Cranberry Tipworm, Dasineura oxycoccana:

(I) Toward Identification of Sex Pheromone;

and

(II) Abundance of Parasitoids

Report to BC Cranberry Marketing Commission
Submitted December 10, 2010

Principal Investigator
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Principal Investigator Dr. Sheila Fitzpatrick, Research Entomologist, Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre (AAFC-PARC), Agassiz, BC.

Assistants Dan Peach (100% time; SFU Co-op student); Sneh Mathur (10% time; technician, AAFC-PARC),.

Collaborators Dr. Gerhard Gries and Regine Gries, Department of Biological Sciences, Simon Fraser University; Dr. Bradley Sinclair, Canadian Food Inspection Agency, Ottawa; Drs. John Huber and Lubomir Masner, AAFC Eastern Cereals and Oilseeds Research Centre, Ottawa.

Start and end date of project May 1, 2010, to November 30, 2010.

Location of work Cranberry farms in Pitt Meadows; Simon Fraser University in Burnaby; Pacific Agri-Food Research Centre in Agassiz.

Amount contributed by BCCMC $18,110.20, of which $10,290 was allocated to salary of student Dan Peach; $458 to student's meal expenses and mileage; $5,000 to supplies and operating expenses; and $2,362.20 to AAFC administration cost.

Other funding In-kind from AAFC: use of fleet vehicle; technical support; growth chambers and growth rooms; microscopes, vials and other supplies; computer, software, network access and computer support. In-kind from SFU: technical support, gas chromatograph, electroantennograph, and related supplies.

Executive Summary

- This study is the first to successfully use GC-EAD and GC-MS technology to tentatively identify candidate pheromone of cranberry tipworm, *D. oxycoccana*, thanks to expert work by Regine Gries and technicial diligence by Dan Peach. Further details remain undisclosed until more progress has been made.

- The candidate pheromone was field-tested for the first time in 2010. During the three-day test on a cranberry farm in Pitt Meadows, BC, lures loaded with candidate pheromone were 6 times more attractive to cranberry tipworm males than were blank lures. Traps baited with female tipworms attracted male tipworms for the duration of pheromone-release behaviour by females, which was only a few hours due to the short lifespan of tipworm adults under hot, dry conditions.

- Two species of hymenopteran parasitoids of cranberry tipworm were present on two farms in Pitt Meadows, as reported in 2009. It is very encouraging to find that two species of parasitoids endure despite insecticide treatment. On one farm, *Platygaster* sp. predominated in 2010, whereas *A*. nr. *marylandensis* predominated in 2009. The schedule of insecticide sprays differed in the two years and may be partly responsible for the pattern of parasitoid species abundance. It is also possible that competition between the two parasitoid species favoured *Platygaster* sp. in 2010, or that hyperparasitoids reduced the population of *A*. nr. *marylandensis* in 2010.

Introduction and Original Objectives

Cranberry tipworm overwinters as a pupa in the trash layer or soil on the floor of the cranberry bed. As the cranberry plants come out of dormancy, the tiny adult midges begin emerging from the pupal stage. The adults mate and the female lays eggs in the tender buds at the tips of uprights. The larvae that hatch from the eggs are so tiny that they complete most of their development undetected. The cupped leaves indicating tipworm presence only become apparent when the larva within has almost finished its development, by which time the tip has been damaged or killed by the larva's rasping mouthparts.

Monitoring for cranberry tipworm involves examining upright tips under a microscope to detect eggs and early stage larvae. This is an excellent, accurate way of monitoring eggs and larvae, but it is time-consuming. It would be helpful to have a way of monitoring the adult midges, so that there could be some foreshadowing of the appearance of eggs and larvae in the tips. The most specific way to monitor adult insects is to attract the males to a trap baited with a synthetic version of the female's sex pheromone.

Here I report progress toward identification of cranberry tipworm pheromone, and observations on abundance of parasitoids of cranberry tipworm. The basis for this research is explained in last year's report (Fitzpatrick, 2009).

