Organic Management of Late Blight on Potatoes

Report to: Organic Sector Development Program (I-144), Lower Mainland Horticultural Improvement Association, Plant Protectants Inc. and Fraserland Organics

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Executive Summary
Late blight continues to be a major limiting factor for organic potato production world-wide. Growers in the Fraser Valley are dependant on regular applications of foliar copper sprays for control. However, there are increasing concerns around the world about the negative impacts of copper on soil biota. In previous work, none of the commercially available biofungicides have had efficacy against the local strain of late blight. For this study we conducted two trials for alternatives to copper. First we examined the efficacy of weekly foliar sprays of local strains of the fungus Trichoderma spp. in conjunction with late blight resistant/tolerant varieties. Second, we examined the efficacy of seed piece treatments with the Heads Up, a product that can induce an systemic acquired resistance (SAR) response in treated plants. For both experiments, treatments were compared against a water only Control and a weekly foliar copper treatment. In neither experiment did we find products that were effective as copper in suppressing the development of late blight on potato foliage. A number of factors including rate of application and application technique itself may contribute to the efficacy of biofungicides and reduced risk products like Heads Up. These factors may be more effectively explored in greenhouse trials before larger scale trials against late blight are repeated.

Introduction

Globally potatoes are one of the largest food crops grown today (Vleeshouwers *et al.* 2011, Haverkort *et al.* 2009). British Columbia is no exception as potatoes account for a large proportion of the organic vegetable acreage grown annually. Potatoes are susceptible to various diseases that can reduce yield significantly (Vleeshouwers *et al.* 2011). Late blight is the most serious disease for both conventional and organic operations global yield losses from late blight have been estimated at 16% (Vleeshouwers *et al.* 2011, Haverkort *et al.* 2009). Late blight is caused by the oomycete *Phytophthora infestans* the pathogen can infect stems, leaves and tubers of the potato plant (Vleeshouwers *et al.* 2011, Fry 2008). If late blight is left unmanaged it can destroy entire potato fields in a matter of days. One of the reasons for this is that many commonly grown potato varieties (eg Russet Norkotah) are highly susceptible to late blight infection. Currently local organic potato production has only one fungicide for late blight control, copper hydroxide, which is applied on average every 5-7 days. The use of copper has been made illegal in Scandinavia and the Netherlands (Dorn *et al.* 2007). This indicates the European Union is moving toward an exclusion of copper based fungicides so an alternative for late blight control is required (Bouws and Finckh 2008).

One way to address the problem of high susceptibility to late blight infection is to replace susceptible varieties with varieties that display some resistance to infection. Some work investigating resistance to late blight has concluded that it is the most promising option for the control of late blight (Struik 2010). Growing varieties with late blight resistance can decrease the number of fungicide applications needed and the amount applied each time (Mekonen *et al.* 2011). The resistant variety used in the present trial was a russet variety known as Innovator. Locally Innovator has displayed significantly lower levels of foliar late blight infection compared to Russet Norkotah (Glover and Prasad 2011). While resistant varieties may not completely eliminate the need for copper-based fungicides they could be part of an integrated approach to late blight control.

Biological control of late blight may also be an option for management of the disease. Biological control utilizes organisms such as fungi or plant extracts to manage disease (Axel *et al.* 2012, Monte 2001). Biological control fits well into an integrated pest management system because in can result in a reduction of the use of chemical pesticides (Monte 2001). *Trichoderma harzianum* is a fungus that has shown significant reduction of late blight on tomato foliage (Abdel-Kader *et al*. 2012). Another strain of *Trichoderma*, *Trichoderma atroviride* has displayed significant reduction of onion white rot (McLean *et al*. 2005, McLean and Stewart 2000). Inducing the potato systemic acquired resistance to disease before plants are infected with late blight may have the potential to control infection. A product that is thought to induce a systemic acquired resistance in plants is HeadsUp (Soleimani and Kirk 2012). HeadsUp contains extracts known as saponins from *Chenopodium quinoa* and can be used as a seed piece treatment for control of fungal diseases of plants (Soleimani and Kirk 2012).

