Insect management tools for organic cranberry production in the Pacific Northwest

Final Report

To:
Organic Sector Development Program
Organic Farming Research Foundation
B.C. Cranberry Growers Association

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Executive Summary

The expansion of organic cranberry production in British Columbia, Washington and Oregon is constrained in part by the limited availability and familiarity with pest control tools suitable for organic production. This study focused on organic methods for the control of the three main insect pests for the region – blackheaded fireworm, cranberry girdler and black vine weevil. We examined products that are currently available and those that are pending use in cranberries. We focused on either developing use protocols for effective product use in cranberries (especially for products requiring delivery via chemigation) or on efficacy alone. Key findings of this multi-year study were as follows.

Blackheaded Fireworm

- Reducing the duration of chemigation with Entrust™ and repeat applications appear to be critical factors for achieving effective control
- *Trichogramma sibericum* releases targeting the summer generation of fireworm resulted in up to 40% parasitism. These findings suggest that June releases may be an additional tool for the fireworm control tool box. Efficacy of releases could most likely be improved by increasing the release rate and timing releases better, e.g. based on detection of adult males in traps. A major challenge however is that *T. sibericum* is currently only available on a research basis and import permits would be required for use of the product in Oregon and Washington, unless the species can be identified from these areas as well.

Cranberry Girdler

- 1 hour of irrigation following nematode application appeared to be sufficient for washing nematodes off of foliage and into the soil root zone. Growers should follow supplier recommendations with regards to irrigation protocols in the days prior to and post application (in order to ensure adequate soil moisture for nematode survival).
- In preliminary bioassays, girdler larvae were shown to be susceptible to the *Metarhizium anisopliae* strain Met52. However in field trials with the granular and emulsifiable concentrates of this entomopathogenic fungus, we did not see a significant reduction in the recovery of live larvae. However the trend in two years of trials was for fewer girdler larvae or feeding tunnels in Met52 treated plots. We suggest *M. anisopliae* is a promising tool for organic cranberry production and efficacy studies should continue.

Black vine weevil

- In small plot assays the nematode *Heterohabditis bacteriophora* provided the most effective kill of larvae compared to the Control. In field plot studies we found that spring applications of all three commercially available nematode species--*H. bacteriophora* on its own, *Steinernema carpocapsae* on its own, and *H. bacteriophora* combined with *S. kraussei*--were all effective in reducing the number of live black vine weevil individuals. Spring applications of nematodes against black vine weevil are the current practice among growers in southern Oregon but not for B.C. growers.

- Trials with granular *Metarhizium anisopliae* (Met52) against black vine weevil did not cause reductions in the recovering of live individuals. However, in other systems Met52 has been shown to be effective against black vine weevil, so the issue in cranberries is most likely getting spores to wash below ground. Effective delivery of the newly available emulsifiable concentrate of Met52 or other bioinsecticide still needs to be developed for cranberries.

Introduction and Objectives

One of the main challenges for cranberry production in Oregon, Washington and British Columbia are insect pests, which are able to survive the mild west coast winters. Disease pressure is high for cranberry production in other areas but less so on the west coast, making it more feasible to grow organic cranberries in this area. Weeds are difficult to control and hand weeding is often used by both organic and conventional growers. There has been a considerable amount of research on different biological methods for controlling the three main insect pests - blackheaded fireworm, cranberry girdler and black vine weevil. However, use protocols are lacking and growers experience variable efficacy using the products that are available (e.g. nematodes). Some of the biological tools needed are not commercially available or are just recently available. Another problem is that protocols for many commercial biological products are for row or orchard crops - cranberries are a unique agricultural environment growing as interwoven vines on the ground. Chemigation is used commonly, especially in B.C. ,for pesticide delivery. Thus the issues to be addressed by this project are (1) identifying effective management tools for blackheaded fireworm, cranberry girdler, and black vine weevil and (2) developing use protocols for these tools that are appropriate for the conditions of cranberry production.

Original Project Objectives - To evaluate the efficacy and develop use protocols for organic methods of controlling the three key insect pests of cranberry in the Pacific Northwest

1. Blackheaded fireworm (*Rhopobota naevana*):
    - Efficacy trials with the spinosad-formulation Entrust (Dow AgroSciences Canada Inc.). Develop guidelines for use via chemigation system.
    - Evaluate the use of *Trichogramma sibericum* for spring generation blackheaded fireworm eggs.
2. Cranberry girdler (*Chrysoteuchia topiaria*)
    - Evaluate the efficacy and application methods of the commercially available *Metarhizium anisopliae* strain Met52 (Novozymes Biologicals Inc., Salem, VA) with and without additional products to improve efficacy (e.g. diatomaceous earth).
    - Develop a protocol for delivering nematodes to the target area under various field conditions.
3. Black vine weevil (*Otiorynchus sulcatus*)
    - Evaluate the efficacy and application methods of the commercially available *M. anisopliae* strain Met52 with and without additional products (e.g. diatomaceous earth).
    - Compare the efficacy of commercially available nematode species.

Variations from Original Work Plan

The main variation from the work plan was that we did not include treatments with diatomaceous earth in our evaluation of *M. anisopliae* for girdler and black vine

weevil. This was because we did not have enough pest pressure to support additional plots for extra treatments.

Objective 1a: Blackheaded fireworm tools - Entrust

Rationale: - Larvae of the blackheaded fireworm (*Rhopobota naevana*) feed on cranberry buds, foliage and berries. If left unchecked, defoliation of cranberry uprights and vines results in severe damage. This is the key pest of cranberry production in the Pacific Northwest and conventional growers spray multiple times in the growing season with organophosphates to manage fireworm damage. In small plot assays we have observed efficacy of the organic formulation of spinosad (Entrust) against blackheaded fireworm. The objective of this component of the study is to develop a protocol for applying Entrust via chemigation.

2008 Methods: Trials were set up at two farms in Oregon and one farm in B.C. Trials were developed collaboratively with both growers so methods were different at each farm.

Oregon Farm 1: Plots were $1m^2$ with a 1-m buffer between plots. Plots were located along an edge of field with a heavy fireworm infestation. Plots were randomly assigned to treatments. Treatments were Control (water only), Pyganic (1.2L/ha) and Entrust (87.2 grams active ingredient (g a.i.)/ha). There were 5 plots per treatment for a total of 15 plots. Treatments were applied with a hand pump sprayer, with 1 L of spray solution applied to each plot. A cardboard screen was used to prevent drift between plots. 72 h after treatment, 30 tents/plot were examined and the number of dead or sick fireworm larvae were counted along with the total number of larvae. The effect of insecticides on the proportion of dead and sick larvae was examined using one-way ANOVA (JMP-IN, SAS Institute, Chicago, IL).

Oregon Farm 2: Plots were $2m^2$ and were located along edges of the field, again in areas with heavy fireworm pressure. Plots were randomly assigned to treatments. There were two treatments at this farm: Control and Entrust. Entrust was applied via chemigation at a rate of 120 g a.i./ha with 550 gallons of water used to apply the product (sprinklers ran for 3 hours and 45 minutes). Control plots were covered with a $3m^2$ sheet of thick clear plastic that was held in place with wooden pegs in each corner. The plastic sheets were to prevent any insecticide from being applied to foliage in the Control plots. Plastic sheets were removed after the sprinklers were turned off. Post treatment assessment and data analysis were done in a similar manner as that described for Farm 1 (above).

B.C. Farm 1: For the Entrust chemigation trial, twelve $1m^2$ plots were staked out along the south and east edges of a field known to have high populations of fireworm. Plots were located between two sprinklers and between 1.5 and 2.0 m from the edge of the field. There were two treatments at this farm: Control and Entrust. To obtain an untreated Control, two sprinkler heads were blocked on either side of the Control plots. Entrust was applied at a rate of 175 g a.i/ha with sprinklers running for 27 minutes (pressure was 1000 to 1800 g/minute). A pre-treatment count was made on July 8 and post-treatment counts were made on July 11, approximately 64h after treatment. Both pre

and post-treatment assessments consisted of opening 20 fireworm tents in each plot and counting the number of live fireworm larvae. During treatment we observed that the sprinklers in two of Control plots were not sufficiently blocked to prevent chemigation. Therefore we removed these two plots from our analysis. The effect of treatment on the proportion of dead larvae/plot was analyzed using Student's T-test, with data arcsine transformed prior to analysis (JMP-IN, SAS Institute, Chicago, IL).

2009 Methods:

B.C. Farm 2: A 0.6 acre cranberry field with a widespread blackheaded fireworm infestation was chosen for this trial in 2009 (this site had not been sprayed with conventional pesticides in three years). There were two treatments at this farm: Control and Entrust. The field was sprayed twice, first on June 15 and again on June 25. Larval counts were done three times - June 14, June 22, and July 2. Fireworm counts were done by examining 20 tents in ten different locations in the field. The total number of fireworm found were recorded and categorized as live or dead/sick. Larva were not always found in the ten locations checked. Entrust was applied via chemigation at a rate of 140 g a.i./ha (or 42.5 g of product / 0.6 acre). The rate 175 g a.i./ha is already registered, in Canada, for cranberry fruitworm in cranberries. However, the rate of 140 g a.i./ha was recommended for fireworm by the Provincial Minor Use Pesticide Program (C. Bedard, British Columbia Ministry of Agriculture, personal communication). Sprinklers distributed the product in 6-7 minutes. Since the whole field was treated as a demonstration we did not have any data for statistical comparison.

2008 Results and Discussion:

Oregon Farm 1 and 2: At both Oregon farms the application of Entrust either via chemigation or through a hand-pump sprayer resulted in a significant increase in the proportion of dead or sick fireworm larvae compared to the Control (Fig. 1; Farm 1 – $t = 3.16$, $df = 9$, $p = 0.012$; Farm 2 – $F_{2,12} = 46.69$, $p < 0.001$). At Farm 2, both Pyganic and Entrust treatments had significantly more dead and sick larvae than the Control, with no difference between these two treatments (Fig. 1). Results with Pyganic are surprising given that our other co-operator in Oregon has not had success in controlling fireworm with Pyganic. In this trial, however Pyganic was used at 2.6X the label rate, which could account for the improvement in efficacy. The average kill (dead or sick) of the single Entrust treatment, however, ranged between 50 and 60%.

B.C. Farm 1: There was significantly more mortality in Entrust plots than Control (Fig. 2; $t = -2.91$, $df = 8$, $p = 0.02$). However, the average mortality was only 41%. This low level of mortality is most likely a reflection of the dilution of Entrust when applied via the sprinkler. In preliminary small plot trials with a backpack sprayer, we observed 92% mortality of larvae following application of much lower rate of Entrust (48 g a.i/ha, i.e. a third the rate used for B.C. Farm 1 trial) applied with 500 mL of water. At B.C. Farm 1, the chemigation needed to run for 27 minutes in order for the product to reach the furthest set of sprinklers. This is the minimum duration this farm has used in the past for applying conventional pesticides, e.g. Diazinon. Our findings suggest that products like Entrust

would need to be applied for a much shorter duration via chemigation in order to achieve the levels of efficacy needed to justify the product costs.

