Disease Management in Organic Blueberries

Final Project Report to:

BC Blueberry Council
Organic Sector Development Program

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Executive Summary

Organic production of highbush blueberries in British Columbia is challenging due to fungal diseases such as mummyberry, *Monolinia vaccinii-corymbosi* and botrytis fruit rot, *Botrytis cinerea*. In recent years, numerous organic-certified products, including fungicides, have come onto the market. However, many of these products have not been tested in the growing conditions and with the varieties of blueberries common to southern BC. The objective of this research was to evaluate the efficacy of organic fungicides for primary mummyberry infections at bud break, and botrytis fruit rot infections at green berry (pre-harvest) and ripe berry (post-harvest). Treatments were tested on 3 year-old potted blueberry plants. Organic fungicides tested for suppression of mummyberry included Actinovate (*Streptomyces lydicus*), Serenade Max (*Bacillus subtilis*), Serenade Aso (*Bacillus subtilis)* and Sonata *(Bacillus pumilis).* These products were compared to conventional industry standards of Topas (propiconazole) and Funginex (triforine), and an untreated control. All fungicide treatments resulted in reduced levels of primary mummyberry infection relative to the untreated control plots; however there were no differences among treatments. The two currently registered fungicides, Serenade Max and Serenade Aso, can be used with confidence by organic growers, but additional registrations should be pursued to delay development of resistance to these products.

Botrytis fruit rot fungicide treatments included Actinovate (*Streptomyces lydicus*), Pre-stop (*Gliocladium catenulatum*), Serenade Aso (*Bacillus subtilis)* and Sonata *(Bacillus pumilis).* These products were tested against the industry standard of Pristine (boscalid and pyraclostrobin) and a water control. Fungicide treatments were applied starting at 20% bloom and continued at 14 day intervals through green berry development. Botrytis levels did not significantly differ by treatment at early or late green berry development. Fruit rot levels were highest at the early green berry stage, when 30-60% of unpollinated ovaries showed symptoms. The mean number of ripe berries, the total weight of ripe berries, and the development of post-harvest fruit rot did not differ between treatments.

Introduction

Blueberry production in British Columbia has expanded rapidly over the last 5 years. Many blueberry growers are interested in organic production but feel that it is too risky to transition to organic production without reliable pest control tools. Two of the fungal diseases of concern include mummyberry and *Botrytis* fruit rot. In recent years, numerous organic-certified products, including fungicides, have come onto the market. However, many of these products have not been tested in the growing conditions and with the varieties of blueberries common to southern BC.

Mummyberry is serious disease of blueberries in the Fraser Valley and on Vancouver Island. It is caused by the fungus *Monolinia vaccinii-corymbosi*. Primary infection from this disease occurs at bud break in March when ascspores are released from mummyberries on the ground and infect the new bud tissue (Batra 1983). Secondary infection occurs when the spores spread from infected leaves to the open flowers in May. If unmanaged, mummyberry can significantly reduce yield. Once established in a field, the mummyberry disease cycle is difficult to break as the infected berries, "mummyberries" overwinter on the ground and release spores the following spring.

Recent research into biological control of mummyberry has evaluated microbial products for suppression of secondary mummyberry infection at bloom (Thornton et al. 2008, Scherm et al. 2003). However, it seems more desirable to prevent the disease from becoming established in the field by reducing primary infection at bud break. Conventional growers currently apply 2-3 fungicide treatments during the month of March to prevent primary mummyberry infection.

Botrytis cinerea is a fungal disease that can cause severe yield loss when wet weather coincides with blossom. Botrytis overwinters as mycelium or sclerotia on blueberry branches and as plant debris on the ground (Lambert 1995). In the spring, conidia spores are carried by the wind and can infect blueberry blossoms when the flowers are fully open (Hildebrand et al. 2001). Infected flowers wilt and the infection often spreads to the other flowers in the cluster. Unpollinated flowers are particularly susceptible to Botrytis infection (Hildebrand et al. 2001). Botrytis can infect the flowers but remain dormant in the berry until harvest, when it develops fruit rot symptoms in storage (BC Production Guide). For this reason, packers encourage all of their blueberry growers to apply 2-4 fruit rot fungicides during bloom.