The objectives of this trial were to evaluate efficacy of several novel alternatives to copper, in particular:
A. Two locally derived species of the fungus Trichoderma spp. as weekly foliar treatments
B. HeadsUp as a seed piece treatment

The efficacy of these alternatives to copper was examined using a late blight resistant (Innovator) and susceptible (Norkotah) potato variety.

Materials and Methods

Study site – The trial was located in an agricultural field in Cloverdale BC in an area that is used for potato production on a yearly basis and had a history of late blight infection. The trial was located along the eastern edge of the field that had been planted with (early-season) potatoes and harvested prior to the planting of the trial. The adjacent crop was squash. Potatoes were planted on June 29, 2012. Each plot was hand planted with 10 Russet Norkotah or Innovator seed potatoes with 25cm spacing between potatoes. The trial area consisted of a sandy type soil and the area was not fertilized or irrigated during the trial. Weeds were controlled in the trial by spraying once with Linuron at the label rate on July 11, 2012.

Pest infestation – The trial area had a history of late blight infection and natural late blight infection was used because of sufficient disease pressure. Natural late blight infection was confirmed by weekly visual observations of all plots. The 2012 field season was considered to be a moderate year for late blight (H. Meberg, E.S. Cropconsult Ltd. personal communication) and the pressure in the trial area overall was severe.

Plot layout and Experimental design – Plots were laid out following a completely randomized design. All plots were 3.5m long and 0.4m (or 1 row) wide and were laid out in a 2 X 48 grid. There was a buffer row planted with Russet Norkotah seed potatoes between the two rows containing plots. There were also two Russet Norkotah seed potatoes used as buffers between the plots within each row. The trial consisted of two *a priori* sets of planned contrasts. Because of limited space and tubers the water Control and weekly Parasol treatments for both trials were shared and the treatment plots for both sets of planned contrasts. Treatments, for both sets of planned contrasts, were randomly assigned to plots across the entire study area.

For the first set of contrasts (Experiment A) we examined the efficacy of five different foliar treatments (Table 1) in combination and two potato varieties for a total of 10 treatments. Each treatment was replicated eight times for a total trial N of 80. The second set of contrasts (Experiment B) was on the efficacy of HeadsUp seed piece treatments on a single variety Norkotah. We examined the efficacy of HeadsUp on it's own and in combination with foliar Parasol applications (Table 2). These two treatment were compared to the water Control and the industry standard (weekly Parasol foliar treatments). Each of these four treatments was replicated eight times for a total N of 32 for this trial.

Application of treatments - Application of HeadsUp (Plant Protectants Inc.) was made just prior to the planting of the trial on June 29, 2012, by submerging all of the seed potatoes for all eight HeadsUp plots in the HeadsUp solution (Table 2) for approximately 5 minutes. Tubers were allowed to air dry and were completely dry prior to planting. *Trichoderma harzianum*, *Trichoderma atroviride* and Parasol weekly treatments began on July 18, 2012 and continued until September 12, 2012. For this trial *T. harzianum* and *T. atroviride* were prepared by the Institute of Sustainable Horticulture, Kwantlen Polytechnic University (KPU) (see below). Parasol treatments were applied as foliar sprays using a SOLO backpack sprayer equipped with XR Teejet 8003VS nozzles hand pumped to maintain full pressure. The amount of water used to apply treatments varied from week to week in order to ensure adequate coverage of the growing plants (Table 3). *Trichoderma* treatments were applied to run-off using a SOLO backpack sprayer.

Table 1: Summary of product rates and amounts applied per plot for the evaluation of the impact of late blight resistance and biological controls for late blight control.

Treatment	Active ingredient	Area of each plot (m^2)	Rate used (*Trichoderma*)/ Label rate (parasol)	Amount of Product applied/plot (g)
Water once every week	NA	$1.4m^2$	NA	NA
Parasol once every week	Copper hydroxide	$1.4m^2$	2.5kg/ha	0.35
T. harzianum once every week	*T. harzianum*	$1.4m^2$	10^8 spores/L	NA
T. atroviride	*T. atroviride*	$1.4m^2$	10^8 spores/L	NA

once every week				
T. harzianum week 1/ Parasol week 2	*T. harzianum* week 1/ copper hydroxide week 2	1.4m^2	10^8 spores/L / 2.5kg/ha	NA/0.35

Table 2: Summary of rates and amounts applied per plot for the evaluation of the impact of late blight resistance and biological controls for late blight control.