Objectives as written in the original proposal:

1. Using GC-EAD technology, analyze female tipworm sex pheromone to detect chemical compounds that stimulate response by male antennae.

2. Using GC-MS technology, identify these compounds and estimate their ratio in sex pheromone produced by female tipworms.

3. Using an olfactometer in the laboratory and pheromone traps in cranberry fields, determine if single compounds or blends of compounds attract male tipworms.

4. Choose the most attractive compound or blend of compounds as the basis for synthetic sex pheromone. Begin the process of developing a commercial pheromone lure with the local company Contech Enterprises, Inc. (formerly Phero Tech, Inc.)

5. As time and resources permit, gather supplementary data on behaviour of females in the afternoon and at night. Write a scientific manuscript reporting results on periodicity of calling behaviour.

6. As time and resources permit, continue to study tipworm parasitoids and to assist with Melissa Cook's study of cranberry tipworm behaviour on cranberry and blueberry.

Methods

Simon Fraser University (SFU) Co-op student Dan Peach was hired to work from May through mid-September, 2010, on this project. With assistance and supervision from the principal investigator and from collaborators Gerhard and Regine Gries, Dan carried out most of the field and laboratory work described here.

Gerhard and Regine Gries provided a large, new, walk-in growth chamber to use for rearing and maintaining field-collected cranberry tipworm. This was an unexpected and greatly appreciated in-kind contribution from SFU. The growth chamber was set to a photoperiod of 16 hours of light and 8 hours of dark. Lights came on at 09:00 AM and went off 01:00 AM. Temperature was 21 ^{o}C during photophase and 20 ^{o}C during scotophase; relative humidity was 70% throughout.

Beginning on May 17, Dan collected cranberry uprights weekly from two farms in Pitt Meadows. Uprights were placed in a cooler and transported to SFU. Fifty to 150 uprights per week were collected from each farm until August 26, when collection ceased. In the growth chamber at SFU, the stem of each upright was immersed in water in a glass scintillation vial (2 uprights per vial), and the leaves of the uprights were covered with an inverted scintillation vial, as reported last year (Fitzpatrick 2009). A layer of parafilm over the lower vial prevented emerged tipworms from drowning in the water. When adult tipworms emerged from pupation in the tips, each adult was placed individually in an inverted glass scintillation vial with moistened filter paper in the cap.

Most of the research effort was dedicated to extracting sex pheromone from adult female tipworms and using antennae from adult male tipworms as pheromone detectors. Dan extracted ovipositors from 1-, 2- or 3-day-old females during the first three hours after lights came on. Most females were calling during this time. Between June 4 and July 14, ovipositors from approximately 500 females were extracted, placed in hexane, and used by Regine Gries for GC-EAD and GC-MS analysis, as described in Fitzpatrick's 2010 research proposal and in Gries et al. (2000, 2002, 2005).

Field tests of a candidate pheromone were conducted at one of the cranberry farms, in an area of vines that supported an evenly distributed population of cranberry tipworms. On July 23, delta-style traps (approx 15 cm long by 8.5 cm high by 10 cm wide) were baited with lures containing either hexane ("blank") or 300 µl of candidate pheromone dissolved in hexane ("treatment"). Traps were attached to wooden stakes and suspended just above the vine tips (Fig. 1). A total of 12 blank and 12 treatment traps were placed in pairs as shown in Fig. 1, with 2 m between each blank and treatment trap in a pair, and approximately 10 m between pairs. At the north end of the trial area, four traps baited with cranberry tipworm females were installed. The purpose of these traps was to attract cranberry tipworm males that could be compared with males caught in the blank and treatment traps. Each female-baited trap contained one female (from the SFU colony) on a mesh-covered cranberry upright in a water-filled scintillation vial, as shown in Fig. 1. Three of the females were one day old; the fourth emerged on the morning of July 23.

