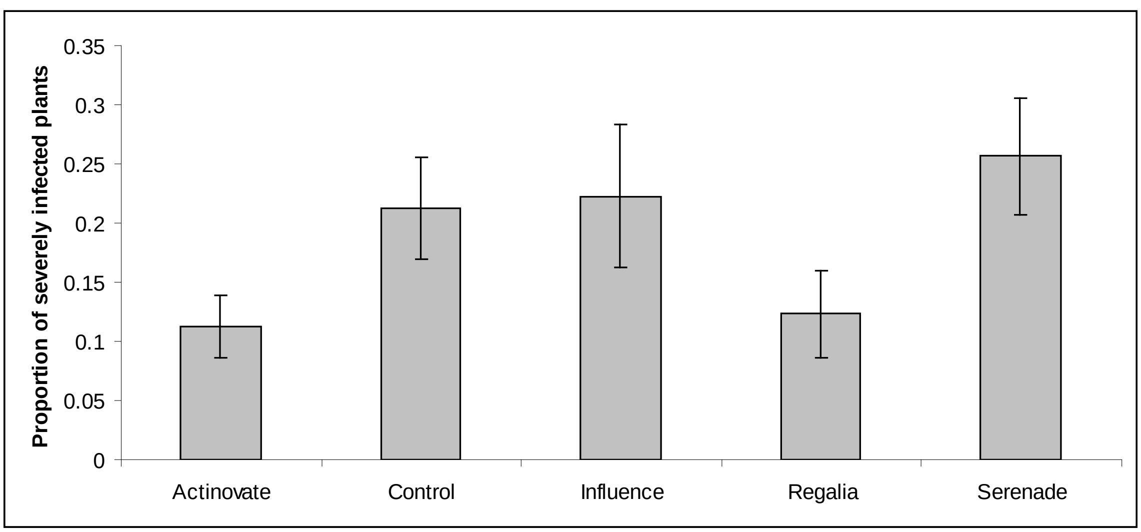

Figure 7. Effect of foliar fungicides on the proportion of bean plants severely (lesions throughout the stem and plants with stunted root system) infected with *Rhizoctonia solani* harvest in Field 3. Each bar is the mean ± s.e. of eight replicates/ treatment.

Table 3. Summary of statistical results for efficacy trial of foliar (Trial A) and seed (Trial B) treatments to control bean diseases in organic production.

	Treatment	Field	Treatment X Field
Foliar Treatments (Trial A)			
Proportion of Botrytis infected pods	F(4,105) = 0.92 p =0.46	F(2,105) = 45.44 p < 0.0001	F(8,105) = 1.02 p =0.42
Rhizoctonia incidence	F(4,105) = 1.40 p =0.24	F(2,105) = 0.82 p = 0.44	F(8,105) = 0.60 p =0.77
Proportion of plants with severe Rhizoctonia	F(4,105) = 0.56 p =0.69	F(2,105) = 6.45 p = 0.002	F(8,105) = 0.91 p =0.52
Plant stand	F(4,105) = 0.76 p =0.56	F(2,105) = 1.75 p = 0.18	F(8,105) = 0.93 p =0.49
Yield	F(4,105) = 0.41 p =0.80	F(2,105) = 15.01 p < 0.0001	F(8,105) = 0.96 p =0.47
Seed Treatments (Trial B)			
Proportion of Botrytis infected pods	F(1,54) = 0.53 p =0.47	F(2,54) = 2.42 p = 0.10	F(2, 54) = 0.19 p =0.82
Rhizoctonia incidence	F(1,54) = 2.86 p =0.10	F(2,54) = 17.68 p < 0.0001	F(2, 54) = 0.45 p =0.64
Proportion of plants with **severe** Rhizoctonia	F(1,54) = 0.004 p =0.95	F(2,54) = 0.19 p = 0.83	F(2, 54) = 0.41 p =0.67
Plant stand	F(1,54) = 2.58 p =0.11	F(2,54) = 0.12 p = 0.89	F(2, 54) = 1.63 p =0.21
Yield	F(1,54) = 0.35 p =0.56	F(2,54) = 7.69 p = 0.001	F(2, 54) = 0.92 p =0.41