Treatment	Active ingredient	Area of each plot (m^2)	Rate used Heads Up/ Label rate (Parasol)	Amount of Product applied/plot (g)
Water once every week	NA	1.4m^2	NA	NA
Parasol once every week	Copper hydroxide	1.4m^2	2.5kg/ha	0.35 Parasol
HeadsUp seed piece treatment then water once every week	Saponins of *C. quinoa*	1.4m^2	1g/1L H_2O	0.02 Heads Up
HeadsUp seed piece treatment then ½ rate Parasol once every week*	Saponins of *C. quinoa* then copper hydroxide once every week	1.4m^2	1g/1L H_2O 1.25kg/ha	0.02 Heads Up /0.175 Parasol

*Treatment requested by Registrant.

Preparation of Trichoderma treatments: Inocula for both strains of Trichoderma were produced through static liquid fermentation in Malt Yeast Broth (MYB) (15g/L Torula Yeast, 20g/L Molasses, 1mL/L Gentamycin). 250mL of MYB was aseptically added to autoclaved polypropylene trays with lids. Cultures were incubated at 24°C in the dark until significant sporulation was observed (typically 1 week). Cultures were inoculated using spores harvested from 7-10 day old potato dextrose agar (PDA) subcultures. MYB media was inoculated initially at $1x10^6$ spores/mL ($2.5x10^8$ spores per tray), but due to contamination issues, the inoculation rate was increased to $1x10^8$ spores/mL ($2.5x10^{10}$ spores per tray). All spore counts were made using a Hausser Scientific "Bright-Line" hemocytometer. To harvest cultures, the entire contents of the tray were placed in a blender and homogenized on high speed for 30 seconds. The final spore concentration was determined using the hemocytometer. Treatments were prepared in the laboratory less than 2 hours prior to application. Treatments were prepared by diluting the homogenized Trichoderma liquid cultures with tap water to $1x10^7$ spores/mL in the final spray volume, which was subsequently increased to $1x10^8$ spores/mL due to increased disease pressure

Table 3: Summary of water volumes used to apply treatments to test the efficacy of late blight resistance and biological controls for late blight control.

Date	Week	Amount of water applied/full	Amount of water

		and 1/2 rate Parasol plots (mL)	applied/control plot (mL)
July 18	1	169	188
July 25	2	182	190
August 1	3	194	200
August 8	4	255	213
August 15	5	254	213
August 22	6	253	213
August 29	7	235	194
September 5	8	216	175
September 12	9	185	175

Assessment and Analysis- Plots were assessed weekly for incidence and severity of late blight infection, starting on July 18, 2012 (pre-treatment) and continuing until September 19, 2012 (1-week post last treatment). Incidence was determined by counting the number of infected plants out of the total numbers of plants within each plot. A single severity score was assigned to each plot by visually determining the total percentage of infected tissue for all plants infected with late blight in the plot. The percentage of infected tissue was then converted to a severity grade using the Horsfall-Barratt scale where percentages are converted into grades of 0 to 11 (Table 4). . Disease severity data were analyzed by calculating the AUDPC (Area Under Disease Progress Curve) using the following formula:

$$AUDPC = \Sigma_{i=1}^{n-1} [(t_{i+1} - t_i)(y_i + y_{i+1})/2]$$

Where t = the time interval between assessments (Weeks) and y = severity score.

Yield and disease incidence on tubers were assessed at harvest on September 19, 2012. From each plot two plants were randomly chosen. All tubers from the two plants were harvested, counted, and weighed. The surface of each tuber was examined for any signs or symptoms of late blight. The proportion of tubers with presence of late blight was recorded.

A post-harvest assessment was conducted to determine if any of the treatments had an effect on late blight incidence after a period of storage. After all harvest parameters for tubers were measured, 10 symptom-free tubers from each plot were put into an onion sack (1 sack/plot) that was then placed in a plastic tote. Totes were placed in a cool dry storage area at 9.0 - 18.3^{o}C, 54 - 88% relative humidity and minimal light. Tubers were stored for four weeks (28 days) and then disease incidence on all 10 tubers in each sack was assessed by visually examining each tuber for late blight symptoms.