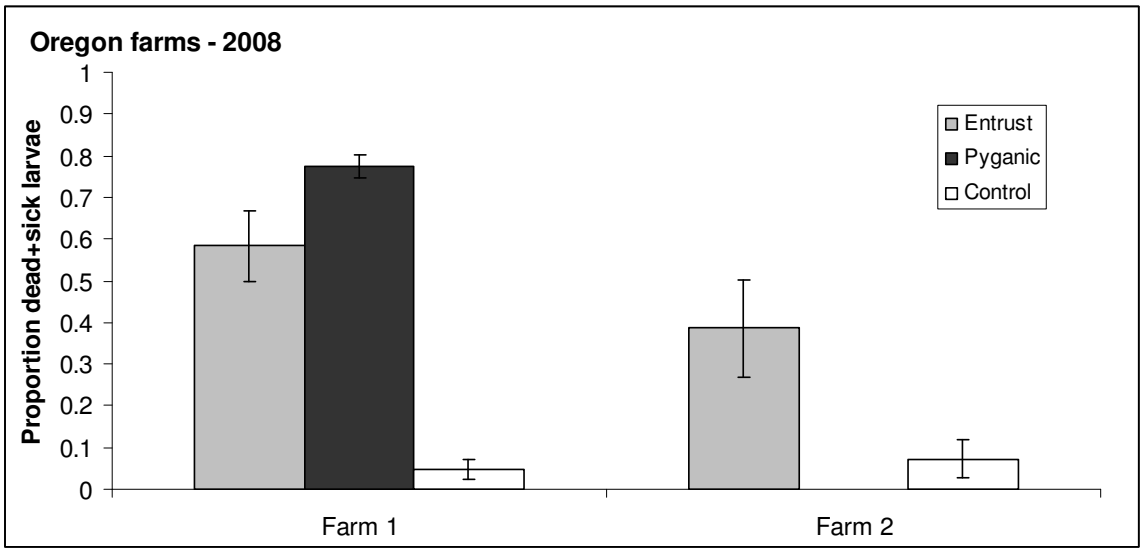

Figure 1. Effect of OMRI approved insecticides Entrust and Pyganic on the proportion of dead or sick blackheaded fireworm larvae, 72 h after treatment at Oregon Farm 1 and 2. Each bar represents the mean ± standard error of 5 replicates per treatment at each location.

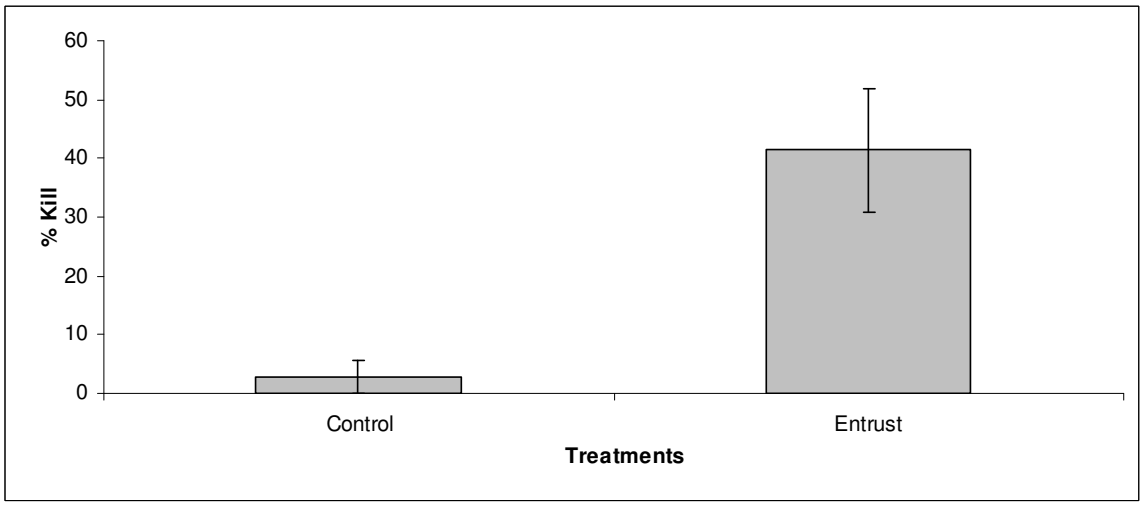

Figure 2. Effect of Entrust chemigation on survival of fireworm larvae at B.C. Farm 1. Bars represent the mean ± standard error of N=6 for Entrust and N = 4 for Control.

2009 Results and Discussion: Kill rates achieved at B.C. Farm 2 were the highest observed on any of the four farms - 70% after one treatment and 100% after the second treatment (Fig. 3). This field had a lower rate of product applied than B.C. Farm 1 (140 vs. 175 g a.i./ha), but higher than either Oregon farm. However, more importantly the duration of chemigation was the shortest at this farm – 7 minutes compared to 27 minutes at B.C. Farm 1 and 3+ hours at Oregon Farm 2. The duration of chemigation is

determined by many factors unique to each farm and field, including pump capacity, water pressure and size of fields. The trend in our findings suggests that a product like Entrust is most effective when applied with the least amount of chemigation. Excess chemigation dilutes the amount of active ingredient reaching the target pest. Our 2009 results also indicate that repeat applications with Entrust may be needed in order to reach satisfactory levels of control.

2010 Update: As of July 2010, Entrust has had a label expansion in Canada to include blackheaded fireworm, however at a much lower rate than tested in our trials - 87.4 g a.i./ha. Repeat applications (up to three) are recommended at 7-10 day intervals. A limitation however is that application via chemigation has not yet been approved. One of our Oregon growers has reported improved fireworm control (based on reduced trap catches of male moths) after implementing suggestions to reduce the duration of chemigation with Entrust.

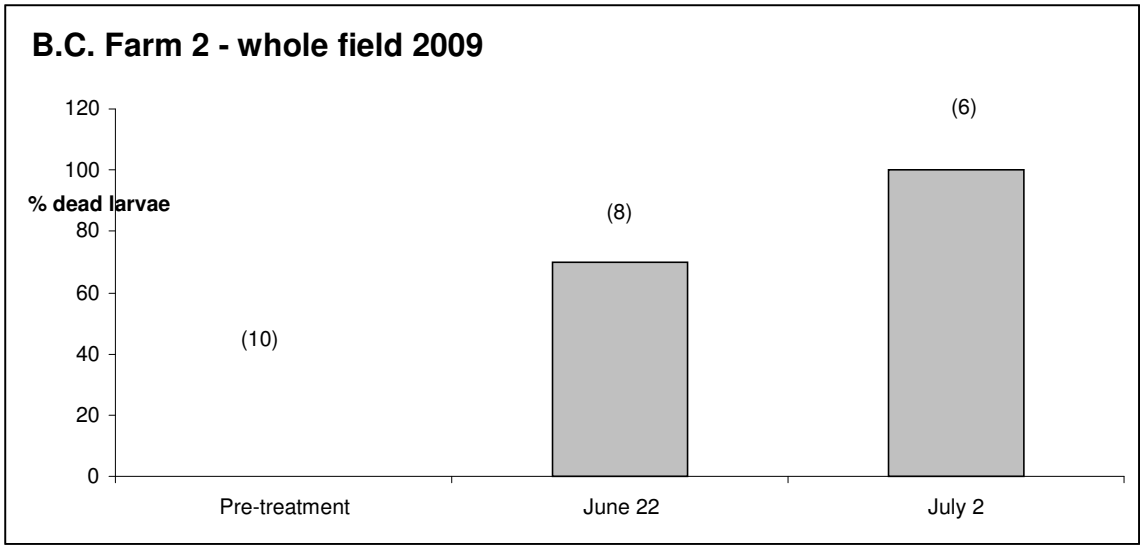

Figure 3. Fireworm larva mortality following two Entrust applications at B.C. Farm 2. Numbers above the bars indicate the number of hot spots checked to determine the % of dead larvae. The field was sprayed on June 15 and 25 and assessments took place seven days after each spray.

Objective 1b: Blackheaded fireworm tools: *Trichogramma sibericum*

Rationale: Blackheaded fireworm has two generations in the Pacific Northwest, with the first larval generation emerging in late April from overwintering eggs. Adults of these first generation larvae lay eggs in June (Cockfield et al. 1994). The egg parasitoid, *Trichogramma sibericum*, has been shown to parasitize up to 93% of overwintering fireworm eggs when it is released in late summer at a rate of 800,000 females/acre (Henderson *et al.* 2002). Overwintering eggs do not hatch until the following spring, giving *T. sibericum* wasps enough time to find eggs. In contrast, fireworm eggs laid in June hatch within days and *T. sibericum* may not have enough time to find and parasitize fireworm eggs before they hatch. Thus we do not know how effective *T. sibericum* is

against the fireworm eggs that are laid in June. For organic production, having a tool to control this generation would be an added benefit since parasitism of June eggs will help reduce the number of larvae in July. Insecticide sprays, even organic ones, in late June and July could interfere with pollination.

2008 Methods: The trial was conducted at a small organic field in Delta, B.C. Twelve plots, approximately $1m^2$ identified with fireworm eggs, were located and flagged. There was at least 10 m of buffer between adjacent plots. *Trichogramma sibericum* were released into six of the areas, with *T. sibericum* pupae sprinkled directly onto the foliage at a rate of 1 million/acre. Seven days later parasitism of eggs was assessed in plots. Parasitism in plots was compared using Student's T-test and all data were analyzed using JMP-IN (SAS Institute, Chicago, IL).

2010 Methods: A field-wide release was undertaken on June 10, 2010, in a 30-acre field located in Delta, B.C.. The release areas were the north and east edges of the field, which was where the main areas of fireworm larvae activity (hotspots) were located during the first generation larval hatch. Ten days following release we assessed the level of parasitism around the two release edges and the two edges where releases had not been made (20 areas checked along each edge).

Results and Discussion: Releasing *T. sibericum* in June 2008 resulted in approximately 40% parasitism of June eggs (Fig. 4; $t = 0.329$, $df = 12$, $p < 0.001$). In June 2010, fireworm eggs were only recovered on the release edges (north and east) and, of these, 30% (or 4 out of 12 eggs) were parasitized. Although the total number of eggs recovered was low, the results from both years confirm that *T. sibericum* releases in June can result in parasitism of fireworm eggs. These results are a good indication that two sets of releases--June and again in late August--could be used as part of the organic management strategy for blackheaded fireworm.