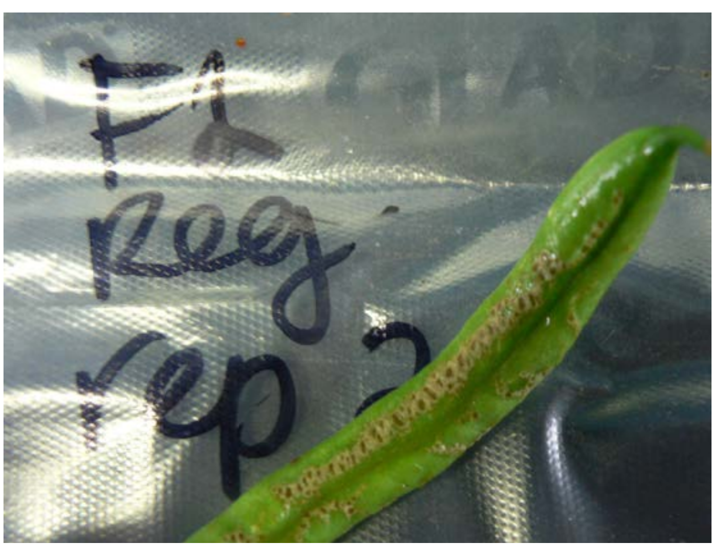

Figure 8. Phytotoxicity symptoms on green beans observed in Regalia plots

Efficacy of Seed Treatments - We did not see a significant effect of Heads Up seed treatment on the proportion of beans with Botrytis or on the proportion of plants with Rhizoctonia (Table 3, Fig. 9). There were significant differences between the three fields in terms of Rhizoctonia incidence (Table 3), with Field 3 again having the most disease pressure. However, there were no obvious trends in seed treatment performance even in fields with more disease pressure (Fig. 10). There were no field or treatment differences in terms of the proportion of plants with severe levels of Rhizoctonia, and the overall level of severely infected plants was low (mean ± s.e. proportion of plants with severe Rhizoctonia symptoms - Control: 0.089±0.02; Heads Up: 0.087±0.02). Finally, there were no significant effects of seed treatment on plant stand or yield (Table 3, Fig. 11 and 12) yield was higher in Field 1, which was the field with least amount of Rhizoctonia.

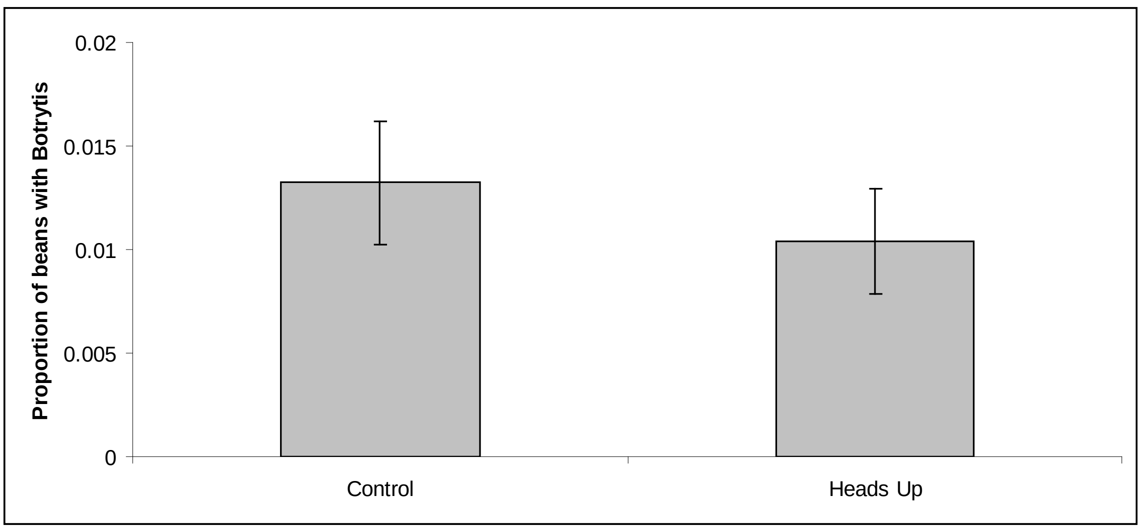

Figure 9. Effect of bean seed treatment on the proportion of bean pods infected with *Botrytis cinerea* at harvest. Each bar is the mean ± s.e. of 30 plots (10 plots in three fields).