Two organic fungicides, Serenade Max and Serenade Aso (*Bacillus subtilis)*, have recently been registered for mummyberry and Botrytis suppression in highbush blueberries. However, local experience with these products is limited. Three additional organic fungicides, Sonata (*Bacillus pumilis),* Actinovate (*Streptomyces lydicus)*, and Pre-stop (*Gliocladium catenulatum)* are registered for disease suppression on blueberries in the US. Actinovate has shown some efficacy against mummyberry in field applications to lowbush blueberries in Nova Scotia (Langdon et al. 2006). It is important for organic blueberry growers to have more than one biological product in their toolbox for disease control in order to prevent the development of disease resistance. We propose to test these organic fungicide products against conventional fungicides and untreated control for efficacy against primary mummyberry and botrytis infections.

Objective

To evaluate the efficacy of organic fungicides for primary mummyberry infections at bud break and Botrytis fruit rot infections at green berry (pre-harvest) and ripe berry (post-harvest).

Methodology

Mummyberry

In late March 2009, one hundred and forty-four, three-year-old potted blueberry plants, *Vaccinium corymbosum* cv. Reka, were acquired from Gaskin Farms in Port Coquitlam. Blueberry plants were transferred to an organic blueberry farm in Surrey BC with a history of high mummyberry pressure.

Potted blueberry plants were paired by tying two plants together, loosely at the base of the stems with flagging tape. A pair of plants constituted a single "plot" or replicate. Pairs of plants were spaced 1 metre apart along the eastern edge of the blueberry field, so as to be out of the grower's way and upwind from the field. Treatments were randomly assigned to each plant pair. There were nine treatments, each replicated eight times (N=72). Treatments were as follows:

Table 1. Trade name, active ingredient, and application rate of treatments for mummyberry based on a standard planting density of 3658 plants per hectare.

Treatment	Active and Registrant	Registrant	Rate	Amount of product per pair of plants
Actinovate	*Streptomyces lydicus*	Natural Industries Inc	425g/1100 litres water per hectare	0.23 g
Serenade Max	*Bacillus subtilis*	AgraQuest Inc	3.0kg/ha at 1% dilution	1.64 g
Serenade Aso	*Bacillus subtilis*	AgraQuest Inc.	9.3 litres per hectare at 1% dilution	5.1 ml
Sonata (half rate)	*Bacillus pumilis*	AgraQuest Inc	4.7 litres per hectare at 1% dilution	2.6 ml
Sonata (label rate)	*Bacillus pumilis*	AgraQuest Inc	9.3 litres per hectare at 1% dilution	5.1 ml
Sonata (2X rate)	*Bacillus pumilis*	AgraQuest Inc	18.6 litres per hectare at 1% dilution	10.2 ml
Topas– industry standard	propiconazole	Engage Agro Corp	500ml/200 litres water per hectare	0.3 ml
Funginex – industry standard	triforine	Summit Agro Corp	3 litres/1000 litres water per hectare	1.6 ml
Untreated Control				

Fungicide treatments commenced when 2-5mm of new leaf tissue was exposed on the buds as this is the stage when highbush blueberries are susceptible to primary infection. Treatments were applied at 7 day intervals following the advice of Agraquest Inc. as many of the organic fungicides are not rainfast. Treatments were applied on March 27th, April 3rd, April 10th, April 17th and April 24th. Each treatment was applied with a 4 litre hand pump sprayer. Application rates were calibrated based on a planting density of 3658 blueberry plants per hectare, which is the mean planting density suggested in the BC Production Guide.

Mummyberries with open apothecia were collected from a heavily infected blueberry field in Ladner, BC on March 18th, April 2nd and April 9th. These mummyberries were individually pressed into the surface of the soil of the potted blueberry plants at a rate of one mummyberry per plot. Mummyberries were added to the plots immediately following treatment.

Blueberry plants were transferred to Abbotsford at the end of April after the period of primary infection had passed. Pots remained in their pairings and were spaced 1 m apart from other pairs once transferred to Abbotsford. Primary infection was assessed on May 14th by counting the number of infected leaf shoots and flower clusters per plot.

