

# Field testing of *Metarhizium anisopliae* (strain Met52) for control of cranberry girdler

Final Report to:  
BC Cranberry Growers Association  
BC Cranberry Marketing Commission

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## Executive Summary:

Cranberry girdler is a difficult pest to control and more options are needed for its management. One possible tool could be the entomopathogenic fungus *Metarhizium anisopliae*, which has shown some promise for girdler control in previous studies conducted in Washington. A field study using microplots was conducted to determine the efficacy of an emulsifiable concentrate formulation of *M. anisopliae* strain F52 (Met52). Cranberry microplots were treated with Met52 at two time intervals - one week prior to egg infestation and at egg infestation and at two different rates - 1X (12 L/Ha) and 2X (24 L/Ha), for a total of four different Met52 treatments. Plots for these four treatments and the untreated Control were infested with cranberry girdler eggs, which were obtained from moths collected from commercial cranberry fields during late June and early July. Microplots were placed out in research cranberry beds to expose larvae and the fungus to environmental conditions similar to a commercial field. Although fewer larvae were recovered from the 2X treatments and the treatments where Met52 was applied one week prior to egg infestation than in untreated Control - these differences were not statistically significant. We examine the combined evidence for *M. anisopliae* against both cranberry girdler and black vine weevil and suggest that while it is still a potential tool for cranberries, the experimental work needed to obtain robust efficacy data to support a product registration could be substantial.

Introduction: Cranberry girdler (*Chrysoteuchia topiaria*) is a difficult to control pest of cranberries in BC. Below ground feeding damage by girdler larvae can result in areas of poor growth or possibly vine death. There are no insecticides registered for girdler control in cranberries in Canada (AAFC 2007) and BC growers are using a combination of cultural control and entomopathogenic nematodes for management of this pest. Although nematodes can effectively control girdler larvae if used correctly, application timing and maintenance of optimal field conditions pre and post application can be challenging. Another control tool, the entomopathogenic fungus *Metarhizium anisopliae* may be a good candidate for the pest management tool box in cranberries. Various strains and formulations of *M. anisopliae* have been shown to control black vine weevil and other soil dwelling insect pests (Ansari *et al.* 2010, 2009, Fisher and Bruck 2008, Bruck *et al.* 2005), the fungus appears to remain active in the soil for several months after application (Bruck 2005, Booth *et al.* 2000) and it is considered a reduced-risk product with some formulations approved for organic agriculture. In terms of *M. anisopliae* efficacy against cranberry girdler the results have been promising, but not conclusive.

Small field-plot assays to test the efficacy of a local (to Washington) strain of *M. anisopliae* against cranberry girdler demonstrated significant reduction in adults (Booth and Shanks 1998) and a non-significant reduction in larvae (Booth *et al.* 2000) compared to Control plots. Similarly, in our previous work there was a trend towards fewer feeding tunnels in roots and fewer larvae overall in Met52 treatments than Control, however this result was also not significant (Prasad and Soto, 2009 Project Report to Cranberry Commission). Met52 is a commercially available strain of *M. anisopliae* that is registered in Canada for black vine weevil control in containerized nursery stock. In preliminary work, we found that directly sprinkling Met52 granules (60kg/ha) on to cranberry foliage, caused a non-significant reduction in survival of weevil larvae in experimental cranberry beds (E.S. Cropconsult unpublished data; Fig. 1). In our 2009 trials with girdler we also directly sprinkled Met52 granules onto the soil surface, as was the protocol for Booth *et al.* (2000). So one challenge of effectively using Met52, or other *M. anisopliae* strain, in cranberries may be to effectively deliver the product (fungal spores) to the area where the pest can become exposed. Recently a liquid formulation of Met52, which could be suitable for delivery via chemigation, has been developed. The objective of this trial was to continue to evaluate the efficacy of *Metarhizium anisopliae* strain Met52 for control of cranberry girdler. If effective such a tool would be useful to both organic and conventional cranberry production.