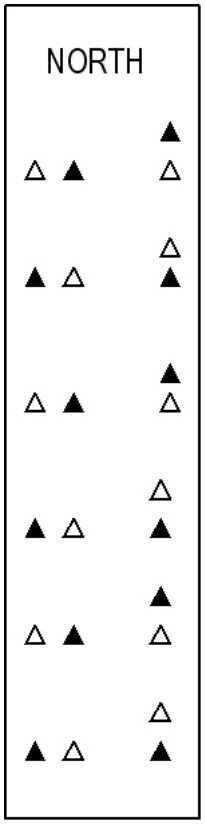

Figure 1. Top left: Delta-style trap baited with gray lure containing either hexane ("blank") or 300 µl candidate pheromone dissolved in hexane ("treatment"). Bottom left: Delta-style trap containing tipworm female on mesh-covered cranberry upright in water. Top right: Trial plot where blank (open triangles) and treatment (black triangles) traps were arranged in pairs, with 2 m between traps in each pair and 10 m between pairs. Four female-baited traps (not shown in diagram) were placed at the north end of the plot.

All traps were retrieved from the field on July 26, placed individually in clear plastic bags, and taken to SFU. On July 27 and 28, Dan identified the male and female cranberry tipworms and parasitoids of tipworms that were stuck to the floor and walls of each trap. Numbers of tipworms in blank vs. treatment traps were compared using paired t-test analysis.

As in 2009 (Fitzpatrick 2009), Dan collected and preserved parasitoids that emerged from tipworm larvae collected from the two farms. All collected uprights were maintained in the growth chamber at SFU for five weeks, and the numbers of tipworms and parasitoids that emerged from each upright (or pair of uprights) were recorded.

Results

Objective 1. Using GC-EAD technology, analyze female tipworm sex pheromone to detect chemical compounds that stimulate response by male tipworm antennae.

GC-EAD analysis of hexane extracts of almost 500 female tipworm ovipositors successfully resolved compounds that elicited responses from male tipworm antennae. Further details of the results remain undisclosed until the pheromone has been positively identified by Regine Gries.

Objective 2. Using GC-MS technology, identify these compounds and estimate their ratio in sex pheromone produced by female tipworms.

Compounds in the pheromone blend have been tentatively identified by GC-MS. Further details remain undisclosed until the pheromone has been positively identified by Regine Gries.

Objective 3. Using an olfactometer in the laboratory and pheromone traps in cranberry fields, determine if single compounds or blends of compounds attract male tipworms.

A field test, conducted as described in "Methods", was the most time-effective method of testing the candidate pheromone blend. Olfactometer studies might be done in future.

In the field test, traps baited with the candidate pheromone lure ("treatment") caught approximately 6 times more male cranberry tipworms than did traps baited with the blank lure. Therefore, the candidate pheromone blend is attractive to cranberry tipworm males.

Lure	Mean # males	Standard error	t-value	P-value
"Treatment"	18.8	3.5	5.03	<0.001
"Blank"	3.3	0.8		

A few females were caught in all traps. There was no difference in the numbers of females caught in traps baited with the candidate pheromone ("treatment") or the blank lure. Therefore, the candidate pheromone blend is not attractive to cranberry tipworm females. Raising the traps 10 or 20 cm above the vine tips might reduce catches of female tipworms.

Lure	Mean # females	Standard error	t-value	P-value
"Treatment"	1.8	0.5	0.12	0.91
"Blank"	1.8	0.4		

A total of two parasitoids, *Aprostocetus* nr. *marylandensis*, were caught in traps baited with the candidate pheromone lure (one parasitoid per trap). It is possible that parasitoids are attracted to the candidate pheromone.