For Experiment A, AUDPC score, yield and tuber disease at harvest and post-harvest disease incidence was analyzed using two-way ANOVA (foliar treatment X variety). For Experiment B, all response parameters were analyzed using one-way ANOVA. *Post hoc* means comparisons were done with Tukey Kramer HSD test. All data were analyzed using JMP-In Version 5.1 (SAS Institute, Chicago, IL).

Table 4: Grades used for determining late blight disease severity using the Horsfall-Barratt scale.

Grade	Range of plant tissue infected by late blight lesions
0	0%
1	0-3%
2	3-6%
3	6-12%
4	12-25%
5	25-50%
6	50-75%
7	75-88%
8	88-94%
9	94-97%
10	97-100%
11	100%

Results

Experiment A- Foliar assessments

Both Variety and Foliar treatment and the interaction of the two had significant effects on the late blight AUDPC scores (Foliar treatment: $F(1,70)=196.5$, $p< 0.0001$; Foliar treatment: $F(4,70)=16.57$, $p< 0.0001$; Variety X Foliar treatment: $F(4,70)=16.56$, $p< 0.0001$). As in previous trials we observed a significant reduction in late blight AUDPC score in Innovator plots compared to the Norkotah plots (Fig. 1). Since the foliar treatments performed differently on the two different varieties (as per the significant interaction term) we examined the performance of foliar treatment on each variety separately.

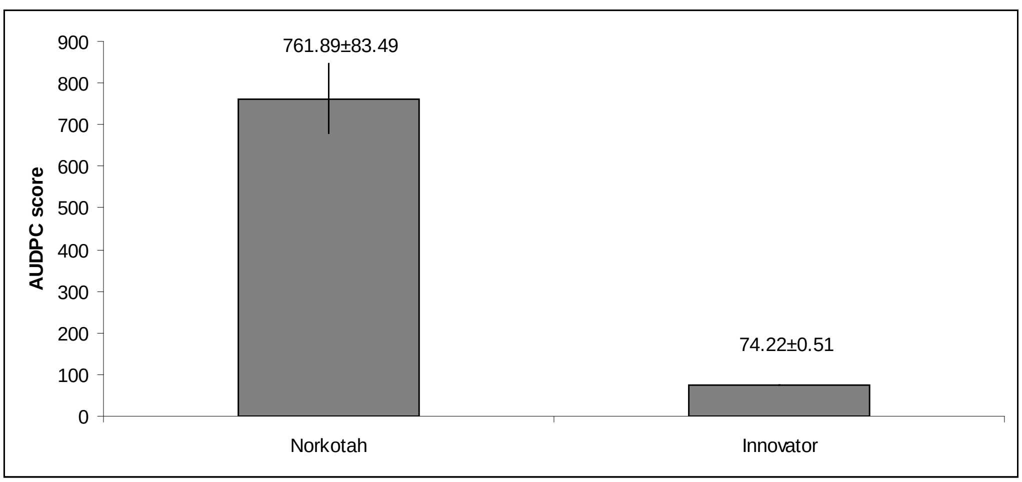

Figure 1. Comparison of late blight development (based on Area Under Disease Progress Curve - AUDPC) two potato varieties. Data are from the water Control treatments for both varieties and bars are the mean ± s.e. of eight replicates/treatment. (NB: the lowest possible score using the Horsfall-Barratt grade to calculate AUDPC is 73.71).

Norkotah - There were significant differences in the AUDPC score for late blight among the five foliar treatments (Fig. 2; Foliar treatment: $F(4,35) = 16.56$, $p<0.0001$). Treatment containing copper had significantly lower AUDPC scores (or less blight) than treatments without copper. Both Trichoderma strains on their own did not cause a reduction in blight compared to the water Control (Fig. 2).