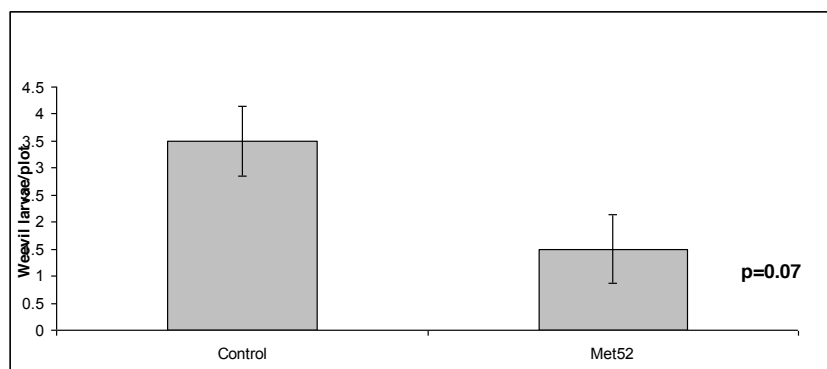


Figure 1. Preliminary trial results of Met52 efficacy against black vine weevil in cranberries. Trial conducted in small experimental beds. Bars represent the mean ( $\pm$  s.e.) of 4 replicates/treatment.

## Methods:

*Moth and egg collection* - Cranberry girdler adults were collected from commercial cranberry fields in Pitt Meadows, Langley and Richmond, BC 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 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. 2a) and 1 m X 1m "field" plots that were inside our research cranberry beds (Fig. 2b). All of the plots were located outdoors in Abbotsford, BC and exposed to ambient environmental conditions. Microplots consisted of 9 cm diameter (0.64 m<sup>2</sup>) 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 has 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 2. 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 or within a 10-cm diameter area around each flag in field plots (Fig. 2b). 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 of 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* Met 52 (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<sup>2</sup>) and 1.2 ml of product in 3 L of water for the field plots (1 m<sup>2</sup>). For the higher (2X rate) this worked out to 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 - on the same day that eggs were added to plots and one week before eggs were added to plots. Microplots were all treated with Met 52 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. 3) 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 3. Cranberry girdler frass 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). All raw data from microplot trials are presented in Appendix 1.

*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 Met52EC. 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  $19 \pm 1^\circ\text{C}$  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.

**Results and Discussion:** 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. 4;  $F(4,25) = 1.41$ ,  $p = 0.26$ ). 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 1; 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 (Fig. 5). In contrast the two Control larvae were either still alive or dead but not covered with any fungal growths.

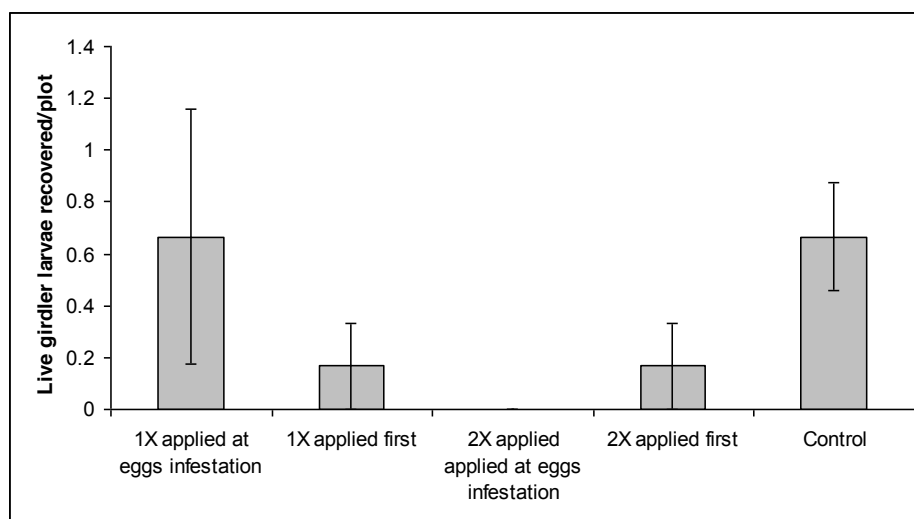


Figure 4. Effect of rate and application timing of *Metarhizium anisopliae* (strain F52) on the recovery of cranberry girdler (*Chrysoteuchia topiaria*) larvae from cranberry microplots. Bars represent the mean  $\pm$  s.e. of 6 replicates/treatment (total N = 30).

Table 1. 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                                  |

In our 2009 trials, conducted with the granular formulation of Met52, we also saw a similar trend towards fewer cranberry girdler larvae in Met52 treated pots than the untreated Control, but again this trend was not statistically significant. Finally, in preliminary trials conducted in research cranberry beds, we observed a similar non-significant trend towards fewer black vine weevil larvae recovered from Met52 granular treated plots than untreated Control (E.S. Cropconsult Ltd. unpublished data). 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. Although there are

several reports confirming the efficacy of granular formulation of Met52 against black vine weevil (Bruck and Donahue 2007, Bruck 2005), 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>).



Figure 5. Dead cranberry girdler larvae (above arrow) from Met52 soil bioassay.