As traps were being placed in the field on July 23, male tipworms were observed in "casting" flight (indicating attraction to a pheromone-releasing female) outside two female-baited traps. Bait females in the four traps were deceased when collected on July 26. Female-baited traps caught 10, 3, 3 and 3 males; and 2, 3, 1 and 2 females. These data suggest that only one female remained alive and calling on July 23. Cranberry tipworm and other midges are very short-lived insects (Fitzpatrick 2009; Gagné 1989; Harris and Foster 1999).

Objective 4. Choose the most attractive compound or blend of compounds as the basis for synthetic sex pheromone. Begin the process of developing a commercial pheromone lure with the local company Contech Enterprises, Inc. (formerly Phero Tech, Inc.)

This objective will be pursued in 2011.

Objective 5. As time and resources permit, gather supplementary data on behaviour of females in the afternoon and at night. Write a scientific manuscript reporting periodicity of calling behaviour.

Work on the preceding objectives expended most of the available time and resources. However, calling behaviour of five females was observed for the first nine hours of photophase. As previously observed (Fitzpatrick 2009, 2010), females began calling at Lights On and continued for 5, 6 or 7 hours, depending on the female. The scientific manuscript is in preparation.

Objective 6. As time and resources permit, continue to study tipworm parasitoids and to assist with Melissa Cook's study of cranberry tipworm behaviour on cranberry and blueberry.

Two species of parasitoids were recovered from tipworm-infested uprights. These two species, *Aprostocetus* nr. *marylandensis* and *Platygaster* sp., identified by Dr. John Huber, were also collected in 2009 (Fitzpatrick 2009; Peach and Fitzpatrick 2009; Peach et al. 2010). The first parasitoids emerged from uprights collected on June 14, 2010, and the greatest number (mean of 0.6 per upright) emerged from uprights collected on July 5, 2010 (Figure 2). The diazinon sprays on July 15 and 27 may have

killed ovipositing female parasitoids. On July 17, and in the following weeks, the mean number of parasitoids that emerged from collected uprights was approximately 0.1 per upright, while the mean number of tipworms that emerged ranged from 0.4 to 0.9 per upright.

In 2010, *Platygaster* sp. predominated early in the season, and *A*. nr. *marylandensis* was more numerous in late July through August (Fig. 3). By contrast, in 2009, *A*. nr. *marylandensis* appeared early and predominated until late July, whereas *Platygaster* appeared in July and predominated in August (Fitzpatrick 2009; Peach and Fitzpatrick 2009, 2010; Peach et al. 2010).

Dan assisted Melissa Cook (Master of Science student, SFU) in collecting tipworm-infested uprights for her thesis project. Melissa's data show that cranberry tipworms do not mate with blueberry gall midges, although morphological characters identify both as *Dasineura oxycoccana (*Cook et al. 2009, 2010).