Botrytis Fruit Rot

Ninety-eight, three-year-old potted blueberry plants, *Vaccinium corymbosum* cv. Duke, were acquired from Gaskin Farms in Port Coquitlam. Blueberry plants were transferred to E.S.Cropconsult's Abbotsford research site.

As with the mummyberry trial, a single replicate for this study consisted of a pair of blueberry plants. Potted blueberry plants were tied together at the base with flagging tape. The 49 pairs of plants were placed in a 7 X 7 arrangement and spaced 50 cm apart. Plants were irrigated by overhead sprinkler for 40 minutes every 3 days in accordance with watering restrictions in the City of Abbotsford. Fungicide treatments were randomly assigned to pair of plants. There were six fungicide treatments, each replicated eight times (N=48). Treatments were as follows:

Table 2. Trade name, active ingredient, and application rate of treatments for botrytis fruit rot based on a standard planting density of 3658 plants per hectare.

Treatment	Active and Registrant	Registrant	Rate	Amount of product per pair of plants
Actinovate	*Streptomyces lydicus*	Natural Industries Inc	425g/1100 litres water per hectare	0.23 g
Prestop	*Gliocladium catenulatum*	Plant Products Co	5kg/1000 litres water per hectare, in 0.5% aqueous suspension	2.74 g
Serenade Aso	*Bacillus subtilis*	AgraQuest Inc.	9.3 litres per hectare at 1% dilution	5.1 ml
Sonata	*Bacillus pumilis*	AgraQuest Inc	9.3 litres per hectare at 1% dilution	5.1 ml
Pristine	boscalid and pyraclostrobin	BASF Canada	1.3kg/1000 litres water per hectare	0.71 g
Untreated Control				

Fungicide treatments commenced at 20% bloom. Treatments were applied at 14 day intervals. Treatments were applied on May 22nd, June 4th, June 18th, and July 2nd. Each treatment was applied with a 4 litre hand pump sprayer. Application rates were calibrated based on a planting density of 3658 blueberry plants per hectare, which is the mean planting density suggested in the BC Production Guide.

Branch tips infected with *Botrytis cinerea* were collected from an established blueberry field in Langley on May 18th. Infected branch tips were held at room temperature in a sealed bag with moist paper towel for 6 days to encourage spore germination. Each pair of plants was infested with two Botrytis-infected branch tips on May 25th. Infected branch tips were paperclipped to healthy stems adjacent to open flower clusters. The number of healthy green berries and the number of green berries with symptoms of Botrytis-infection was assessed in each plot at the early green berry stage on June 11th and at the late green berry stage on June 25th.

Ripe berries were harvested on July 24th . The number and weight of ripe berries from each plot was recorded. Ripe berries from each plot were placed in individually labelled hard plastic containers in the refrigerator (6ºC, 38% humidity) and re-examined for fruit rot development 4, 6, 8, 13, 20, 25 and 32 days after harvest (DAH).

Statistical Analysis

Data were analyzed statistically, using JMP-IN Version 4 software. Mummyberry infection counts were analyzed with a one-way ANOVA. A Tukey-Kramer HSD analysis was performed to compare differences between the means. The percentage of green berries with symptoms of Botrytis infection was arcsine-transformed prior to analysis with a one-way ANOVA. Ripe berry yield and weight in Botrytis plots were analyzed with a one-way ANOVA. Post harvest fruit rot development was analyzed with repeated measures MANOVA. An arc-sine transformation was performed on proportion berries with fruit rot symptoms prior to analysis.

Results

<u>Mummyberry</u>

The number of leaf and flower cluster infections was significantly lower in the fungicide-treated plots than in the untreated Control plots (Fig 1. $F_{8,63} = 9.06$, $P < 0.001$), however there were no differences among treatments. The conventional fungicides Funginex and Topas provided complete suppression of mummyberry. Phytotoxicity was observed on the plants treated repeatedly with Funginex. Considerable spray residue was visible on the plants treated with Serenade Max.