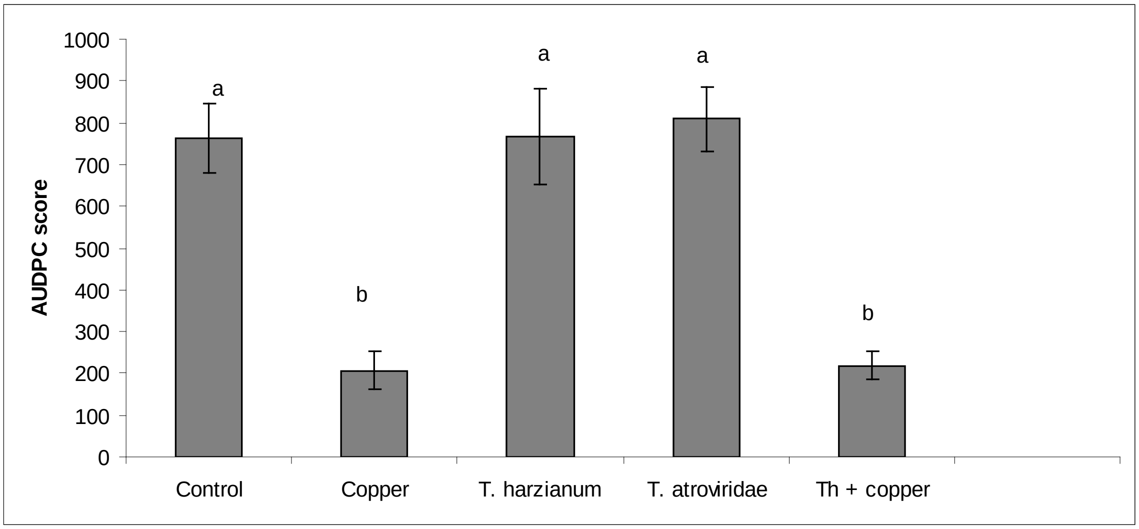

Figure 2. Effect of foliar treatments on late blight development (AUDPC score) on Norkotah potatoes. Bars are the mean ± s.e. of eight replicates/treatment; bars with same letter are not significantly different based on Tukey Kramer HSD.

Innovator - In contrast to the blight-susceptible Norkotah, there was no significant difference among foliar treatments on the blight-resistant Innovator (Fig. 3; $F(4,35)=1.00$, $p=0.42$). This is because there was no blight development on the Innovator plots.

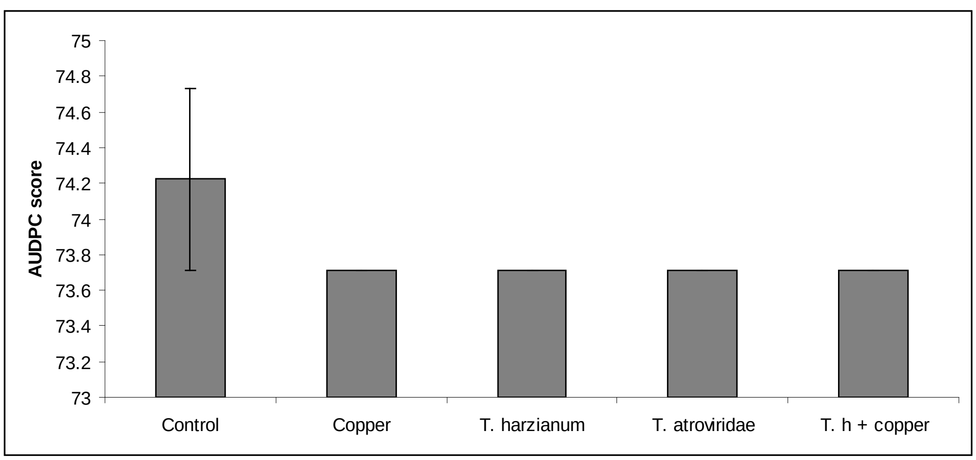

Figure 3. Effect of foliar treatments on late blight development (AUDPC score) on Innovator potatoes. Bars are the mean ± s.e. of eight replicates/treatment - bars without standard errors have an s.e. value of 0. (NB: the lowest possible score using the Horsfall-Barratt grade to calculate AUDPC is 73.71).