In 2008, the timing of the release for June was based on observations of eggs in the field. Given that the first generation eggs hatch quickly, many of the eggs may not have been suitable for parasitism by the time wasps were released. Timing releases in 2010 was based on captures of male moths in pheromone traps (i.e. release following 2 consecutives weeks of male captures), however this approach did not result in higher rates of parasitism. Additional modifications such as an earlier release date (following the first male capture in traps for example), increased release rate (higher than 800,000 females/acre), or split releases in June may improve parasitism. These modifications, however, may also increase the costs of applying *T. sibericum*. Currently, there are no commercial suppliers of *T. sibericum* thus it is not possible to estimate costs. One of our grower cooperators (Oregon) has expressed interest in mass rearing local Trichogramma species collected from local fireworm populations. In November 2009, during a visit to the farms of our Oregon cooperators we found some evidence of parasitized fireworm eggs in organic cranberry fields. So this route (growers mass-rearing their own local Trichogramma species) may be possible, although would require substantial investment in research and development. Alternately, if *T. sibericum* can be found and confirmed in

Oregon then colonies from B.C. could be exported to Oregon to help start colonies for mass production.

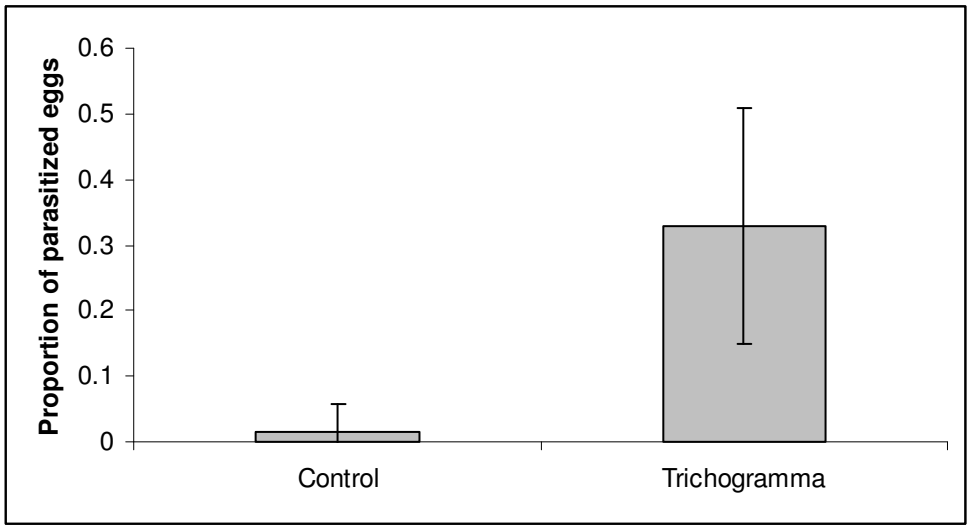

Figure 4. Comparison of blackheaded fireworm parasitism in plots with and without (Control) *Trichogramma sibericum* releases in June 2008. Bars are the mean ± standard error of N= 7 plots/treatment.

Objective 2a– Tools for Cranberry Girdler: *Metarhizium anisopliae*

Rationale: The larvae of the cranberry girdler (*Chrysoteuchia topiaria*) feed on roots of cranberry vines. Severe feeding damage can result in death of vines and patches of dead vines which are then difficult to re-plant as weeds can establish quickly. The cranberry girdler is difficult to control both conventionally and organically, in part due to inadequate monitoring tools for the cryptic larvae. There is one generation per year and since eggs are laid in the duff layer where larvae feed initially before moving into the root zone, it is challenging to deliver pest control products to these stages. We conducted small plot trials to assess the efficacy of *M. anisopliae* against the currently used tool, nematodes, for cranberry girdler control.

2009 Methods:

Girdler Collection and Rearing – Girdler moths were collected from cranberry farms on June 24 and July 3. Girdler moth presence became scarce in cranberry bogs early in July, and moths collected until then did not produce enough eggs required for this trial. Girdler moths were then collected from a blueberry farm in which a large population was present until mid-July. On June 30, July 4, 11, 18 and 21, girdler moths were collected from a grassy area adjacent to the blueberry field. Moths were collected on sunny days, with minimal wind, using a small hand held Insect Vac (Bioquip Products, Rancho Dominguez CA). In order to collect mostly female moths, the hose of the insect vac was kept low in the cranberry or grass canopy as females tend to be weaker fliers than males. All moths were placed in an oviposition cage (on loan from AAFC – Agassiz) to collect eggs. Moths were fed a dilute honey and water solution via a cotton wick. Eggs were collected and once they turned orange (indicating viability) were placed in the fridge in

order to stockpile enough eggs to run the trial. Eggs were held in the fridge for up to one week before running trials. Adult moths placed in oviposition cages were pinned and submitted to confirm identification.

Plants & Experimental Units – The trial was conducted using reed canary grass (*Phalaris arundinacea*), which has been used successfully for rearing girdler larvae (Fitzpatrick *et al.* 2001, Fitzpatrick 2007). Grass seeds were planted into 4-inch and 6-inch pots using Sunshine Mix #1 as the growth medium. Pots were kept in a greenhouse (UFV – Chilliwack campus) with a regular watering and fertilizer schedule. The experimental unit for the trial was a 4-inch pot stacked on top of a 6-inch pot. This was to ensure that if larvae moved out of the 4-inch pot they would have a ready source of food and hopefully prevent escape from mesh bags (see below). The pair of pots was placed inside an acetate sleeve (made by taping together two overhead transparency sheets). The pots and acetate sleeve were then placed inside a fine mesh bag (made out of drapery sheer fabric) and the opening to the bag was folded over and clipped shut. The mesh was fine enough to prevent larvae from escaping and predators from entering but we were able to water plants through the mesh bag. Plants were watered every other day unless otherwise needed.

Egg infestation –Approximately 70 girdler eggs were added to the 4-inch pots. Orange eggs were counted out under a dissecting microscope using a fine paintbrush. Eggs were gently brushed onto a piece of paper and then sprinkled on the surface of the soil in each pot. Blades of grass were pushed aside to ensure that eggs landed on the soil surface. A thin dusting of potting mix was sprinkled over each pot, after eggs were added. All pots were thoroughly watered prior to adding eggs and pots were not watered again until 72-h later. This was to ensure that eggs weren't disturbed or washed out of pots by irrigation water. All pots were kept under shade cloth in the greenhouse (average day-time temperature was 29^{o}C). Although the greenhouse temperature was very hot, we did not place pots outside because in our previous attempts to run this trial, pots kept outdoors become heavily infested with predators (including spiders and rove beetles) even when pots were protected by an enclosure. Haase-Statz (1997) reported that rove beetles can eat cranberry girdler eggs.

Treatment Application & Study Design - Although our original proposal was to test the efficacy of Metarhizium and nematodes at different application timings, we did not have enough girdler eggs to do this. Thus we picked one time interval –three weeks after egg infestation - to run our trial. Treatments were randomly assigned to experimental units. Pots received treatments 3 weeks after egg infestation. The trial was done in two time blocks or runs. For the first run (Run 1) we had 12 replicates for each treatment and for the second run (Run 2) we only had 6 replicates for each treatment. The total number of replicates was higher than what we originally proposed (5 replicates/treatment) because, after consulting with Dr. Sheila Fitzpatrick (Agriculture and Agri-Food Canada – Agassiz), we felt that more replicates were needed as results were expected to be highly variable.

Nematodes (*Steinernema carpocapsae*, Becker Underwood, Inc., Ames, IA) were applied at a rate of 3 billion/acre and Metarhizium (Met52) granules at a rate of 60 kg/ha (the highest rate used in Booth et al. 2000). The surface areas of both the 4-inch and 6-inch pots were used to calculate the total treatment surface area ($0.31m^2$) for experimental units/plots. Following application of both treatments, pots were watered with a small amount of water to help spores and nematodes move into the soil, but not to run off (water coming out of the bottom of the pot). Control plots also received a small amount of water. All treatments were randomly assigned and after treatments were applied, plots were placed back on greenhouse benches in a random order.

Assessment & Data Analysis – To determine the impact of treatments on girdler larvae survival, plots were assessed 5 weeks (Run 1) and 8 weeks (Run 2) after treatment. Table 1 summarizes the timing of trial events for each run. The soil from both the 4 and 6-inch pots were sifted through by hand and the bag was examined carefully for any escaped (and dead) larvae. When the grass and soil was removed from pots we noticed that some samples had heavy tunnelling in the roots. This tunnelling was associated with girdler feeding (i.e. when live or sick girdler larvae were found they were in tunnels). Thus we also recorded whether a sample had tunnelling or not.

Table 1. Summary of the timing of trial activities for the two time runs.

	Moths collected	Infestation of pots with eggs	Application of treatments	Assessment
Run 1	July 3, 4, 11	July 16	Aug 6	Sept 10 (5 weeks)
Run 2	July 18, 21	July 28	Aug 18	Oct 13 (8 weeks)

As very few larvae (live or dead) were recovered upon completion of this study we scored data categorically based on two responses – 1) larvae and/or tunnels observed and 2) no larvae or tunnels observed. Data were analyzed using a 3 X 2 contingency table with 3 levels of X-variable (Control, Metarhizium and Nematode) and 2 levels of response variable (larvae/tunnels observed or not). Analysis was conducted with JMP-In Version 4.0.3 (SAS Institute).

2009 Results: Overall very few live or dead larvae were recovered from samples at the end of the trial (Table 2). One possible reason why very few larvae were recovered despite infesting pots with approximately 70 eggs was due to poor quality of grass. Although grass plants were initially quite lush and green at the start of the trial (egg infestation and treatment application), grasses began to die in some pots, possibly due to greenhouse temperatures and/or becoming root bound in pots. Waiting 5 and 8-weeks for post-treatment assessment may have been too long and larvae may have escaped prior to assessment. Another possible reason why very few girdler larvae were recovered from pots may be that few of the eggs survived transfer. Because of the low number of larvae recovered we decided to combine the larva counts and tunneling data for analysis.

Fewer Metarhizium-treated pots had observations of larvae and/or tunnelling (Table 2; Fig. 5); however these differences were not statistically different from the untreated control. Using either Metarhizium or nematodes did not result in a significant reduction in the number of larvae or tunnels observed in samples at the end of the study (Fig. 5; Likelihood Ratio $\chi^2 = 4.73$, df = 2, p = 0.094). However, it must be stressed that this trial failed as a demonstration of the efficacy of either product because we had such poor survival of girdler larvae in our control plots. Thus our findings should not be used to make conclusions on the efficacy of either product.