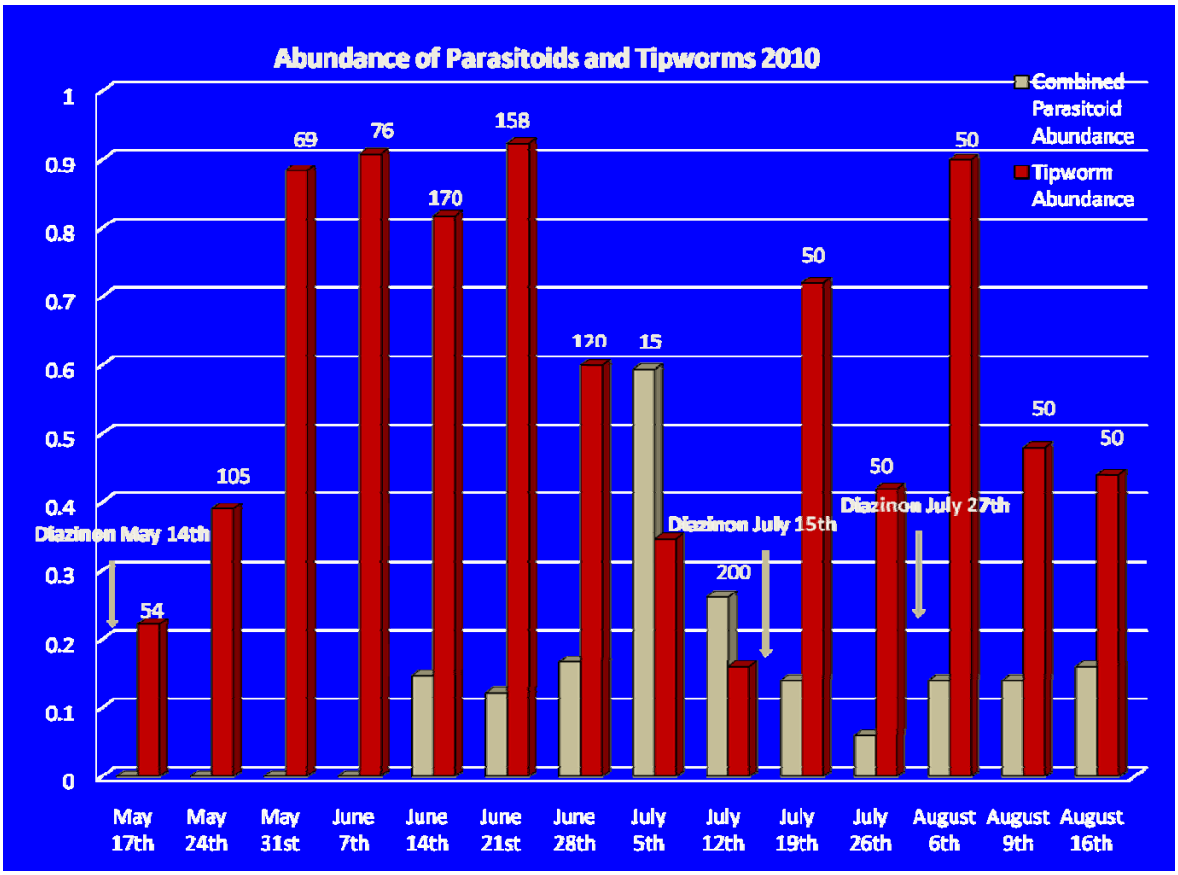

Figure 2. Mean number of insects (parasitoids or tipworms) that emerged from uprights collected on May 17, May 24, etc. Number of uprights collected on each date is shown above the bars of the graph. (July 5 should be 150, not 15.) Three diazinon sprays were applied, as indicated by the arrows.