Experiment A - Harvest and Post-Harvest tuber assessment

Overall levels of tuber blight were quite low at harvest and there were no difference between treatments in tuber blight incidence (Fig. 4; Variety: $F(1,70)=1.63$, $p=0.21$; Foliar treatment: $F(4,70)=0.91$, $p=0.46$; Variety X Foliar treatment: $F(4,70)=1.03$, $p=0.40$). Similarly, there were no significant treatment effects on the weight of tubers at harvest (Variety: $F(1,70)=1.18$, $p=0.28$; Foliar treatment: $F(4,70)=1.08$, $p=0.37$; Variety X Foliar treatment: $F(4,70)=0.34$, $p=0.85$) or on the development of late blight during harvest (Variety: $F(1,70)=1.65$, $p=0.21$; Foliar treatment: $F(4,70)=0.15$, $p=0.96$; Variety X Foliar treatment: $F(4,70)=0.88$, $p=0.48$), which was minimal (i.e. less than 2% of tubers in developed blight after four weeks of storage).

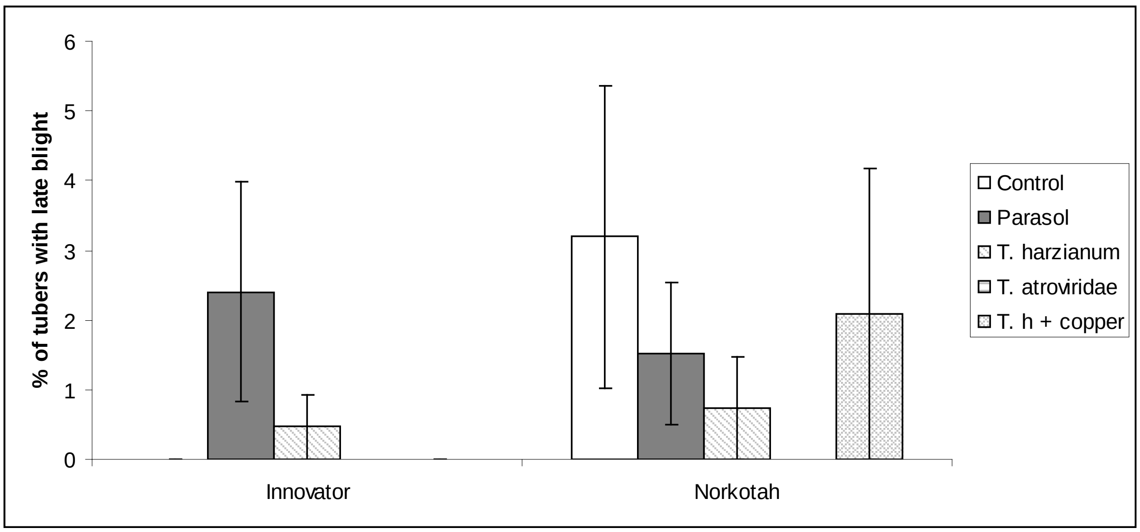

Figure 4. Effect of foliar treatments for late blight on incidence of late blight on Innovator and Norkotah tubers at harvest. Bars are the mean ± s.e. of eight replicates/treatment - missing bars have values of 0.

Experiment B

There was a significant difference among treatments in the AUDPC score, with treatments containing copper having lower scores than those without copper (Fig. 5; $F(3,28) = 24.99$, $p < 0.0001$). In other words, treating potato seed with Heads Up prior to planting did not reduce the incidence or severity of foliar late blight. As well, tubers treated with Heads Up and then the 1/2 rate of copper on foliage had levels of late blight similar to plots only treated with copper at the full rate. Although this might suggest that Heads Up can be used to reduce reliance on copper without compromising blight control, it is important to note that in previous work we have demonstrated that a 1/2 rate of copper without any additional products is as effective as the full rate (E.S. Cropconsult Ltd. unpublished data). Given that our plots were not fertilized and plants were growing slowly the 1/2 rate of copper probably provided sufficient coverage. For well fertilized and rapidly growing plants this may not be the case. Also 1/2 rates of pest control products increase the risk of resistance development in the target pathogens. There was no difference in yield ($F(3,28)=0.88$, $p=0.46$) or tuber blight incidence at harvest ($F(3,28)=0.69$, $p=0.57$) or post-harvest ($F(3,28)=0.67$, $p=0.57$) (Fig. 6).