Table 2. Summary of the number of girdler larvae recovered from pots and number of pots where larvae and/or feeding tunnels were observed (N = 18 for each treatment)

	No larvae or tunnels observed	Larvae and/or tunnels observed	Mean number of larvae (live or dead) (± s.e.)
Control	8	10	0.17±0.09
Metarhizium	13	5	0.05±0.06
Nematode	7	11	0.33±0.11

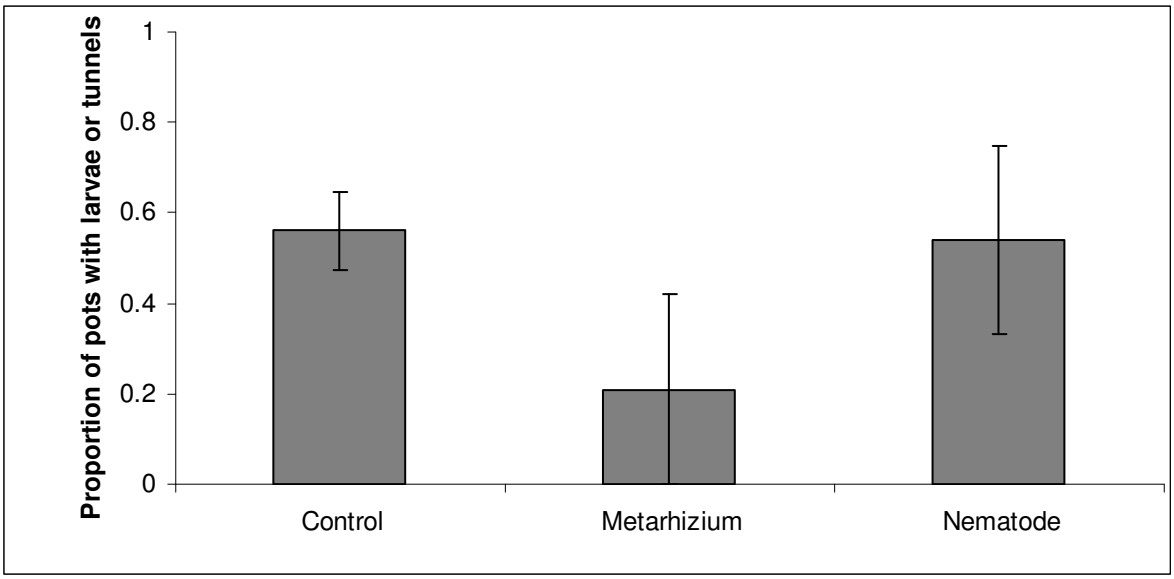

Figure 5. Effect of the biological treatments *Metarhizium anisopliae* (Met52) or nematode (*Steinernema carpocapsae*) on proportion of pots with either live or dead girdler larvae and/or tunnelling in the roots of host plants. Bars represent the mean (± s.e.) of two runs of the trial.

2010 Methods:
Moth and egg collection - Cranberry girdler adults were collected from commercial cranberry fields in Pitt Meadows, Langley and Richmond, B.C. from June 26 to July 13,

2010. Moths were collected directly from foliage and into 100 ml vials (1 moth per vial). Initially moths were placed inside a rearing cage with a sugar and water solution to promote egg laying (Fitzpatrick et al. 2001). However, we noticed that many of the moths collected on later dates (June 29 and onwards) laid eggs within a day of capture directly into the vials. Thus we modified our egg collection approach - leaving moths inside their vials for 24 to 48 h during which time eggs were laid. A major advantage of this approach for collecting eggs is that we avoided overstocking the rearing cage with males and thus having poor egg production in rearing cages. Vials where the majority of eggs turned orange were then placed in the fridge (most females by this time had died and could be easily removed). This is was another advantage of using the vials for oviposition - less handling of eggs to separate fertilized (orange) and unfertilized (white) eggs from each other. The eggs used for the trial were from three different batches of moths collected on 1) June 30 to July 1, 2) July 7 to July 8 and 3) July 12 to July 14. These eggs were placed in the fridge for 24 to 72 h prior to being used in the trial.

Preparation of test plots and infestation with girdler eggs - For this trial we had two types of test plots - cranberry microplots (Fig. 6a) and 1 m X 1m "field" plots that were inside our research cranberry beds (Fig. 6b). All of the plots were located outdoors in Abbotsford, B.C., and exposed to ambient environmental conditions. Microplots consisted of 9 cm diameter (0.64 m^2) plastic cup planted with cranberry plugs (roots and shoots) and filled with potting media (Sunshine Mix 1). To increase the root material available for girdler larvae to feed on we also sprinkled 2 grams of reed canary grass seeds on the surface of the soil. Cranberry plugs were planted into cups 21 days prior to the start of the trial and grass seeds added 2 days prior. The soil in microplots was watered so that the soil was moist, but not saturated, prior to the introduction of eggs. There were no drainage holes in the cups. Subsequently, microplots were irrigated carefully to keep soil evenly moist but standing water was avoided in order to prevent girdler larvae from drowning (Fitzpatrick 2007).

Research cranberry (var: Stevens) beds were four years old at the time of the trial. Vines were obtained from a commercial cranberry farm. No girdler damage had been observed in these beds since they were planted. The individual field plots were marked off with flagging tape and the area where eggs were added to plots was marked with a flag. We selected an area within each field plot where vine establishment was healthy and upright growth was lush. Field plots were irrigated for two hours prior to the start of the trial and then for approximately 30 minutes every two to four days after depending on weather and rainfall.

Figure 6. A (Left): Microplots - cranberries were planted in cups 3 weeks prior to start of trial. After treatment and egg infestations cups were dug into research cranberry beds. B (Right): "Field" plots placed inside research cranberry beds. Cranberry girdler eggs were sprinkled in the area around flags.

Girdler eggs were gently sprinkled over the entire soil surface of microplots and within a 10-cm diameter area around each flag in field plots (Fig. 6b). We used 90 eggs/microplot and 75 eggs/field plot. We used such a large number of eggs for infestation, even for our relatively small microplots, because our plots were open to other arthropods and we were concerned that egg predation from rove beetles (Haase-Statz 1997) and other predators like centipedes (Mahr 2005) could occur. Previously we conducted this trial in a greenhouse to avoid the predator problem, but even in a greenhouse beetles and centipedes managed to infest pots (R. Prasad and M. Soto, personal observations, September 2009).

Girdler eggs were handled with a fine paint brush, counted out under a microscope and transferred to wax paper envelopes from which they were sprinkled. Thus the physical handling off eggs was kept to a minimum. There were two rounds of egg infestation based on treatment (see below). For microplots the first and second rounds of egg infestation occurred on July 5 and July 11. In the field plots, the first and second rounds of egg infestation occurred on July 10 and July 16.

Application of treatments - Originally our work plan was to test the granular formulation of *Metarhizium anisopliae* Met52 (Novozymes Biologicals Inc., Salem VA) at two rates: 60 kg/ha (used by Booth et al. 2000) and 120 kg/ha. However in discussion with Novozymes a newer emulsifiable concentrate (EC) formulation of Met52 was recommended for the trial (J. Leland, Novozymes Biologicals, Inc., personal communication June 2010). Met52 EC is five times more concentrated than the granular formulation and the rates recommended for this trial were 12L/Ha and 24L/Ha. For the lower (1X rate) this worked out to be 0.76 ml of product in 50 ml of water for the microplots (0.64 m^2) and 1.2 ml of product in 3 L of water for the field plots (1 m^2). For the higher (2X rate) this worked out be 1.5 ml of product in 50 ml of water for the microplots and 2.4 ml of product in 3 L of water for the field plots. For control microplots only 50 ml of water was added and 3 L of water was added to control field plots.

Treatments were applied at two different time intervals - one week before eggs were added to plots and on the same day that eggs were added to plots. Microplots were all treated with Met52 on July 5. Thus the trial consisted of four combinations of rate X application timing and a control treatment for a total of five treatments:

1) 1X rate - at egg infestation (eggs added July 5 (microplots) & July 10 (field))
2) 1X rate - one week before egg infestation (eggs added July 11 (microplots) & July 16 (field))
3) 2X rate - at egg infestation (eggs added July 5 (microplots) & July 10 (field)
4) 2X rate - one week before egg infestation (eggs added July 11 (microplots) & July 16 (field))
5) Untreated control (eggs added July 5 (microplots) & July 10 (field))

Each treatment was replicated six times for a total of 30 microplots and 30 field plots. After treatment microplots were planted into field plots with the same treatment regime. Cups were buried so that 3 cm of the top of the cup was above the ground. This ensured that microplots were exposed to the same environmental conditions of the field plots but hopefully minimized predator movement into cups. Field plots were treated on July 10.

Assessment - Microplots were assessed on August 31, 8 weeks after they were infested with girdler eggs. Plots were assessed by turning over cup contents and sifting through soil and roots to find frass (Fig. 7) and larvae. Field plots were assessed on September 6, when larvae are still expected to be active and 8 weeks from when eggs were added to plots. Plots were assessed by removing a 40 cm x 40 cm x 5 cm deep patch of soil around the area where eggs were released. These soil samples were then sifted through by hand and signs of frass, root feeding or girdler larvae were recorded.

Figure 7. Cranberry girdler frass (light brown granules in middle of soil) was easily detected from the potting media and plant roots in microplots.

Analysis of data - Only microplot data were analyzed as only one girdler larva was recovered from field plots. The impact of the four different Met52 treatments on the recovery of girdler larvae was analyzed using one-way ANOVA. Since frass was also

recovered from microplots we also analyzed these data, in combination with larval counts, using a 5 X 2 contingency table with presence/absences of frass + live larva(e) as our two levels of response variable and treatments as five levels of the X-variable. Data were analyzed using JMP-IN (Version 5.1, SAS Institute Chicago, IL).

Direct mortality bioassay (preliminary trial) - The four larvae recovered from the control plots were used for a very preliminary Petri dish bioassay to determine if Met52 can cause direct mortality to girdler larvae. We followed the soil bioassay protocol provided by Novozymes Biologicals, but at a much smaller scale and only at a single rate (the highest). To 100 g of sterile moist soil we added 1 mL of Met52 EC. The soil was then mixed and placed in a container and a single girdler larva was added. This protocol was repeated twice for a total of two larvae in two dishes with Met52. For the control treatment a single larva was placed in a container with 100g of moist sterile soil. There were two replicates of the control treatment. All four containers were sealed with tape and placed individually in Ziploc bags to minimize movement of spores. Containers were kept at under low light conditions at 19± 1^{o}C and were checked after 7 and 10 days. Soil was checked every other day but remained moist throughout the trial so no additional water was required. All larvae used for the bioassay were obtained from the control microplots - thus had no prior exposure to Met52 and were approximately 1 cm to 1.5 cm in length at the time of the trial.