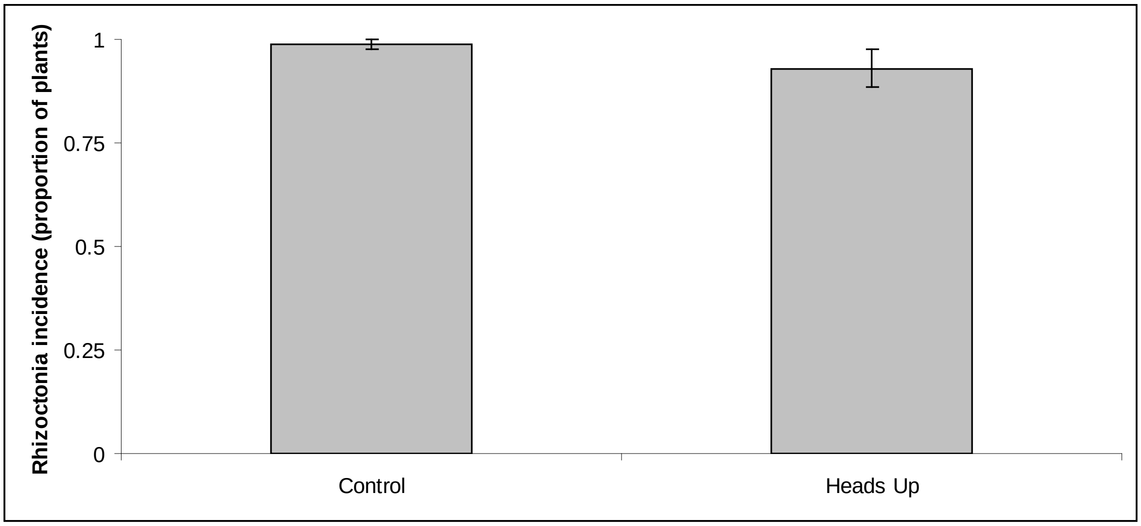

Figure 10. Effect of bean seed treatment on Rhizoctonia incidence (proportion of plants with presence of the disease) at harvest, in Field 3. Each bar is the mean ± s.e. of 10 plots/treatment

Figure 11.Final plant stand in the Heads Up treated plot (pink flag) and untreated Control (blue flag).

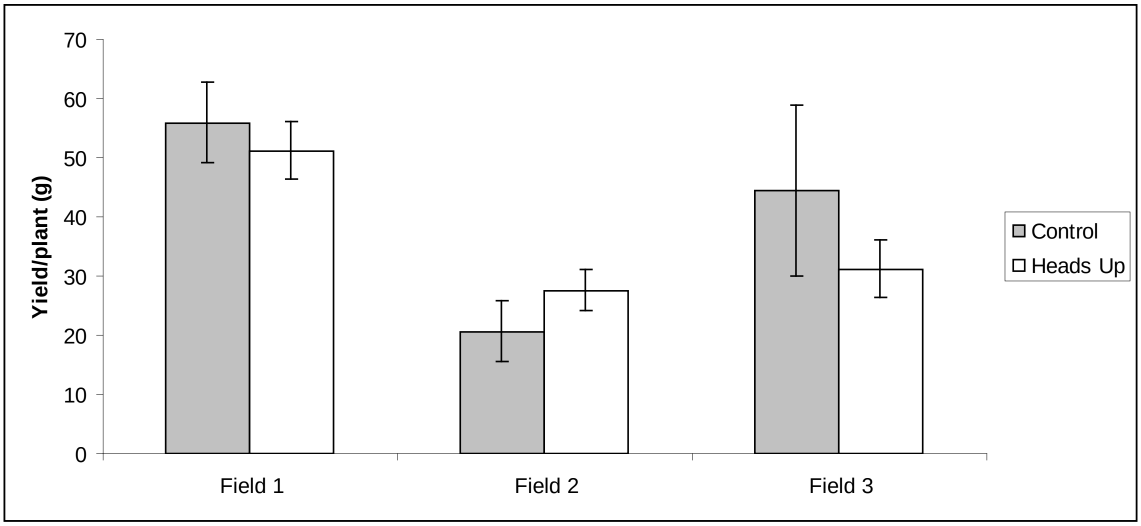

Figure 12. Effect of bean seed treatment on bean yield (grams) in bean plots. Each bar is the mean ± s.e. of 10 plots/treatment/field.

DISCUSSION

The objective of this study was to evaluate the efficacy of potential foliar (Actinovate, Serenade, Influence and Regalia) and seed treatment (Heads UP) options for control of diseases on organic green beans. The two diseases that we focused on were *Botrytis cinerea* and *Rhizoctonia solani*. We did not observe any significant treatment effect of any of the treatments (foliar and seed) on control of either Botrytis or Rhizoctonia, nor did we see any effect on plant stand or yield. While Rhizoctonia was present in all three of our fields (with almost 100% of plants infected), Botrytis pressure was lower than what we experienced in previous trials. The lower than normal Botrytis pressure in 2012 may have influenced the outcomes of our trial.