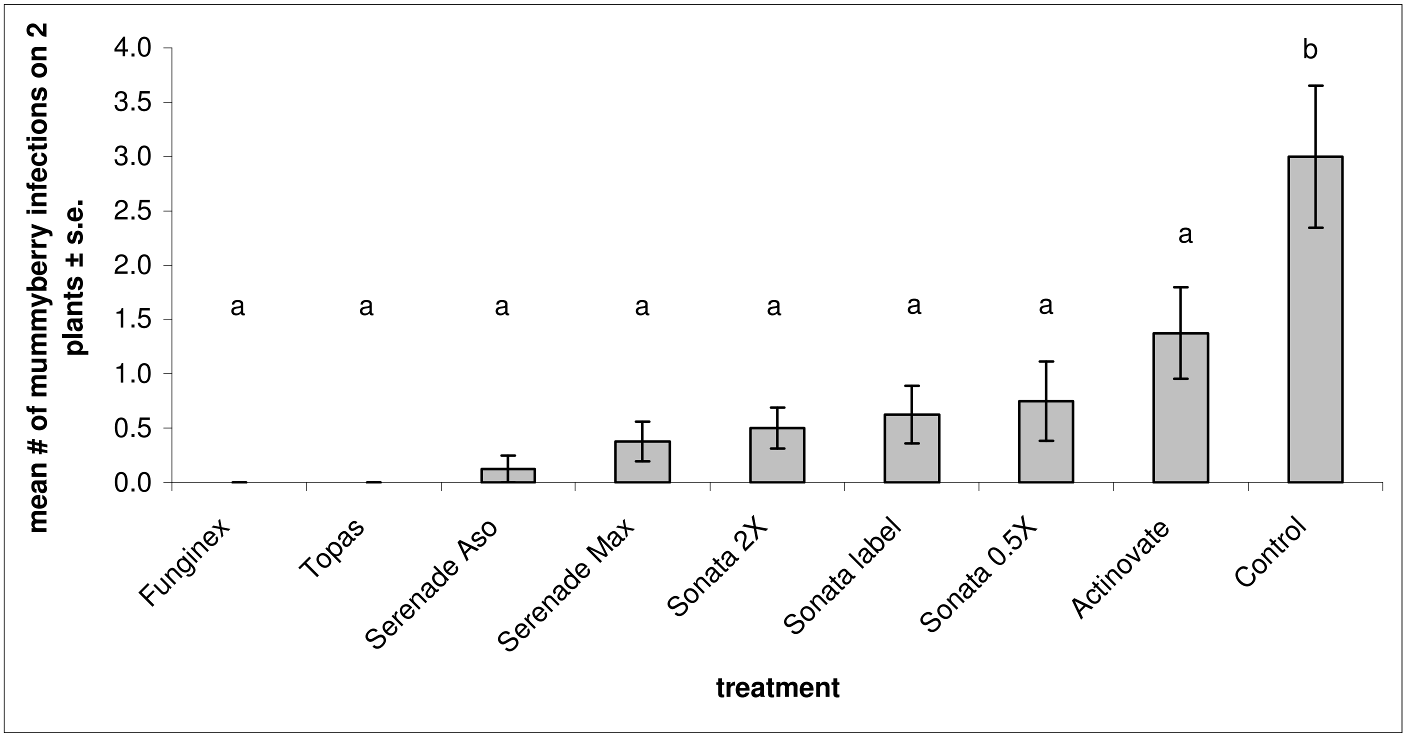

Figure 1. Effect of fungicide treatment on the mean number of primary mummyberry leaf and flower infections (± s.e.). (N=8 for each treatment).

Botrytis: Pre-harvest

At the early green berry stage, 30-60% of unpollinated ovaries showed symptoms of Botrytis fruit rot. Although the untreated control plots had a greater percentage of infected green berries, Botrytis levels did not significantly differ by treatment (Fig 2. $F_{5,42}$= 1.49, P = 0.21). When green berries were fully sized, fruit rot levels were much lower, ranging from 1-11% of berries infected, and there was no significant difference between treatments (Fig 3. $F_{5,42}$= 0.67, P = 0.65). When harvested, the mean number of ripe berries (Fig 4. $F_{5,42}$= 0.48, P = 0.79) and the total weight of ripe berries (Fig 5. $F_{5,42}$= 0.66, P = 0.65) also did not differ between treatments.