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<sup>2</sup>). In follow up work (Booth *et al.* 2000) conducted with an improved application process for *M. anisopliae*, a lower and more affordable rate of granule application (6 g/m<sup>2</sup>) was used. 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 like our results some sites did have fewer larvae in *M. anisopliae* treated plots than Control. Thus the previous work by E.S. Cropconsult and others 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 (see below) and this may be another reason why very few published studies have followed up on the findings of Booth and Shanks (1998).

**Suggestions for Next Steps:** While the results of the past two studies have not been conclusive, we suggest that trial work with *M. anisopliae* not be abandoned. The granular formulation of Met52 does have demonstrated efficacy against black vine weevil (Bruck and Donahue 2007) and the preliminary results of the soil bioassay from this study suggest that there is direct mortality to cranberry girdler larvae. Thus *M. anisopliae* (Met52 or other formulations) have the potential to provide control of two cranberry pests. The advantage of Met52 is that the granular formulation is already registered for (non-food) use in Canada and the EC formulation could be suitable for chemigation.

- Repeat at a larger scale the soil bioassay for girdler to confirm that cranberry girdler is susceptible to Met52 EC formulation. If soil bioassays are conclusive and promising *then* consider field trials (microplot, field plot or other) for cranberry girdler.
- Field trials to determine efficacy of Met52 EC formulation against black vine weevil in cranberry fields (Issues to consider are timing of application, rate of EC formulation, and water volumes accompanying application).
- However, there are some major challenges for any future trials with girdler (and weevil). These challenges include
  - producing a sufficient amount of eggs to adequately infest plots. Although an individual female can lay many eggs (reportedly 300 on the first day (Mahr 2005)) we have found it challenging to obtain this many eggs from a single female or to infest pots/plots at a level that yields more than 4 larvae. Egg predation may be a big factor in trials.
  - rearing a sufficient number of larvae for bioassays
  - conducting the trial in conditions that are representative of a typical cranberry field
  - obtaining a site large enough to have a sufficient number of replicates and permission to introduce either or both pest

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Appendix 1 - Raw data from microplots (note that only 1 larvae was recovered from field plots thus raw data are not presented for that portion of the trial).

| Treatment & Replicate | Frass present  | # of cranberry girdler larvae | <i>Other</i>            |
|-----------------------|----------------|-------------------------------|-------------------------|
| Control 1             | Present        | 0                             |                         |
| Control 2             | Present        | 1                             |                         |
| Control 3             | Present        | 1                             |                         |
| Control 4             | Present        | 1                             |                         |
| Control 5             | Absent         | 0                             |                         |
| Control 6             | Present        | 1                             |                         |
| 1X - at infestation 1 | Present        | 1                             | <i>2 cutworm larvae</i> |
| 1X - at infestation 2 | Present        | 3                             |                         |
| 1X - at infestation 3 | Absent         | 0                             | <i>1 cutworm larva</i>  |
| 1X - at infestation 4 | Absent         | 0                             |                         |
| 1X - at infestation 5 | Absent         | 0                             |                         |
| 1X - at infestation 6 | Absent         | 0                             |                         |
| 2X - at infestation 1 | Absent         | 0                             |                         |
| 2X - at infestation 2 | Absent         | 0                             |                         |
| 2X - at infestation 3 | Absent         | 0                             |                         |
| 2X - at infestation 4 | Present        | 0                             |                         |
| 2X - at infestation 5 | Absent         | 0                             |                         |
| 2X - at infestation 6 | Absent         | 0                             |                         |
| 1X - 1 week pre 1     | Absent         | 0                             |                         |
| 1X - 1 week pre 2     | Absent         | 0                             |                         |
| 1X - 1 week pre 3     | Present        | 1                             | <i>1 cutworm larva</i>  |
| 1X - 1 week pre 4     | Absent         | 0                             |                         |
| 1X - 1 week pre 5     | Absent         | 0                             |                         |
| 1X - 1 week pre 6     | Absent         | 0                             |                         |
| 2X - 1 week pre 1     | Present        | 0                             |                         |
| 2X - 1 week pre 2     | Present        | 1                             |                         |
| 2X - 1 week pre 3     | Present        | 0                             |                         |
| 2X - 1 week pre 4     | Absent         | 0                             | <i>1 cutworm larva</i>  |
| 2X - 1 week pre 5     | Absent         | 0                             | <i>1 cutworm larva</i>  |
| 2X - 1 week pre 6     | <i>Present</i> | <i>0</i>                      | <i>1 cutworm larva</i>  |