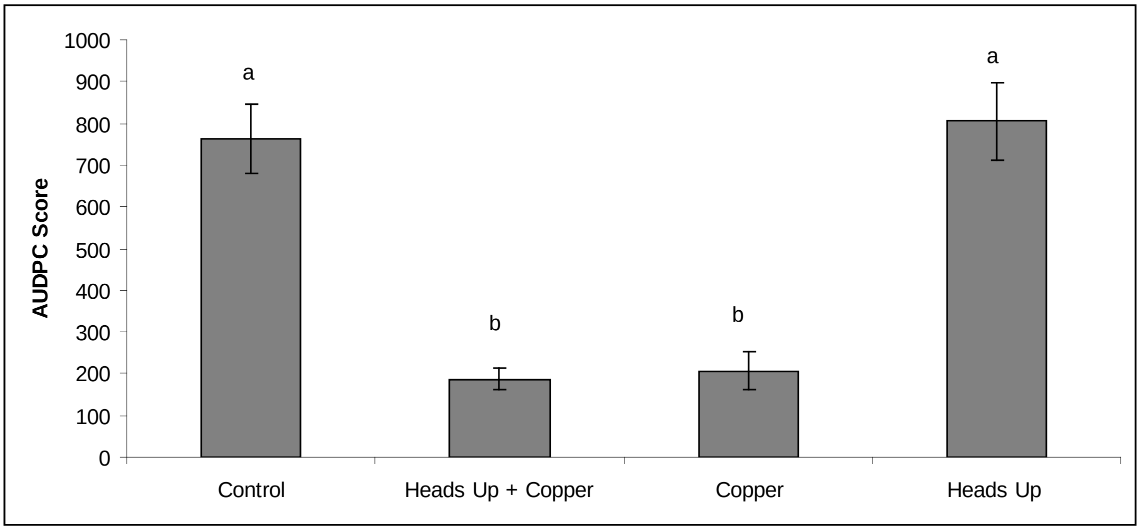

Figure 5. Effect of Heads Up seed treatment alone or in combination with foliar copper on late blight development (AUDPC score) on Norkotah potatoes. Bars are the mean ± s.e. of eight replicates/treatment; bars with same letter are not significantly different based on Tukey Kramer HSD.

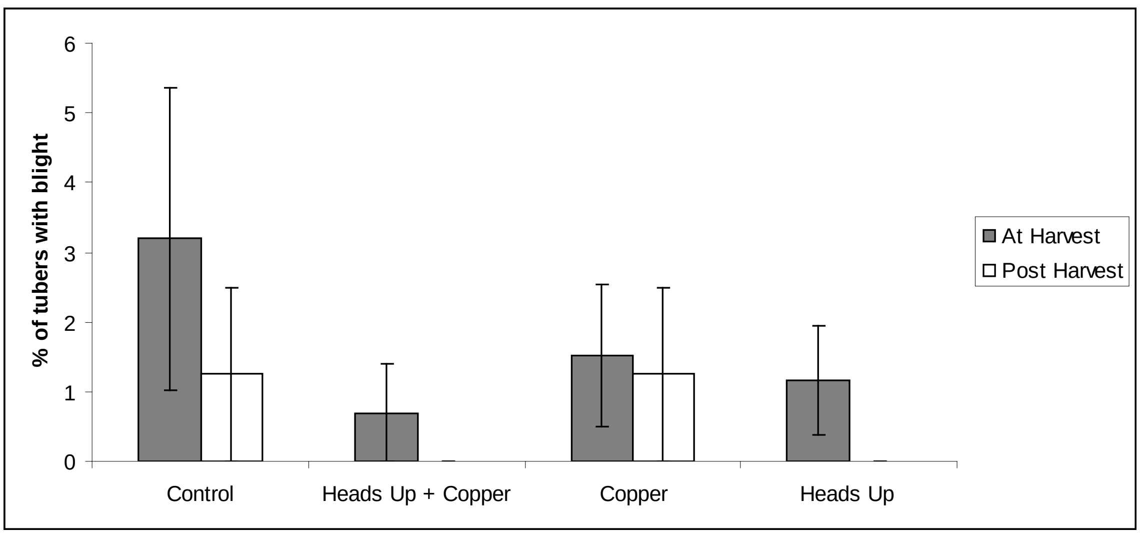

Figure 6. Effect of Heads Up seed treatment alone or in combination with foliar copper on incidence of late blight on Norkotah tubers at harvest and post harvest. Bars are the mean ± s.e. of eight replicates/treatment - missing bars have values of 0.