While we did have Botrytis inoculum in our trial area (both natural and introduced), we did not have enough moisture in September (Fig. 3) for the disease to develop to levels at which an organic grower would need fungicide sprays. Furthermore, we only observed Botrytis in small immature pods and did not observe secondary infection to mature pod, again this suggests that conditions were not conducive to disease development for the majority of the trial. In Field 3, where 9.8% of the Control beans were infected with Botrytis, there was a trend towards lower levels of Botrytis infection in Actinovate, Regalia and Influence treated plots compared to the Control. Furthermore, we observed fewer plants with severe Rhizoctonia infections in the Actinovate and Regalia plots, again in Field 3. Together these findings suggest that Actinovate and Regalia are good candidates for further testing as foliar treatments, (with the caveat that the results were non-significant trends observed in only one of the three fields).

Regalia does not directly act on the pathogen itself but instead induces systemic acquired resistance (SAR) in the treated plants (resulting in plant defense against pathogen attack). Since SAR is usually effective against a wide range of pathogens (Walters *et al.* 2005) it is not surprising that Regalia showed some effect on both Botrytis (above ground disease) and Rhizoctonia (below ground disease). Based on other studies *Streptomyces lydicus* (Actinovate) is also a good candidate for Botrytis and possibly Rhizoctonia control (Lu *et al.* 2008; Sabaratnam and Traquair 2002; Farrag 2011). Experiments showing Streptomyces spp. effect on *Rhizoctonia solani* (Sabaratnam and Traquair 2002; Farrag 2011) were either demonstrated in petri dish (antimicrobial essays) or via seed piece treatment, both allowing direct contact with the pathogen. In 2009 experiment, application of Actinovate on bean plants resulted in a significant increase in bean yield (Fraserland Organics, unpublished data). Furthermore, studies have shown an increase in shoot length and root weight when pea seeds are inoculated with *Streptomyces lydicus* (Tokala *et al*. 2002). Actinovate may be a promising tool to both for foliar and seed treatment on beans.

Greenhouse trials could potentially address the environmental challenges experienced in this trial. The survival and success of bacteria-based biocontrol agents at controlling pathogens in the field depends on environmental conditions such as wind, rain, temperature and solar radiation (Campbell 1989). It is possible that the unusually hot and dry conditions during the majority of our trial were not favourable to the performance of the bacteria-based products (i.e. Actinovate and Serenade). Environmental parameters can be controlled in a greenhouse setting to mimic conditions encountered in the field in a "high Botrytis year" while it can take years of field experiments to finally obtain good conditions.

While we did not see any effect of Heads Up seed treatment on Botrytis and Rhizoctonia in this year study, previous trials showed a significant reduction in Rhizoctonia severity on Heads Up treated plants compared to untreated Control

(Dessureault 2011). This further shows that field conditions can largely vary from year to year. Furthermore, for products known to have SAR effect, seed treatment versus foliar application can largely influence the efficacy of the product. In an experiment conducted on millet using a Chitosan formulation (inducing SAR), seed treatment offered 48% protection again Downy mildew, 67% with foliar spray and 71 % when both seed and foliar treatments were combined (Sharathchandra *et al.* 2004). Finally, the control effect of SAR inducing materials on plant pathogens can potentially be enhance when combined with a fungicide and yield can be successfully improved by the uses of fertilizer combined with a SAR product (Vallad and Goodman, 2004).

In conclusion, based on other studies biofungicides (bacteria-based and plant extracts) appear to be promising tools for integrated disease management in organic green bean production. However, obtaining local results that are field-based is challenging for a number of reasons (primarily ensuring that all components of the disease triangle are optimal for adequate disease pressure during trials).

ACKNOWLEDGEMENTS

We thank Fraserland Organics for supplying field space and for providing financial contributions. We thank E.S. Cropconsult Ltd staff for their various contributions to this trial. This trial was funded by Lower Mainland Horticultural Improvement Association, Organic Sector Development Program and Fraserland Organics.

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Appendix I. Plot layout for each field_ Trial A.

Field 1

Treatment	Rep	Treatment	Rep	Treatment	Rep	Treatment	Rep
Water	1	Reg	2	Ser	4	Act	7
Act	1	Reg	3	Ser	5	Inf	7
Inf	1	Water	3	Inf	4	Reg	7
Ser	1	Act	3	Act	5	Water	7
Water	2	Reg	4	Inf	5	Ser	7
Ser	2	Reg	5	Water	6	Inf	8
Act	2	Inf	3	Act	6	Reg	8
Inf	2	Water	4	Inf	6	Ser	8
Ser	3	Act	4	Ser	6	Act	8
Reg	1	Water	5	Reg	6	Water	8