Botrytis: Post-harvest

Botrytis fruit rot did not develop in harvested berries until 20 days after harvest. However, neither treatment, time nor the interaction of the two were significant (Fig. 6; Treatment X Time: $F_{30,146}$ = 0.63, P = 0.93; Time: $F_{6,36}$= 1.85, P = 0.12; Treatment: $F_{5,41}$= 0.79, P = 0.56).

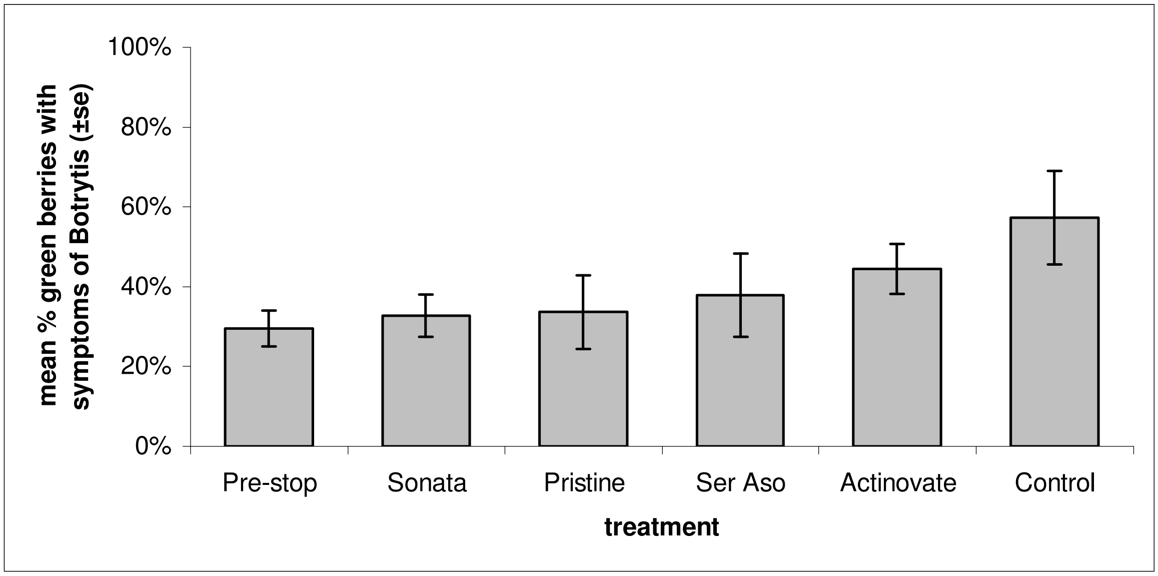

Figure 2. Effect of fungicide treatment on mean percentage of small green berries with symptoms of Botrytis infection (± s.e.). (N=8 for each treatment)