Discussion

The overall objective of this work was to test several possible organic solutions for late blight control. When used with the blight susceptible variety Norkotah, neither the foliar treatments with Trichoderma species or seed piece treatment with Heads Up resulted in a reduction in blight development. With the highly resistant variety Innovator, we also did not see any efficacy from Trichoderma strains but that is because there was no blight development on Innovator. We may see better results for Trichoderma species with potato varieties that have partial resistance to late blight - e.g. Island Sunshine and Krantz. Another reason we may not have seen efficacy with the Trichoderma species tested is because applications were focused on the foliage; most commercially available Trichoderma products are for root or soil applications (Hermosa *et al*. 2012). Both Trichoderma species showed efficacy against *P. infestans* in Petri dish assays (D. Henderson, unpublished data). So the lack of efficacy in our trials could be reflecting issues with application. Three basic requirements of a Trichoderma- crop system that will optimize the benefits are 1) the Trichoderma must be a highly effective strain, able to compete and persist in the environment and colonize and proliferate on plant tissue (whether in the soil or air); 2) there must be a cost effective production method; and 3) there needs to be an application method suitable for the crop, which allows full expression of the benefits of the isolate of Trichoderma. By working with the pre-screened isolates from KPU's earlier screening work and the production system that KPU currently has in place requirements 1 and 2 were met with the Trichoderma strains and the production methods used in this study.

Thus the poor efficacy of foliar applications of the Trichoderma strains may be due to factors like rate of application and application technique itself, for example, time of day of application or exposure to UV (Ignoffo and Garcia 1992). Future work should address the possible factors that limit efficacy of foliar applications of *T. harzianum* and *T. atroviridae*. Additionally, soil applications of Trichoderma species have been shown to enhance plant defenses via systemic acquired resistance (SAR) (Hermosa *et al.* 2012). So it would be interesting to evaluate the efficacy of soil applied or seed piece treatments of *T. harzianum* and *T. atroviridae* on the development of tuber late blight and possibly other soil-borne potato diseases (e.g. scab, silver scurf or Rhizoctonia).

Inducing plant resistance or SAR is the mode of action of HeadsUp, which is a formulation of saponins from *Chenopodium quinoa*; however resistance may not be to a specific pathogen. For example, Soleimani and Kirk (2012) found that seed piece treatment with Heads Up did not reduce the development of brown leaf spot disease on potatoes, in either field or laboratory trials. However, Heads Up treated plots had consistently higher yields than other treatments. Similarly, in previous work we have also found higher yields in Heads Up treated plots for potatoes and beans (E.S. Cropconsult Ltd. unpublished data) but we did not observe improved yield in the current study. Soleimani and Kirk (2012) suggest that while Heads Up may not have had an impact on brown leaf spot disease in their study, it may have been able to confer "broad disease resistance". Efficacy against late blight with Heads Up may be more apparent with varieties with partial resistance to late blight. Additionally, only a single rate (1g/L) of Heads Up was tested in both our study and that of Soleimani and Kirk (2012), efficacy of higher rates should be explored.

The broader objectives of this work are to find replacements for copper for organic late blight management. To date commercially available biofungicides (e.g. Serenade, Actinovate, and Sonata) have not been effective. Similarly, efficacy of compost teas have been inconsistent. Soleimani and Kirk (2012) found that foliar application of chitosan was effective against brown leaf spot on potatoes. To our knowledge chitosan has not been examined against late blight in local trials and we recommend that this be examined in combination with potato varieties with varying levels of susceptibility to late blight. Given the costs of field trials, we recommend that greenhouse trials should be pursued to identify promising candidates (e.g. chitosan, higher rates and different formulations of local Trichoderma species, higher rates of Heads Up) for future field testing.

Acknowledgements

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