North

Field 2

Treatment	Rep	Treatment	Rep	Treatment	Rep	Treatment	Rep
Ser	1	Reg	3	Act	4	Water	7
Water	1	Act	3	Reg	5	Inf	7
Inf	1	Ser	3	Reg	6	Ser	7
Reg	1	Water	3	Inf	5	Act	6
Inf	2	Ser	4	Water	6	Reg	8
Reg	2	Inf	3	Ser	5	Inf	8
Act	1	Water	4	Act	5	Ser	8
Water	2	Reg	4	Inf	6	Act	7
Ser	2	Inf	4	Reg	7	Water	8
Act	2	Water	5	Ser	6	Act	8

East

Field 3

Treatment	Rep	Treatment	Rep	Treatment	Rep	Treatment	Rep
Reg	1	Inf	3	Ser	4	Water	7
Water	1	Act	3	Water	5	Inf	7
Inf	1	Reg	3	Act	5	Reg	7
Ser	1	Water	3	Ser	5	Act	7
Act	1	Reg	4	Inf	6	Water	8
Ser	2	Inf	4	Reg	5	Ser	7
Water	2	Ser	3	Act	6	Ser	8
Act	2	Act	4	Water	6	Reg	8
Reg	2	Water	4	Ser	6	Inf	8
Inf	2	Inf	5	Reg	6	Act	8

West

Actinovate (Act) Influence (Inf) Water Control (Water)

Serenade (Ser) Regalia (Reg)

Appendix II. Plot layout for each field_Trial B

Field 1

Treatment	rep	Treatment	rep	Treatment	rep	Treatment	rep
H	1	H	4	C	5	C	8
C	1	C	3	H	7	H	9
H	2	H	5	C	6	C	9
C	2	H	6	H	8	C	10
H	3	C	4	C	7	H	10

North

Field 2

Treatment	Rep	Treatment	Rep	Treatment	Rep	Treatment	Rep
C	1	H	3	C	6	H	9
C	2	H	4	H	6	C	8
H	1	C	4	C	7	H	10
H	2	H	5	H	7	C	9
C	3	C	5	H	8	C	10

East

Field 3

Treatment	Rep	Treatment	Rep	Treatment	Rep	Treatment	Rep
H	1	C	3	H	6	C	8
H	2	H	4	H	7	H	9
C	1	C	4	C	6	C	9
C	2	H	5	H	8	C	10
H	3	C	5	C	7	H	10

West

Heads Up (H)

Unteated Control (C)

Appendix III. Raw data and summary tables

Table 1. Raw data_Trial A

Field	Treatment	Rep	# Plant	# plant with Rhizoc.	# plant with sev. Rhizoc.	Yield (g)	Botrytis(g)	Botrytis (#)	# of beans/ plot
1	Actinovate	1	5	5	2	268.29	0.06	6	147
1	Actinovate	2	3	3	0	247.71	0.01	3	120
1	Actinovate	3	6	6	1	443.88	0.11	5	218
1	Actinovate	4	8	8	0	366.53	0.03	3	189
1	Actinovate	5	8	7	0	469.71	0.05	7	255
1	Actinovate	6	7	7	0	403.37	0.05	6	191
1	Actinovate	7	7	7	2	428.91	0.09	7	219
1	Actinovate	8	9	9	1	389.7	0.05	11	221
1	Serenade	1	4	4	0	311.09	0.04	4	125
1	Serenade	2	5	5	1	327.77	0.06	6	174
1	Serenade	3	5	5	0	280.04	0.03	5	152
1	Serenade	4	7	7	0	534.59	0.03	5	227
1	Serenade	5	8	7	1	556.68	0.06	9	242
1	Serenade	6	8	8	1	480.01	0.07	12	254
1	Serenade	7	4	4	1	250.98	0.01	3	120
1	Serenade	8	7	7	1	421.93	0.05	9	197
1	Influence	1	3	3	0	288.54	0.01	3	132
1	Influence	2	7	7	0	404.38	0.45	18	229
1	Influence	3	5	5	0	238.82	0.04	16	158
1	Influence	4	6	6	0	442.82	0.05	6	225
1	Influence	5	7	7	1	516.47	0.01	3	214
1	Influence	6	4	4	0	395.83	0.05	6	174
1	Influence	7	8	8	1	636.26	0.04	6	250
1	Influence	8	12	12	2	517.87	0.03	7	246
1	Regalia	1	3	3	0	368.4	0.07	7	167
1	Regalia	2	8	8	1	295.43	0.09	8	175
1	Regalia	3	6	6	0	337.51	0.15	10	176
1	Regalia	4	7	7	1	312.22	0.09	10	189
1	Regalia	5	7	7	0	411.14	0.05	5	205
1	Regalia	6	7	7	1	409.2	0.04	7	239
1	Regalia	7	4	4	0	442.14	0	0	180
1	Regalia	8	8	8	2	311.72	0.08	10	210
1	Control	1	5	5	1	239.54	0.03	3	129
1	Control	2	7	7	0	392.78	0.08	19	207
1	Control	3	6	6	1	345.3	0.09	8	169
1	Control	4	6	6	1	368.62	0.02	8	133