2010 Results: Overall the number of larvae recovered was quite low and there were no significant treatment effects on the number of live larvae recovered from microplots (Fig. 8; $F(4,25) = 1.41$, $p = 0.26$). When we examined the combined response of frass + larvae we still did not see a significant effect of Met52 treatment on reducing cranberry girdler activity (Table 3; Likelihood Ratio $\chi^2 = 8.21$, $df = 4$, $p = 0.084$). Although not significant, the overall trend is for fewer larvae recovered in microplots treated with Met52 either prior to egg infestation or at 2X the rate compared to the untreated control. Further, in our preliminary bioassay we saw indications of *M. anisopliae* infection on the two larvae exposed to Met52 in the soil bioassay--both larvae were dead after 7 days and covered with fuzzy grey-green fungal growths. In contrast the two control larvae were either still alive or dead but not covered with any fungal growths.

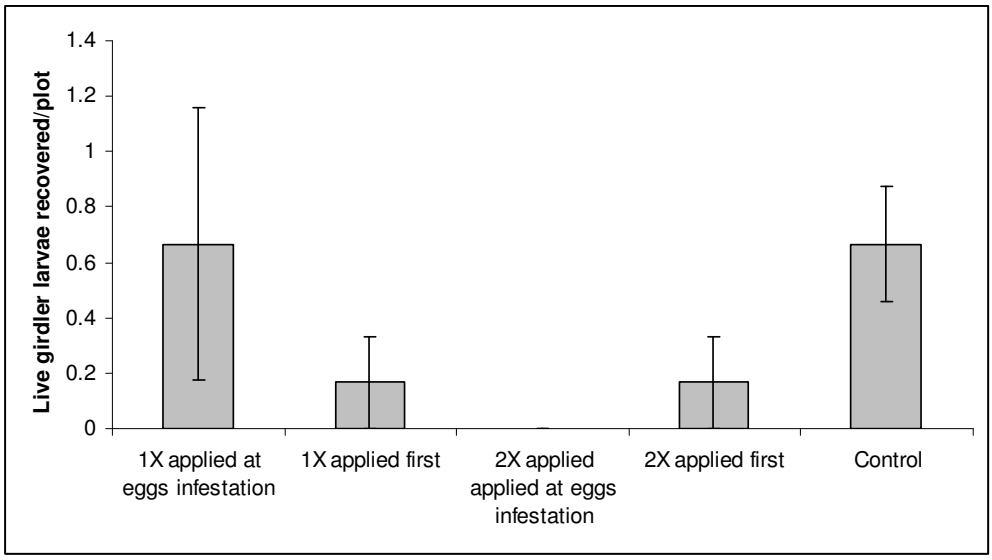

Figure 8. Effect of rate and application timing of *Metarhizium anisopliae* (strain F52) on the recovery of cranberry girdler (*Chrysoteuchia topiaria*) larvae from cranberry microplots. Bars represent the mean (± s.e.) of 6 replicates/treatment (total N = 30).

Table 3. Effect of rate and application timing of *Metarhizium anisopliae* (strain F52) on the recovery of cranberry girdler (*Chrysoteuchia topiaria*) larvae and/or frass from cranberry microplots.

Treatment	**Proportion of pots with frass + larvae (#pots out of 6)**	**Proportion of pots with at least one live larvae (#pots out of 6)**	**Average number of larvae recovered**
Control (eggs only)	0.83 (5/6)	0.67 (4/6)	0.67 (4 live larvae total)
1X Met52 applied before eggs	0.17 (1/6)	0.17 (1/6)	0.17 (1 live larvae total)
1X Met52 applied on same day as eggs	0.33 (2/6)	0.33 (2/6)	0.67 (4 live larvae total)
2X Met52 applied before eggs	0.67 (4/6)	0.17 (1/6)	0.17 (1 live larvae total)
2X Met52 applied on same day as eggs	0.17 (1/6)	0	0

Summary Discussion: In our 2009 trials conducted with the granular formulation of Met52, we also saw a trend towards fewer cranberry girdler larvae in Met52 treated pots than the untreated control but this trend was not statistically significant. Our hope was that the EC formulation of Met52 would be better at penetrating to the root zone of cranberries and we would see better control of cranberry girdler. Unfortunately, we did

not see this with our 2010 results. Again the trends, in 2010, do indicate that Met52 applied either a week prior to egg laying or at 2X the rate increased mortality of larvae.

Since the original work of Booth and Shanks (1998) and Booth *et al.* 2000 there have been no published reports, to our knowledge, examining the impact of *M. anisopliae* on cranberry girdler. This is surprising since cranberry girdler is also an important pest of sod and turf (Booth and Shanks 1998) and earlier crop profiles for cranberries have Metarhizium for girdler control listed as research priorities (e.g. Wisconsin 1998 http://www.ipmcenters.org/cropprofiles/docs/wicranberries.pdf and New Jersey 1997 http://pestmanagement.rutgers.edu/njinpas/CropProfiles/cranberryprofile.pdf).

In their original work, Booth and Shanks (1998) observed a significant reduction in emergence of male girdler moths from plots treated the summer previous with a very high rate of a local strain of *M. anisopliae* (200g of granules/m^2). In follow up work conducted with an improved application process for *M. anisopliae,* a lower and more affordable rate of granule application (6 g/m^2) was used (Booth *et al.* 2000). At one field site there were significantly fewer larvae in the *M. anisopliae* treated plots than the control (see Table 4 in Booth *et al.* 2000). But in the remaining three of the four field sites tested, there were no significant differences in the number of larvae between treated and control plots – but, similar to our results, some sites did have fewer larvae in *M. anisopliae*-treated plots than the control.

Thus the previous work provides indications that Met52 or other strains of *M. anisopliae* may be effective against cranberry girdler but the results have not been robust. Application timing, rate, and delivery of product to the target area may all be factors that could be causing the inconsistent results with *M. anisopliae* for girdler control. Cranberry girdler is a challenging pest to work with and this may be another reason why very few published studies have followed up on the findings of Booth and Shanks (1998).

Objective 2b – Tools for Cranberry Girdler: Nematodes – duration of irrigation

Rationale: While nematodes should be effective for girdler control as moist soil conditions of the bed provide a suitable habitat, results vary (D. Henderson, unpublished). Experiences of both B.C. and Oregon growers confirm that girdler control with nematodes is not always consistent. A major concern is how long to irrigate in order to get nematodes through the crop canopy and the duff layer to the root zone. Not irrigating enough will result in the majority of nematodes remaining in the foliage. The objective of this trial was to compare the amount of nematodes reaching the soil zone after 1, 2, or 4 hours of irrigation.

2008 Methods: We placed 500mL collecting cups out in three farms during the day prior to the nematode application. Cups were placed around three randomly selected sprinklers at intervals of 1 m from the sprinkler. At each distance there were four cups: one in the canopy and three buried to be flush with the soil surface. Cups were then collected at three time intervals after the start of nematode application via sprinklers. The canopy cup and one of the buried cups were collected after one hour, a second buried cup was

collected after 2 hours of irrigation, and the third buried cup was collected after 4 hours of irrigation (at Farm 1 and 3) and 7 hours (at Farm 2). The cups were collected and returned to the lab, where they were held in the refrigerator until they were assessed.

The total volume of water in each cup was measured and then upper layer of water was poured off as nematodes sink when they die. In none of the cups did water volume exceed cup volume (i.e. none of the cups were full to the rim when collected). The remaining water in each cup was poured into a 9mm Petri dish so that there was 3 mm deep layer of water in each Petri dish. The bottom of the Petri dishes were scored into a grid with $1cm^2$ cells. The number of nematodes in 3 randomly selected $1cm^2$ cells was recorded. We examined the effect of irrigation duration and distance from the sprinkler on the number of nematodes washing down to soil level. This was calculated relative to maximum number of nematodes released via irrigation (i.e. the number of nematodes in the canopy after 1hr of irrigation at each distance).

2009 Methods: A tray containing 250 million nematodes was mixed with 31.5 L of water (following label recommendations) and applied through chemigation to a 0.3 acre cranberry field in Richmond, B.C. Sprinklers distributed the nematode solution in approximately 7 minutes. Prior to nematode application, the field was irrigated for 1 hour and collecting cups were distributed in the field after the pre-treatment irrigation but before nematodes were applied. Two sprinkler heads from the irrigation system were randomly selected following nematode dispersal. Three different distances from each sprinkler head were selected for collection of nematodes for a total of 6 "plots". In each plot, 5 collecting cups (500 ml or 10 cm deep) were buried (for a total of 30 cups) so that their rims were flush with the ground surface and then covered with cranberry vines.

Following nematode application, irrigation ran for 2 hours. One cup from each plot was removed at five different time intervals: immediately after nematode application, and then 0.5 hr, 1 hr, 1.5 hr, and 2 hr after irrigation. As in the 2008 trial none of the cups were filled to the rim at collection. Cups were taken to the lab and kept refrigerated until they were assessed. Three samples of 1 mL of fluid was extracted from each cup. The number of nematodes in each 1mL sample was counted and the total number of nematodes per 3 mL of sample solution recorded.

2008 Results and Discussion: Cups collected from Farm 3 were too dirty to accurately count nematodes and so no results are available for that farm. At neither Farm 1 nor 2 did the distance of collecting cups from the sprinklers have any impact on nematode density so we were able to pool the data from different distances and focus on duration of irrigation. The density of nematodes was always highest in the canopy after 1 hour of irrigation. The highest density of nematodes in the soil was found after 1 hour of irrigation. The irrigation effect was strongest at Farm 1 (Fig. 9A, $F_{2,48} = 5.84$, $p = 0.005$) and although not significant at Farm 2 (Fig. 9B, $F_{2,45} = 0.15$, $p = 0.86$), there was still a trend indicating that most nematodes were caught 1 hour after irrigation.