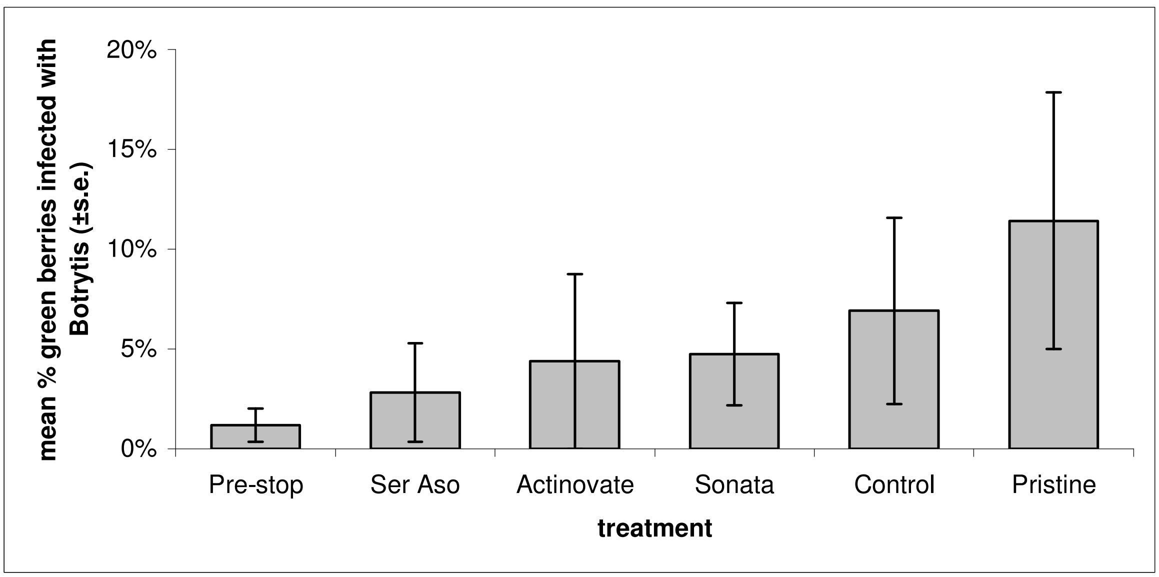

Figure 3. Effect of fungicide treatment on the mean percentage of green berries (± s.e.) with symptoms of Botrytis infection. (N=8 for each treatment)

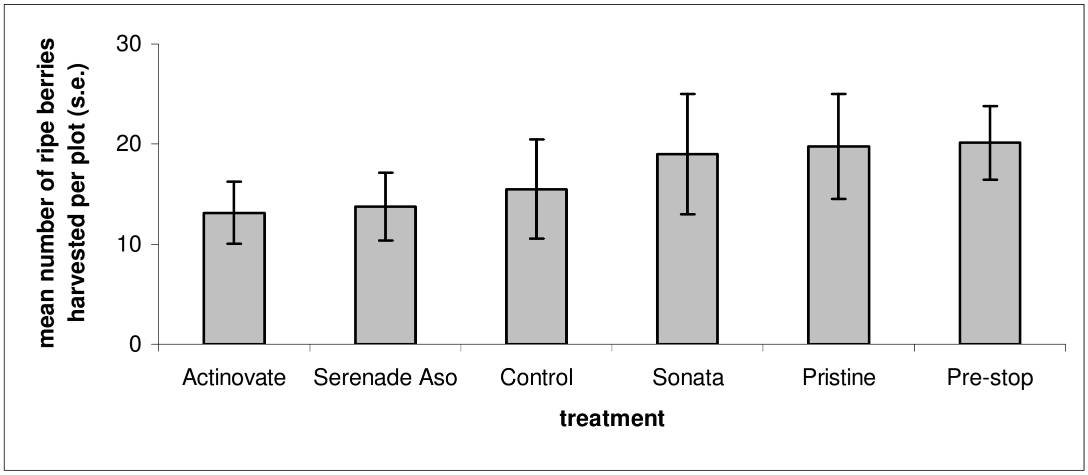

Figure 4. Effect of fungicide treatment on number of ripe berries harvested per plot (± s.e.). (N = 8 for each treatment).