1	Control	5	4	4	0	394.43	0.01	4	177
1	Control	6	7	7	1	410.45	0.02	2	186
1	Control	7	7	7	0	613.21	0.01	2	231
1	Control	8	8	8	0	469.69	0.11	19	269
2	Actinovate	1	8	8	1	547.55	0	0	180
2	Actinovate	2	3	3	0	432.48	0	0	121
2	Actinovate	3	7	7	1	282.96	0	0	106
2	Actinovate	4	3	3	0	421.41	0	0	122
2	Actinovate	5	4	4	1	250.41	0.02	2	95
2	Actinovate	6	7	7	0	414.45	0.08	3	166
2	Actinovate	7	5	5	2	168.99	0.02	10	83
2	Actinovate	8	2	2	0	192.01	0.01	2	63
2	Serenade	1	4	4	0	354.84	0	0	120
2	Serenade	2	6	5	0	322.45	0	0	107
2	Serenade	3	6	6	1	520.68	0	0	159
2	Serenade	4	8	8	2	434.74	0.03	1	166
2	Serenade	5	6	6	0	334.32	0.07	3	148
2	Serenade	6	4	4	0	279.85	0.01	1	107
2	Serenade	7	6	6	0	416.69	0.01	3	172
2	Serenade	8	9	9	1	366	0.13	9	205
2	Influence	1	4	4	0	629.63	0.07	8	201
2	Influence	2	4	4	0	360.85	0	0	119
2	Influence	3	10	10	2	442.73	0.01	1	189
2	Influence	4	9	9	2	168.35	0	0	82
2	Influence	5	6	6	1	408.45	0	0	139
2	Influence	6	6	6	1	246.37	0.09	4	124
2	Influence	7	8	8	2	472.69	0.01	1	145
2	Influence	8	5	5	1	246.14	0.02	3	128
2	Regalia	1	7	7	0	462.23	0.01	3	166
2	Regalia	2	6	6	0	485.86	0.01	1	166
2	Regalia	3	6	6	1	368.32	0.01	2	117
2	Regalia	4	4	4	0	197.58	0	0	68
2	Regalia	5	3	3	0	289.25	0.04	2	118
2	Regalia	6	8	8	1	396.8	0	0	139
2	Regalia	7	8	8	1	395.51	0.03	3	157
2	Regalia	8	6	6	1	432.85	0.05	2	187
2	Control	1	3	3	0	346.06	0.03	1	111
2	Control	2	4	4	1	394.88	0	0	145
2	Control	3	6	6	1	364.24	0.02	1	130
2	Control	4	6	6	1	268.26	0.03	2	110
2	Control	5	6	6	1	424.76	0	0	144

2	Control	6	7	7	0	420.43	0.05	8	161
2	Control	7	6	6	1	531.37	0.03	4	204
2	Control	8	5	5	0	480.17	0	0	152
3	Actinovate	1	7	7	1	292.84	0.26	7	175
3	Actinovate	2	8	8	0	387.88	0.01	1	197
3	Actinovate	3	8	8	1	218.56	0.31	12	137
3	Actinovate	4	7	7	0	504.59	0.11	6	216
3	Actinovate	5	7	7	1	217.96	0.47	14	143
3	Actinovate	6	5	5	1	194.62	0.09	9	117
3	Actinovate	7	6	6	1	194.66	0.04	5	131
3	Actinovate	8	8	8	1	316.18	0.03	2	170
3	Serenade	1	6	6	1	175.6	0.28	20	130
3	Serenade	2	6	6	0	343.2	0.21	16	232
3	Serenade	3	10	10	3	277.34	0.35	14	187
3	Serenade	4	4	4	1	107.26	0.01	2	52
3	Serenade	5	7	7	2	113.97	0.65	20	98
3	Serenade	6	4	4	1	150.92	0.05	8	91
3	Serenade	7	6	6	3	209.65	0.47	19	138
3	Serenade	8	10	10	3	331.57	0.22	15	218
3	Influence	1	10	10	3	542.12	0.09	6	263
3	Influence	2	8	8	2	276.83	0.3	18	178
3	Influence	3	6	6	0	294.71	0.28	7	156
3	Influence	4	7	7	0	268.13	0.48	30	178
3	Influence	5	8	8	1	342.44	0.23	12	180
3	Influence	6	4	4	1	266.36	0.07	5	141
3	Influence	7	7	7	3	195.32	0.14	15	144
3	Influence	8	7	7	3	505.47	0.01	3	185
3	Regalia	1	6	6	0	234.92	0.11	13	158
3	Regalia	2	5	5	1	199.53	0.01	2	104
3	Regalia	3	6	6	1	246.38	0.12	5	126
3	Regalia	4	4	4	1	250.17	0.16	8	154
3	Regalia	5	5	5	1	201.32	0.12	13	123
3	Regalia	6	7	7	0	265.06	0.34	24	161
3	Regalia	7	2	2	0	294.32	0.03	3	130
3	Regalia	8	6	6	1	286.19	0.07	3	169
3	Control	1	9	9	3	317.12	0.11	12	211
3	Control	2	7	7	2	172.43	0.21	9	120
3	Control	3	6	6	0	192.19	0.71	24	165
3	Control	4	6	6	1	310.92	0.19	6	134
3	Control	5	4	4	1	88.44	0.2	11	65
3	Control	6	6	6	1	323.58	0.2	10	165