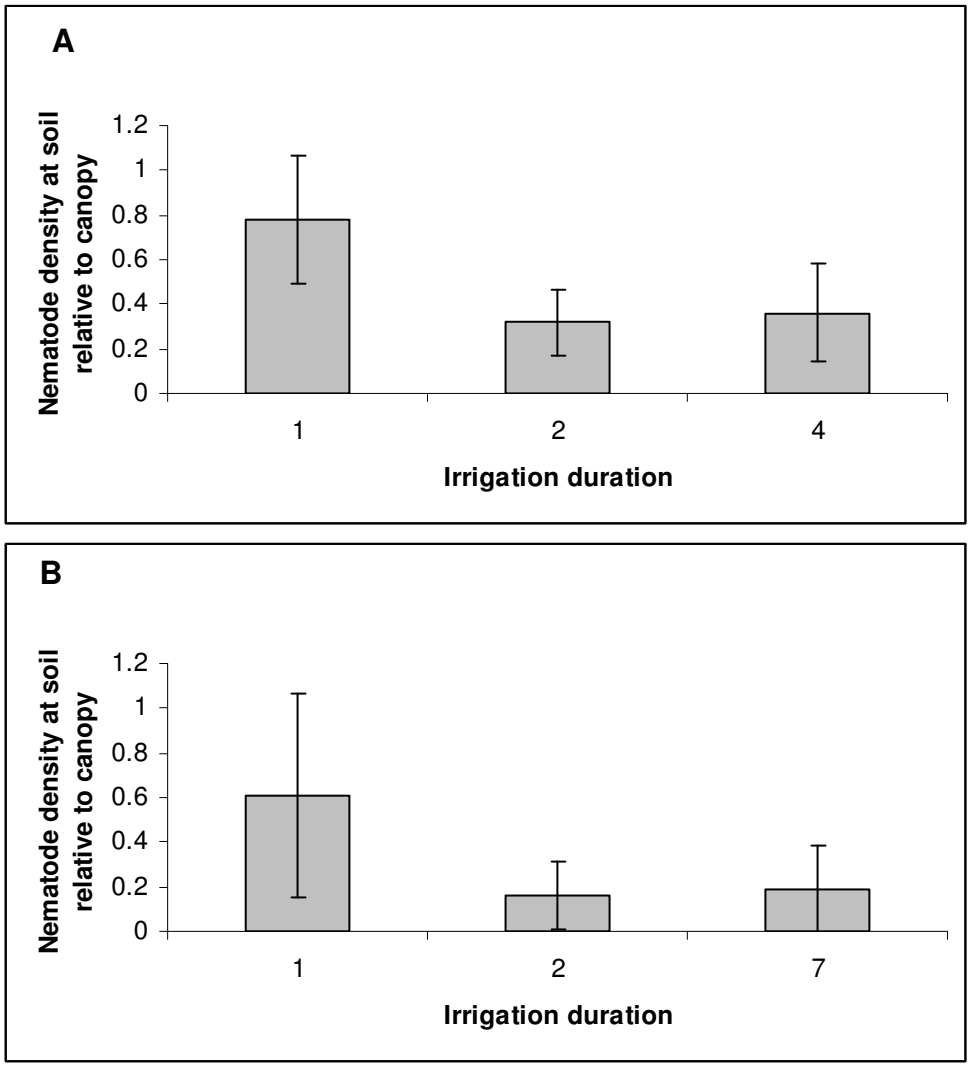

Figure 9. The effect of irrigation duration on the density of nematodes at soil level in A) Farm 1 and B) Farm 2. Nematode density was measured as the proportion of nematodes at soil level after each time interval compared to the nematode density in the crop canopy after one hour of irrigation. Bars are the mean ± standard error of N= 18 cups for each irrigation duration.

2009 Results and Discussion: Our results in the second year of the trial indicated that one hour of irrigation following nematode application was sufficient to wash the nematodes down into the root zone (Fig. 10; $F_{4,24} = 3.14$, $p = 0.03$). After only 30 minutes of post-treatment irrigation, the number of nematodes in the root zone was not significantly different from the amount immediately following treatment (0 minutes of post-treatment irrigation). A general recommendation following nematode application via chemigation is to irrigate daily to ensure that soil remains moist to allow for maximum nematode survival (D. Henderson, Kwantlen Polytechnic University, personal communication). However the duration that growers irrigate immediately following nematode application varies considerably. Our results suggest that an hour is sufficient for washing nematodes

off of foliage and to the soil zone. In sufficiently moist soil, nematodes would then be able to locate pests.

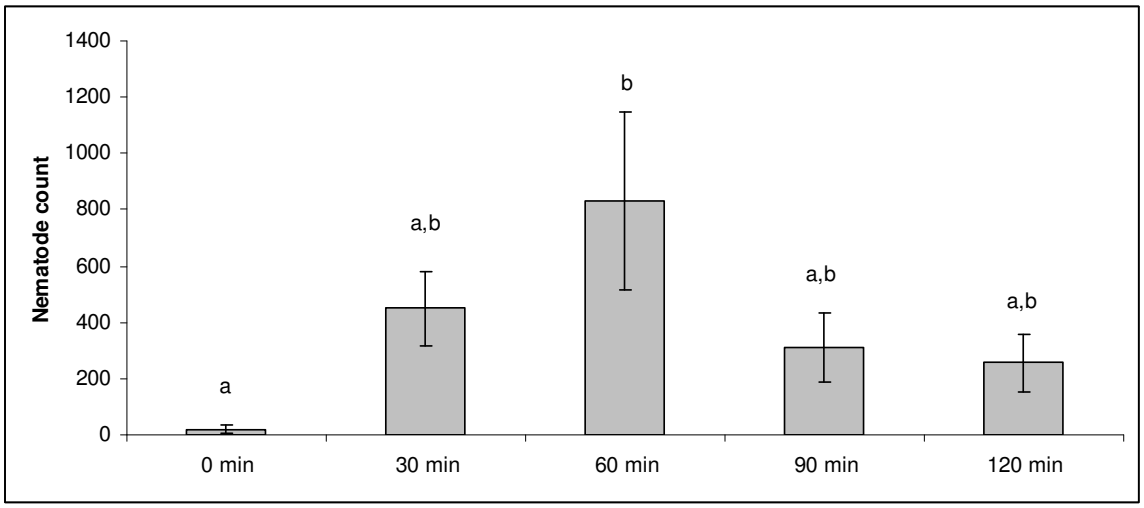

Figure 10. Effect of post-treatment irrigation duration on the number of nematodes found at soil level. Bars represent the mean ± standard error of 6 samples for each duration (5 samples for 30 minutes). Bars with the same letter are not significantly different from each other based on Tukey-Kramer HSD ($\alpha = 0.05$).

Objective 3a –Tools for black vine weevil: Nematodes

Rationale: In several studies, levels of black vine weevil control using different *Heterorhabditis* species of nematodes were comparable to that achieved using broad-spectrum insecticides (Fitters et al. 2001). In previous cranberry studies, a 96 to 100% reduction in black vine weevil larvae was achieved using either *Steinernema carpocapsae* or *S. glaseri*; but this study did not include *Heterorhabditis* species (Booth *et al.* 2002). Heterorhabditids are thought to be better suited for control of sedentary species like black vine weevil larvae because they actively search or cruise in the soil. A comparison with both Steinernemid and Heterorhabditid nematodes in the cranberry system is lacking.

2008 Methods: The study was carried out in 20 plots of potted cranberries, each measuring 0.9 m². Black vine weevil larvae were collected from strawberry fields in May and each cranberry plot was infested with 20 of these larvae. The following treatments were used in the plots and each had 4 replicates:

- Control (C)
- *Heterohabditis bacteriophora* (Hb)
- *Steinernema carpocapsae* (Sc)
- *Steinernema kraussei* (Sk)
- *Steinernema carpocapsae* + *Heterohabditis bacteriophora* (Hb + Sc)

In the weeks prior to treatment, plots were watered daily to keep the soil moist. In addition they were individually irrigated for 1 minute prior to treatment (enough to moisten the soil without there being any runoff from the plots). Control plots were treated

with 100ml water. Each nematode plot was treated with the label recommended rate. All nematode species were packaged in trays containing 6 million nematodes.

- Hb: A tray was diluted into 0.85 L of water and 9 mL of solution were added to plot.
- Sc: A tray was diluted into 7.5 L of water and 8.5 mL of solution were added plot.
- Sk: A tray was diluted into 45 L of water and 337 mL of solution were added to plots.
- Hb + Sc: Using the solutions mentioned above, each plot was treated with half of the recommended rate of each Hb and Sc (i.e. 4.25 mL of Sc and 4.5 mL of Hb) for a 50/50 combination.

Nematodes were mixed with water and constantly stirred during application to ensure an even concentration of nematodes in the solution. Required solution amounts were measured and applied with syringes. Plots were irrigated for 1 minute after treatment to rinse nematodes off the top of the vines and work them into the soil. Conditions during treatment were overcast and 14-16 ° C. Three weeks after treatment plots were taken apart in search of weevils. Each plot was assessed for 0.5 hour. The recovered weevils were assessed (dead, alive, sick) and their development stage (larva, pupa, adult) were recorded. The effect of nematode treatment on the proportion of sick and dead individuals was analyzed using one-way ANOVA, with proportion data arc-sine transformed prior to analysis. Post-hoc means comparisons were done using Tukey-HSD ($\alpha = 0.05$). All data were analyzed using JMP-IN (SAS Institute, Chicago, IL).

2008 Results: The two treatments containing the nematode *Heterohabditis bacteriophora* (i.e. Hb and Hb+Sc) had significantly more sick or dead individuals than the Control treatment (Fig. 11; $F_{4,15} = 6.00$, $p = 0.0043$). All other treatments were not significantly different from each other or the control. Many cranberry growers are already accustomed to using nematodes for girdler control, however very few do so for black vine weevil. One issue may be timing – for girdler nematodes are applied during July prior to fruit ripening. For black vine weevil, larvae would be targeted in August when fruit is ripening and growers may not want to irrigate due to the risks of fruit rot. So while *H. bacteriophora* may be the most effective nematode, a species like *S. kraussei* (effective at lower soil temperatures) may be more practical as it could be applied in the spring when black vine weevil larvae resume feeding. On its own, S. *kraussei* did not perform better than the control, however combining it with *H. bacteriophora* has been suggested as away to achieve black vine weevil control either in fall or spring (D. Henderson, personal communication).