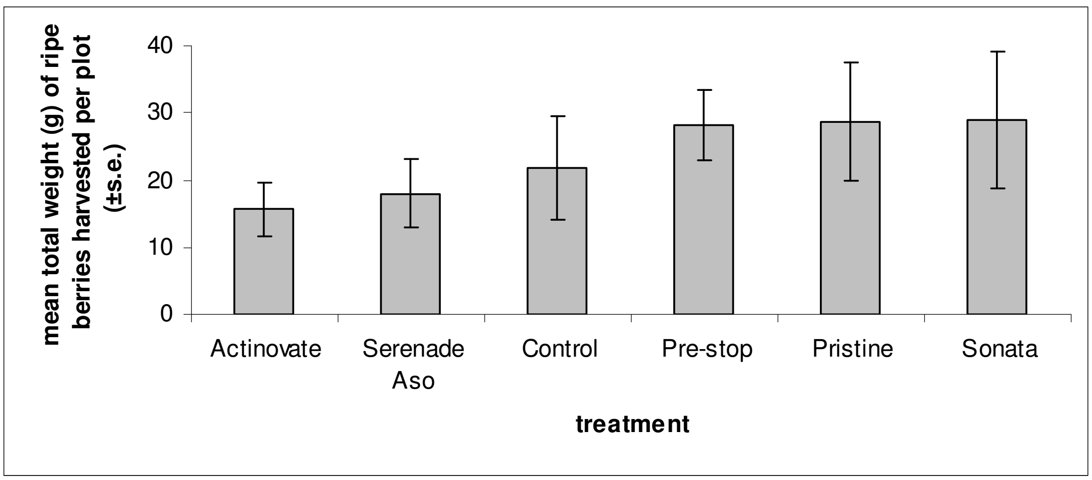

Figure 5. Effect of fungicide treatment on mean total weight of ripe berries harvested per plot (± s.e.). (N = 8 for each treatment).

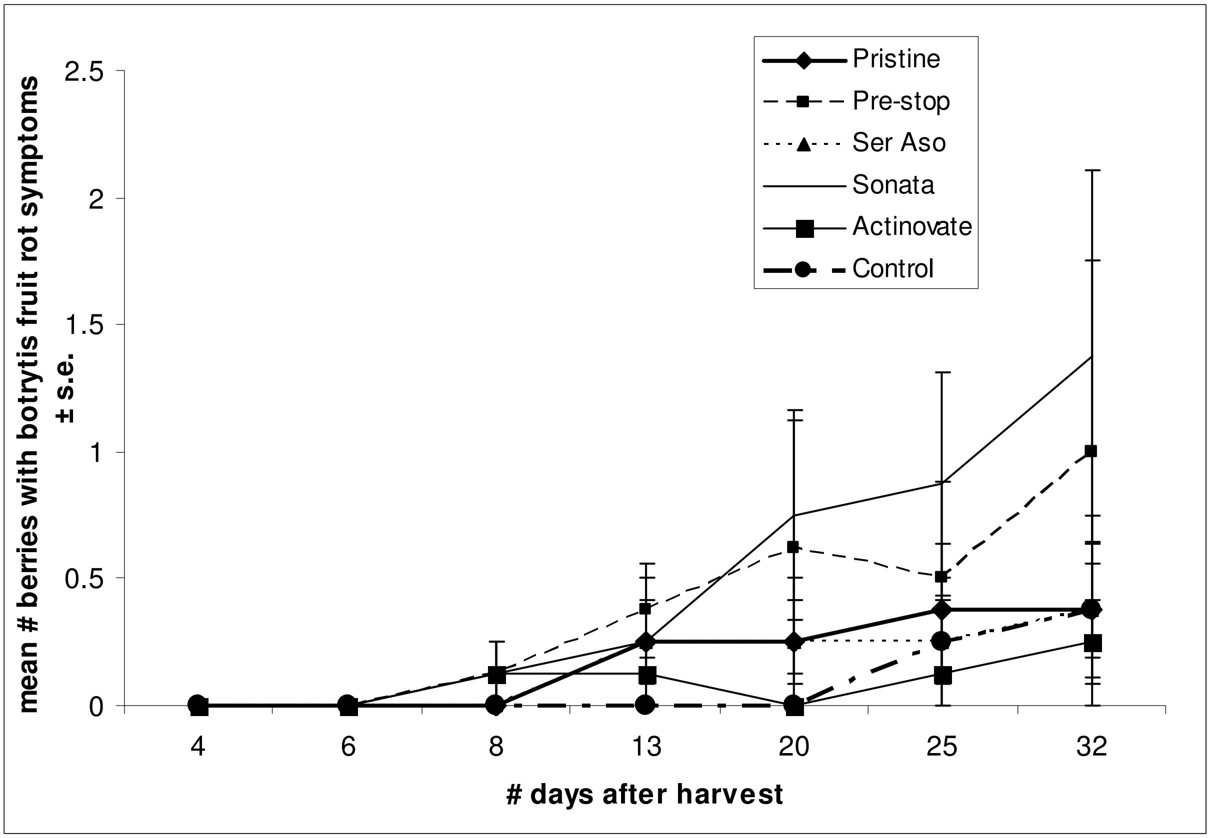

Figure 6. Mean number of berries the developed botrytis fruit rot symptoms 4, 6, 8, 13, 20, 25 and 32 days after harvest (± s.e.). N= 8 for all treatments