3	Control	7	8	8	3	351.6	0.28	12	172
3	Control	8	8	8	1	198.98	0.58	27	176

Table 2. Summary mean ± s.e _Trial A

Treatment	Field	# of reps	Proportion of plant with Severe Rhizoctonia	Total yield	Yield /plant	Proportion of Botrytis (weight in grams)	Proportion of Botrytis (number of bean)
Actinovate	1	8	0.12 ± 0.05	377.26 ± 28.43	59.62 ± 4.69	0.00015 ± 0.00003	0.03 ± 0.00
Actinovate	2	8	0.11 ± 0.05	338.78 ± 47.47	80.64 ± 15.00	0.00006 ± 0.00003	0.02 ± 0.01
Actinovate	3	8	0.11 ± 0.03	290.91 ± 38.91	41.47 ± 4.97	0.00068 ± 0.00027	0.05 ± 0.01
Control	1	8	0.08 ± 0.03	404.25 ± 37.82	65.82 ± 6.20	0.00012 ± 0.00004	0.04 ± 0.01
Control	2	8	0.11 ± 0.03	403.77 ± 28.71	79.37 ± 8.48	0.00005 ± 0.00002	0.01 ± 0.01
Control	3	8	0.21 ± 0.04	244.41 ± 33.21	36.07 ± 4.43	0.00156 ± 0.00044	0.10 ± 0.02
Influence	1	8	0.05 ± 0.03	430.12 ± 45.57	71.37 ± 7.31	0.00021 ± 0.00013	0.04 ± 0.01
Influence	2	8	0.15 ± 0.03	371.90 ± 52.79	66.01 ± 15.00	0.00008 ± 0.00004	0.01 ± 0.01
Influence	3	8	0.22 ± 0.06	336.42 ± 43.45	48.22 ± 5.47	0.00071 ± 0.00020	0.07 ± 0.02
Regalia	1	8	0.08 ± 0.03	360.97 ± 19.43	65.91 ± 11.53	0.00021 ± 0.00005	0.04 ± 0.01
Regalia	2	8	0.07 ± 0.03	378.55 ± 33.52	65.67 ± 6.02	0.00005 ± 0.00002	0.01 ± 0.00
Regalia	3	8	0.12 ± 0.04	247.24 ± 12.39	59.96 ± 13.20	0.00048 ± 0.00014	0.06 ± 0.02
Serenade	1	8	0.11 ± 0.03	395.39 ± 42.07	66.04 ± 2.80	0.00011 ± 0.00002	0.03 ± 0.00
Serenade	2	8	0.07 ± 0.03	378.70 ± 26.85	64.92 ± 5.98	0.00009 ± 0.00005	0.01 ± 0.01
Serenade	3	8	0.26 ± 0.05	213.69 ± 33.11	32.89 ± 4.16	0.00156 ± 0.00064	0.10 ± 0.02