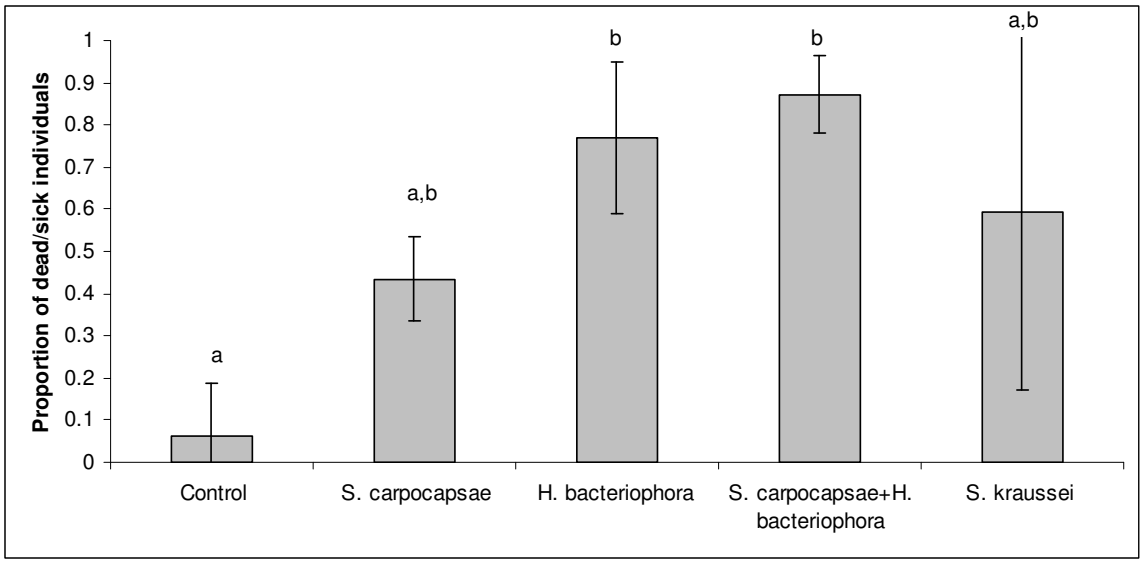

Figure 11. Effect of nematode treatments on the proportion of sick or dead black vine weevil individuals (larvae, pupae or adults) recovered from plots. Bars are the means ± standard error of N = 4 replicates per treatment. Bars with the same letter are not significantly different from each other based on Tukey-Kramer honestly significant difference means comparison.

2010 Methods: Based on our findings in 2008 and in conversation with cranberry specialists in southern Oregon, our 2010 trials focused on small plot spring applications of nematodes to areas of commercial fields with natural infestations of black vine weevil. The trial was conducted at two farms, one in Richmond, B.C., and one in Pitt Meadows, B.C., both with a history of black vine weevil damage.

In the spring, cranberry vines were peeled back manually to search for weevil larvae in sections of each field where weak or dying cranberries were seen. In weevil-infested patches the number of larvae was counted and a 2m² area was flagged around these hotspots. Hotspots where a minimum of 3 weevil larvae were seen were used as plots for this study. In the Richmond farm a total of 24 2m² plots were flagged in five different fields. The treatments applied here were water (untreated control), *H. bacteriophora*, *S. kraussei,* and a combination of *H. bacteriophora* & *S. kraussei.* Each treatment had six replicates. In the Pitt Meadows farm a total of 18 2m² plots were flagged along the sloped edge of one field. The treatments applied here were water (untreated control), *H. bacteriophora*, and *S. carpocapsae* (Table 4).

Plots were not irrigated prior to nematode application at either study site as the constant precipitation the previous week ensured sufficient soil moisture. We used the label-recommended rate for each species of nematodes (Table 4). Nematode trays were mixed with water and stirred constantly during application to ensure an even concentration of nematodes in the solution. Nematode solutions were applied via a 4-litre hand pump sprayer. Control plots were also sprayed with 0.2 L of water/plot. Weather conditions during and after application were 12-14°C during the day and overcast with light rain. The total number of live, sick, or dead larvae, pupae, or adults in each plot was assessed

one and two weeks after the last nematode treatment. Sickness or death due to nematodes was differentiated from other forms of sickness/death by the appearance of the insect body (swollen) and discharge upon squeezing (milky white) which is typical of nematode infection.

Table 4: Spring nematode treatments for the black vine weevil control study at Richmond and Pitt Meadows fields.

Active ingredient	**Fields and dates**	**Manufacturer**	**Label-recommended rate**	**Rate used in study plots**	**Minimum soil temperature for application**
Heterohabditis bacteriophora	Richmond (May 17) Pitt Meadows (May 18)	Becker Underwood	250 million / 350 m² in 35 L of water	1.5 million / 2m² in 0.2 L of water	54 ° F
Steinernema carpocapsae	Pitt Meadows (May 18)	Becker Underwood	3 billion / 4,050 m² in 378.5 L of water	1.5 million / 2m² in 0.76 L of water	57° F
Steinernema kraussei	Richmond (April 20)	Becker Underwood	250 million / 510 m² in 1,892.7 L of water	1 million / 2m² in 7.5 L of water	40° F
Steinernema kraussei + *Heterohabditis bacteriophora*	Richmond (April 20/May 17)	Becker Underwood	N/A	1 million / 2m² in 7.5 L of water 1.5 million / 2m² in 0.2 L of water	40° F/54 ° F

2010 Results: At both farms there was a significant reduction in the proportion of live individuals recovered from some of the nematode-treated plots compared to the water-only control. At the Richmond farm, spring applications of *H. bacteriophora* or the combination treatment of *H. bacteriophora* & *S. kraussei* were the most effective (Fig. 12A; $F_{3,20} = 6.51$, $p = 0.003$). At the Pitt Meadows farm we did not test the combined treatment but did see that both nematode species tested, *H. bacteriophora* and *S. carpocapsae,* provided equal control and were significantly more effective than the untreated control (Fig. 12B; $F_{2,15} = 5.67$, $p = 0.01$).

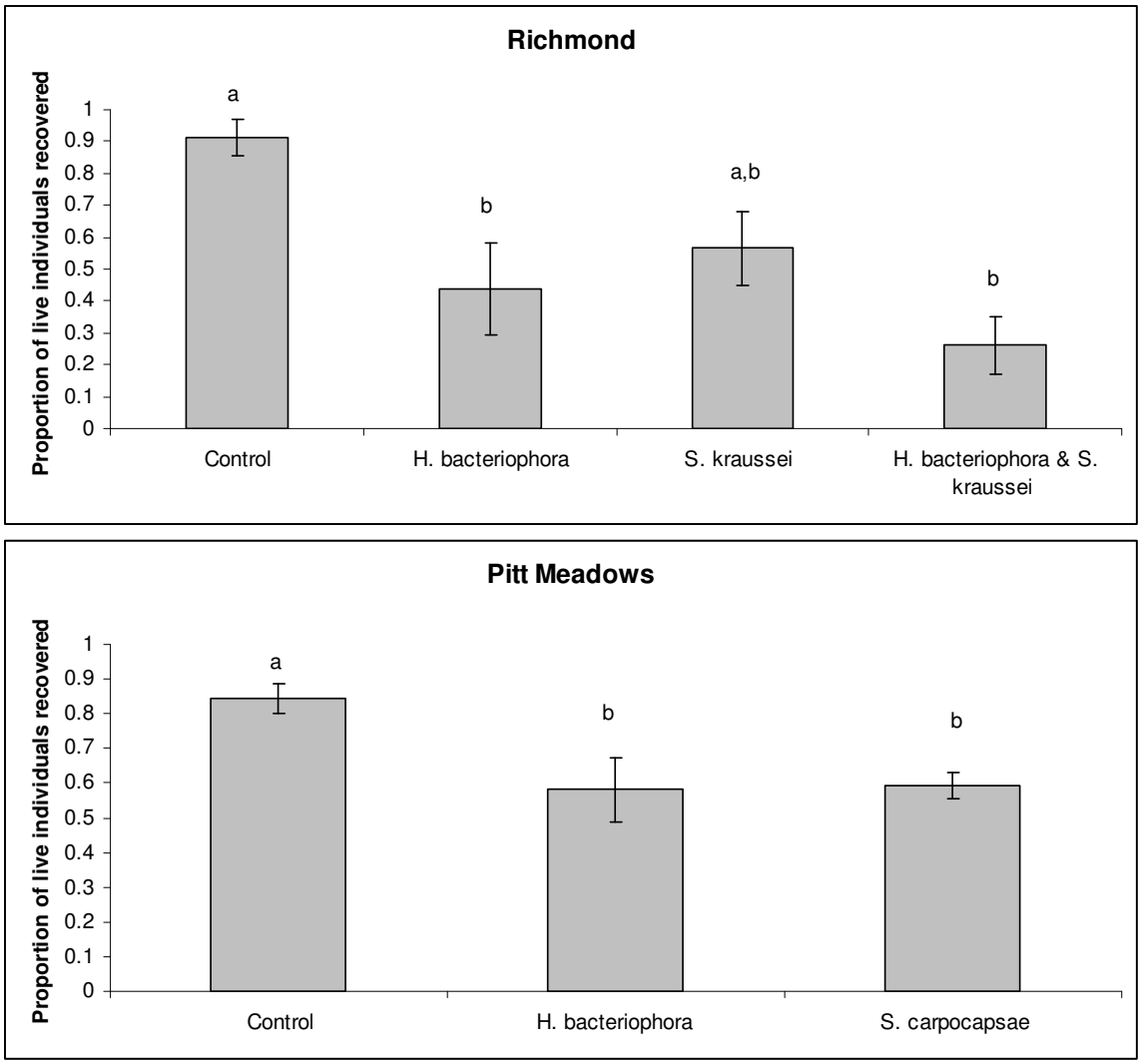

Figure 12. Effect of spring nematode treatments on the proportion of live black vine weevil individuals (larvae, pupae, or adults) recovered from plots in A) Richmond and B) Pitt Meadows cranberry fields. Bars are the means ± standard error of N = 6 replicates per treatment. Bars with the same letter are not significantly different from each other based on Tukey-Kramer honestly significant difference means comparison

Summary Discussion: Our study demonstrates that the single species treatment of *H. bacteriophora* or the combined species treatment of *H. bacteriophora* & *S. kraussei* are effective at reducing the survival of black vine weevil larvae. These findings were also confirmed in our field trials conducted in 2010. Interestingly, *S. kraussei* on its own was not effective in 2008 container trials (Fig. 11). Treatment with *S. carpocapsae* was not effective in small container studies either (2008) but was effective in reducing the number of live individuals in field plot trials (2010). Waiting until the spring will give growers a better chance to locate weevil damage hotspots so that nematodes could be applied with a backpack sprayer and applications targeted. Also, spring applications avoid potential issues around damage to fruit as a result of irrigation needed to keep nematodes alive in the soil. Spring applications will need to be well timed to ensure that

soil temperatures are warm enough to support individual nematode species but that larvae are still present and have not pupated. Booth *et al.* (2002) discussed the potential for variable efficacy of nematodes in the Pacific Northwest because of cooler soil temperatures in the spring when they made their applications. As a cool temperature species, *S. kraussei* would be a good candidate for spring applications.

Objective 3 –Tools for black vine weevil: Metarhizium anisopliae

Rationale: Booth *et al.* (1998) were able to significantly reduce black vine weevil numbers in plots treated with dried *M. anisopliae* mycelium compared to control plots. In containerized nursery stock, *M. anisopliae* has been shown to be most effective when used prophylactically (prior to weevil infestation) when incorporated into the potting media (Bruck 2005). Since weevil damage in cranberries continues to occur around the same area as previous years' damage, prophylactic applications should be explored for cranberries, along with the challenge of how to deliver the product to the root zone. For these trials the main question was how to get the Met52 to the weevil larvae in the cranberry agroecosystem.