Discussion

Mummyberry

Our research showed promising results from numerous organic fungicide treatments for suppression of mummyberry but not Botrytis. Serenade Aso and Serenade Max (*Bacillus subtilis*), Sonata (*Bacillus pumilis*), and Actinovate (*Streptomyces lydicus*) treatments all reduced the number of mummyberry infections by at least 50% compared to the untreated controls. Although a significant difference was not found between treatments, the Serenade products provided similar mummyberry suppression to conventional fungicides. Other studies have found promising results from *Bacillus subtilis* applied at the secondary infection stage. Thorton et al (2008) and Scherm et al. (2004) observed strong antibiotic activity and reduced mummyberry hyphal growth in blueberry flowers treated with *Bacillus subtilis.* Serenade Aso and Serenade Max are currently registered for suppression of mummyberry on highbush blueberries in BC, and organic growers should apply them at the bud break stage to reduce primary mummyberry infections.

The least promising mummyberry treatment in our trial was Actinovate, which was one of the better performing biological controls tested on lowbush blueberries by Langdon et al. (2006). This highlights the importance for testing organic fungicides on highbush blueberries in BC field conditions.

The fluctuations in fruit rot levels at the early and late green berry stages are consistent with the findings of Hildebrand et al. (2001). The unpollinated green berries were very susceptible to Botrytis while the fully pollinated green berries were not. Very few ripe berry infections were observed in this trial which may be due to the hot and dry weather from June to August 2009, which is unfavourable for germination of *Botrytis cinerea* (Hildebrand et al. 2001). Very little Botrytis fruit rot was also observed in commercial fields in 2009 (E.S. Cropconsult Ltd. unpublished data). Although treatment effects were not significant for Botrytis fruit rot, there was a trend towards lower pre-harvest infection levels in Prestop (*Gliocladium catenulatum*)-treated plants. Prestop treatment also showed a trend toward more ripe berries harvested. Prestop is currently registered for control of Botrytis in strawberries in BC. Further field trials should be conducted with this product to identify whether a label expansion would be beneficial to blueberry growers.

One of the principles of organic farming is a reduced dependence on sprays, and a greater reliance on cultural practices to regulate insect and disease pests. Mummyberry can be further suppressed by mulching the ground beneath the bushes between November and February when the previous season's mummyberries have fallen to the ground. By burying the mummyberries, apothecia development and spore release can be reduced the following March. Similarly, the ground beneath the bushes can be raked in late February to flip the mummyberries over after the apothecia have developed. The apothecia are sensitive to freezing at this stage and spore germination will be reduced when apothecia are not protected from cold temperatures (Wharton and Schilder 2005). Levels of Botrytis inoculum can be reduced in the spring by pruning out the dead branch tips. Vigorous pruning of blueberry bushes will improve air circulation and reduce the humid conditions that are favourable to Botrytis infection. Similarly, the installation of drip irrigation instead of overhead irrigation will help to minimize periods of flower wetness which favour Botrytis germination. Some growers in BC are currently experimenting with berry production under tunnels. By excluding rainfall from the field and relying solely on drip irrigation, the conditions for fruit rot development are severely reduced.

By using a combination of cultural practices and organic fungicide sprays, organic blueberry growers will likely be able to reduce yield losses due to mummyberry and fruit rot to economically acceptable levels.

Recommendations for further research

- Evaluate cultural controls of mummyberry (mulching vs. raking) in combination with organic fungicide sprays in the field for best suppression of mummyberry

- Field test Serenade Aso (*Bacillus subtilis*) and Sonata (*Bacillus pumilis*) at longer spray intervals to develop a rotational spray program for mummyberry suppression for organic blueberry growers

- Evaluate organic fungicide treatments for combined control of Botrytis, Anthracnose and Alternaria fruit rots

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