Table 3. Raw data_Trial B

Field	Treatment	Rep	# Plant	# plant with Rhizoc.	# plant with sev. Rhizoc.	Yield (g)	Botrytis(g)	Botrytis (#)	# of beans/ plot
1	Heads Up	1	9	8	0	499.41	0.1	3	246
1	Heads Up	2	9	6	2	482.69	0	0	258
1	Heads Up	3	9	7	3	492.41	0.01	1	234
1	Heads Up	4	10	8	2	361.49	0.01	6	225
1	Heads Up	5	9	6	0	474.94	0.05	5	254
1	Heads Up	6	13	10	0	469.31	0.08	2	255
1	Heads Up	7	12	10	0	485.27	0.07	13	292
1	Heads Up	8	7	5	1	237.75	0	0	143
1	Heads Up	9	6	4	0	505.93	0.06	9	251
1	Heads Up	10	8	6	0	516.06	0	0	231
1	Control	1	7	6	0	552.95	0	0	304
1	Control	2	8	6	1	757.24	0.12	3	334
1	Control	3	10	6	3	410.35	0.35	14	300
1	Control	4	10	9	2	301.92	0.01	1	209
1	Control	5	11	10	2	333.48	0.06	9	206
1	Control	6	10	7	0	531.47	0.06	4	297
1	Control	7	5	3	0	375.52	0.01	1	163
1	Control	8	10	8	0	503.07	0.06	7	277
1	Control	9	9	6	0	524.8	0.02	2	228
1	Control	10	7	6	0	331.52	0.05	4	136
2	Heads Up	1	8	6	0	222.02	0	0	117
2	Heads Up	2	12	11	2	307.67	0.01	6	153
2	Heads Up	3	13	10	0	248.31	0.01	1	125
2	Heads Up	4	9	5	0	293.63	0	0	143
2	Heads Up	5	7	7	0	181.85	0	0	103
2	Heads Up	6	9	8	2	182.49	0	0	89
2	Heads Up	7	12	12	5	639.36	0.01	2	264
2	Heads Up	8	11	9	0	401.8	0	0	185
2	Heads Up	9	10	9	0	188.95	0.05	2	112
2	Heads Up	10	9	8	0	145.1	0	0	107
2	Control	1	10	10	3	208.22	0	0	108
2	Control	2	8	8	0	273.45	0.01	1	139
2	Control	3	8	6	0	290.41	0.01	2	123
2	Control	4	9	9	2	97.07	0	0	73
2	Control	5	7	7	0	72.87	0.01	2	105
2	Control	6	9	9	3	58.92	0.01	2	115
2	Control	7	7	6	1	55.99	0	0	63
2	Control	8	5	4	0	38.66	0	0	89
2	Control	9	10	10	0	159.31	0	0	124
2	Control	10	5	4	1	275.91	0.04	5	86
3	Heads Up	1	5	5	1	139.64	0.01	1	184
3	Heads Up	2	10	10	0	346.07	0.03	7	203
3	Heads Up	3	7	7	0	234.25	0	0	115
3	Heads Up	4	9	9	1	510.48	0	0	234
3	Heads Up	5	7	6	1	91.12	0.01	2	182
3	Heads Up	6	9	5	0	34.98	0	0	151
3	Heads Up	7	6	6	0	251.7	0.01	1	192

3	Heads Up	8	13	13	2	293.3	0.11	5	182
3	Heads Up	9	9	8	1	337.81	0	0	141
3	Heads Up	10	11	11	2	448.3	0.01	1	222
3	Control	1	10	10	0	258.41	0.01	3	172
3	Control	2	11	11	1	291.47	0	0	147
3	Control	3	3	3	0	501.19	0.02	4	258
3	Control	4	9	8	0	1.13	0	0	10
3	Control	5	9	9	0	251.56	0	0	153
3	Control	6	9	9	1	500.73	0.03	2	185
3	Control	7	9	9	0	291.33	0	0	131
3	Control	8	7	7	1	342.62	0.01	1	188
3	Control	9	9	9	2	332.57	0	0	138
3	Control	10	11	11	1	256.79	0.11	6	141

Table 4. Summary mean ± s.e _Trial B

Treatment	Field	# of reps	Proportion of plant with Severe Rhizoctonia	Total yield	Yield /plant	Proportion of Botrytis (weight)	Proportion of Botrytis (number of bean)
Control	1	10	0.08 ± 0.04	462.23 ± 44.12	55.94 ± 6.67	0.07 ± 0.03	0.02 ± 0.01
Control	2	10	0.012 ±0.04	153.08 ± 32.05	20.59 ± 5.13	0.01 ± 0.00	0.01 ± 0.01
Control	3	10	0.07 ± 0.02	302.78 ± 44.60	44.47 ± 14.43	0.02 ± 0.01	0.01 ± 0.00
Control	1	10	0.09 ± 0.04	452.53 ± 27.46	51.21 ± 4.92	0.04 ± 0.01	0.02 ± 0.01
Control	2	10	0.08 ± 0.05	281.12 ± 46.54	27.62 ± 3.50	0.01 ± 0.00	0.01 ± 0.00
Control	3	10	0.09 ± 0.03	268.77 ± 47.89	31.24 ± 4.80	0.02 ± 0.01	0.01 ± 0.00