2009 Methods: For this trial we used cranberries growing in four raised beds at the E.S. Cropconsult research site in Abbotsford, B.C. In each bed, four areas were infested with 30 black vine weevil eggs on September 5. A sampling area measuring 0.3m² was flagged around each infestation point for a total of 16 plots. Plots were treated on October 10 which allowed four weeks for the weevil eggs to hatch. Treatment with granules of *M. anisopliae* strain Met52 was randomly assigned to half the plots and the other half were used as controls (eight replicates per treatment). All plots were irrigated with 2L of water prior to treatment. Met52 was applied at a rate of 40 kg per ha (or 1.2g per plot) following the protocol by Booth *et al.* (2000). Granules were hand-spread uniformly over treatment plots. All plots were manually irrigated with 2L of water following treatment to ensure that *M. anisopliae* spores penetrated the trash layer.

Results and Discussion: There was no significant effect of Met52 treatment on recovery of weevil larvae (Fig. 13; $F_{1,15} = 0.2$, $p = 0.66$). In previous work Met52 has been shown to be very effective for black vine weevil control when the product is incorporated into the soil media prior to weevil infestation (Bruck and Donahue, 2006). We suspect that the lack of efficacy observed in our trial is a reflection of the formulation of the product rather than the efficacy of the product. The fungal spores most likely did not wash down through the cranberry duff layer to the larvae. An emulsifiable concentrate (EC) formulation of *M. anisopliae* (same strain as Met52) is being developed for commercial use. This formulation is more likely to be appropriate for the cranberry environment than granules. Further testing of the EC formulation should be conducted in order to determine rates, viability via chemigation, and to support product use in cranberries.

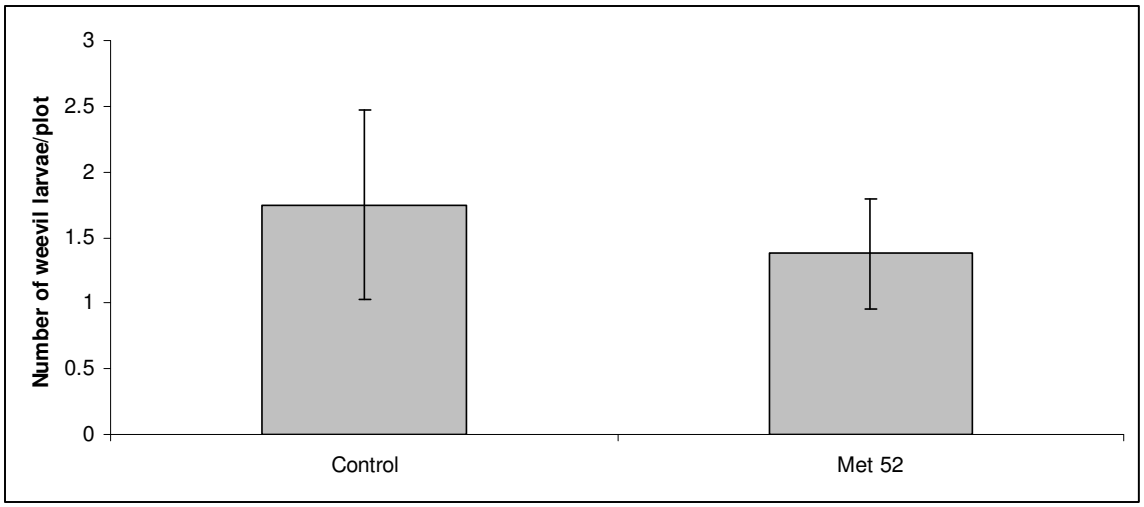

Figure 13. Effect of a granular formulation of *Metarhizium anisopliae* on the survival of black vine weevil larvae in 5-year old experimental cranberry beds. Bars are the means ± standard error of N = 8 replicates per treatment.

Summary

Insect control for organic cranberry production does not appear to be limited by tool availability – for each of three pests studied, at least two tools are either currently available or expected to be available in the near future. However, effective delivery of products appropriate to organic production is a challenge in the cranberry agroecosystem. While conventional insecticides are generally applied via chemigation, the duration of irrigation typical of broad-spectrum synthetic insecticides does not appear to be appropriate for organic formulations like Entrust. We saw the most effective fireworm larva kill rates with Entrust in a small field that required only 7 minutes for full coverage. In addition, repeat applications seem to be necessary in our demonstration to achieve desirable levels of fireworm larva control. Chemigation for longer periods of time may dilute the amount of active ingredient reaching larva, thus limiting the efficacy of a treatment. The experience of one of our Oregon cooperators confirms that reducing the irrigation time appears to improve the efficacy of Entrust application. Biological control with *Trichogramma sibericum* or other locally active species of egg parasitoids could be used in combination with Entrust to provide dual pressure on blackheaded fireworm.

In addition to the challenge of applying an appropriate product via chemigation, another challenge in cranberries is getting products to the pest. This is especially difficult for the two belowground pests cranberry girdler and black vine weevil. We found that nematodes, currently used for girdler control, are effectively washed down from the foliage and into the soil layer after one hour of irrigation. This finding was consistent in all three fields (with different varieties, ages, and densities of vines) where the study was conducted. Our preliminary results with the entomopathogenic fungus *Metarhizium anisopliae* (Met52) granular formulation suggests that fungal spores are not easily washed down to larvae feeding on roots when granules are sprinkled on the surface of the field. Incorporation of granules into the soil, which has been shown to be effective for weevil control in nursery stock, would be difficult in cranberries due to the perennial

nature of the crop. A follow-up study with a new emulsifiable concentrate formulation of Met52 did not result in significant reduction in girdler larvae. Additional aspects such as the rate of product and timing of application are all factors that can further impact the delivery and thus efficacy of fungal spores to the target pest. Further work with *M. anisopliae* and other entomopathogenic products should be pursued, especially as *M. anisopliae* is likely to control both cranberry girdler and black vine weevil.

Extension and Outreach Activities

As part of the extension and outreach associated with this project, we worked closely with all of our grower collaborators, both organic and conventional. We also participated in three extension events.

July 2009 - B.C. Cranberry Growers Field Day - booth set up with preliminary results of our trial. Attended by both B.C. and Washington state growers.

March 2009 - Certified Organic Association of B.C. Annual Meeting - oral presentation highlighting results of this work. Attended by B.C. and Washington state growers.

June 2009 - Cranberry Field Day (Southern Oregon) - informal oral presentation and one-on-one discussion with growers. Attended by Oregon growers.

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References

Booth, S.R. and C.H. Shanks. 1998. Potential of dried rice/mycelium formulation of entomopathogenic fungi to suppress subterranean pests in small fruits. *Biocontrol Science and Technology* 8(2):197-206.

Booth, S.R., L. Tanigoshi, and I. Dewes. 2000. Potential of dried mycelium formulation of an indigenous strain of *Metarhizium anisopliae* against subterranean pests of cranberry. *Biocontrol Science and Technology* 10:659-668.

Booth, S.R., L.K. Tanigoshi, and C.H. Shanks, Jr. 2002. Evaluation of entomopathogenic nematodes to manage root weevil larvae in Washington state cranberry, strawberry, and red raspberry. Environmental Entomology 31:895-902.

Bruck, D.J. 2005. Ecology of *Metarhizium anisopliae* in soilless potting media and the rhizosphere: implications for pest management. *Biological Control* 32:155-163

Bruck, D.J. and K.M. Donahue. 2006. Persistence of *Metarhizium anisopliae* incorporated into soil-less potting media for control of the black vine weevil, *Otiorhynchus sulcatus* in container-grown ornamentals. *Journal of Invertebrate Pathology* 95 (2):146-150

Cockfield, S.D., J.B. Olson, and D.L. Mahr. 1994. Blackheaded fireworm (Lepidoptera: Tortricidae) larval phenology model based on diet specific developmental rates. *Environmental Entomology* 23(2):406-413.

Fitzpatrick, S.M. 2007. Survival of submerged larvae of cranberry girdler, *Chrysoteuchia topiaria*, in the laboratory. *Crop Protection* 26(2007):1810-1816

Fitzpatrick, S.M., J.A. Newhouse, J.T. Troubridge, and K.A. Weitemeyer. 2001. Rearing the cranberry girdler *Chrysoteuchia topiaria* (Lepidoptera: Pyralidae) on reed canary grass *Phalaris arundinacea* (Festucoideae: Panicoideae). *Journal of the Entomological Society of British Columbia* 98:211-215.

Fitters, P.F.L., R. Dunne, and C.T. Griffin. 2001. Vine weevil control in Ireland with entomopathogenic nematodes: optimal time of application. *Irish Journal of Agricultural and Food Research* 40:199-213.

Haase-Statz, S. 1997. Evaluation of *Paederus littorarius* (Coleoptera : Staphylinidae) as an egg predator of *Chrysoteuchia topiaria* (Lepidoptera : Pyralidae) in Wisconsin cranberry bogs. *Great Lakes Entomologist* 30(3): 93-98 (Abstract only)

Henderson, D.E., S.Y. Li, and R. Prasad. 2002 *Rhopobota naevana*, Blackheaded Fireworm (Lepidoptera: Torticidae). Pp. 242-246 in P.G. Mason & J.T. Huber, eds. *Biological Control Programmes in Canada, 1981 – 2000.*

Henderson, D.E. and R. Singhai. 1999. Efficacy study of three species of nematode for control of Cranberry girdler *Chrysoteuchia topiaria* in British Columbia, Canada. E.S. Cropconsult Research Report to The Cranberry Institute, B.C. Cranberry Growers Assn, and Micro Bio Inc. 27 pp.

Koppenhöfer, A.M., and E.M. Fuzy. 2004. Effect of white grub developmental stage on susceptibility to entomopathogenic nematodes. *Journal of Economic Entomology* 97:1842–1849.

Mahr, D. 2005. Cranberry girdler. Fact sheet. http://www.hort.wisc.edu/cran/mgt_articles/articles_pest_mgt/insects/profiles_insects/GIRDLER.pdf. First access May 2009.

Ruisheng, A. and P.S. Grewal. 2007. Differences in the virulence of *Heterorhabditis bacteriophora* and *Steinernema scarabaei* to three white grub species: The relative contribution of the nematodes and their symbiotic bacteria. *Biological Control* 43:310-316.

Rutherford, T. A., D. Trotter, and J. M. Webster. 1987. The potential of heterorhabditid nematodes as control agents of root weevils. *The Canadian Entomologist* 119:67-73.