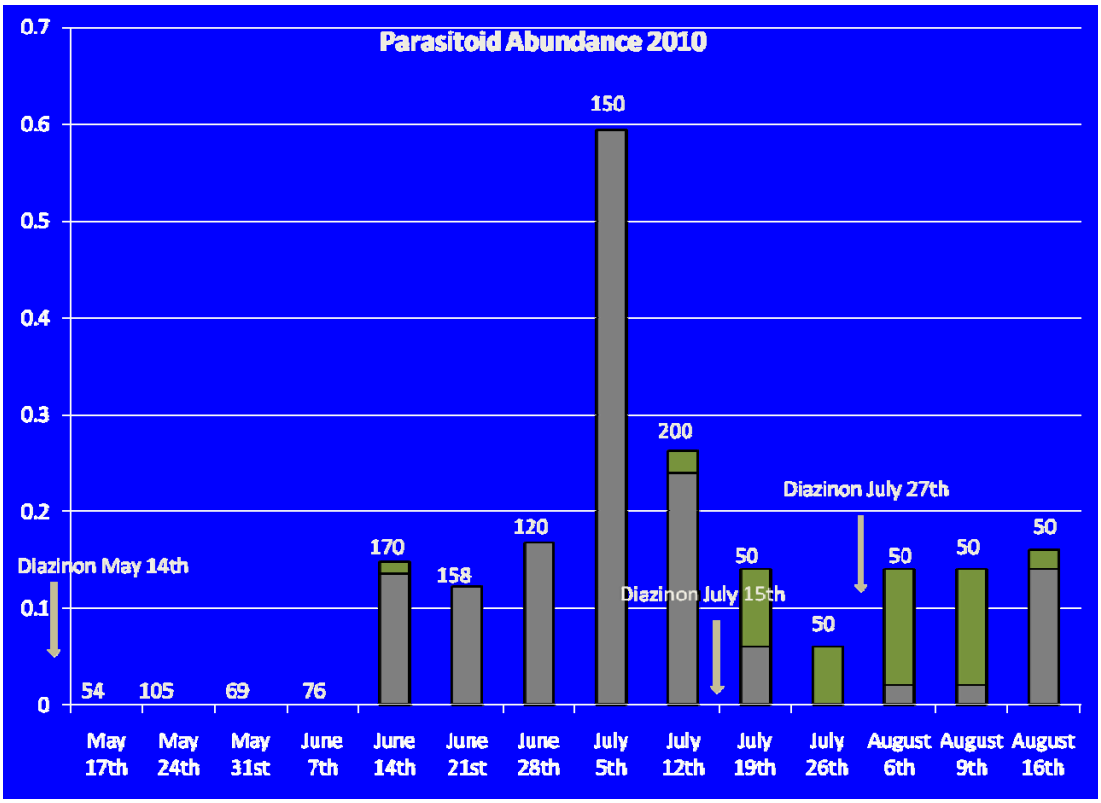

Figure 3. Mean number of parasitoids that emerged from uprights collected on May 17, May 24, etc. The gray bars and top left photo show *Platygaster* sp.; the green bars and top right photo show *Aprostocetus* nr. *marylandensis*. Number of uprights collected on each date is shown above the bars of the graph. Three diazinon sprays were applied, as indicated by the arrows.

Discussion, Deliverables, and Future Work

This study is the first to successfully use GC-EAD and GC-MS technology to tentatively identify candidate pheromone of cranberry tipworm, *D. oxycoccana*, thanks to expert work by Regine Gries and technicial diligence by Dan Peach. Further details remain undisclosed until more progress has been made.

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Two species of hymenopteran parasitoids of cranberry tipworm were present on two farms in Pitt Meadows, as reported in 2009 (Fitzpatrick 2009; Peach and Fitzpatrick 2009). It is very encouraging to find that two species of parasitoids endure despite insecticide treatment. On one farm, *Platygaster* sp. predominated in 2010, whereas *A*. nr. *marylandensis* predominated in 2009 (Peach and Fitzpatrick 2010; Peach et al. 2010). The schedule of insecticide sprays differed in the two years and may be partly responsible for the pattern of parasitoid species abundance. It is also possible that competition between the two parasitoid species favoured *Platygaster* sp. in 2010, or that hyperparasitoids reduced the population of *A*. nr. *marylandensis* in 2010.

Voss (1996) reported parasitoids in the same two families as ours – Eulophidae and Platygastridae – emerging from cranberry tipworms in Wisconsin. Sampson *et al*. (2006) discovered several species of parasitoids, including *Aprostocetus* sp. and *Playgaster* sp., in *D. oxycoccana* and a related midge in blueberry in Florida. César Rodriguez at Rutgers University has also discovered parasitoids emerging from *D. oxycoccana* on blueberry. In BC, it is possible the parasitoid activity can be conserved and enhanced by minimizing insecticide sprays, particularly in late July and August.

Other studies have revealed genetic separation (Mathur et al. 2010) and differences in reproductive behaviour (Cook et al. 2010) between *D. oxycoccana* on cranberry (cranberry tipworm) and *D. oxycoccana* on blueberry (blueberry gall midge). For cranberry growers, the implication of these results is that cranberry tipworm populations probably do not come from nearby blueberry fields.

Posters and presentations generated in whole or in part by this study are listed in "References": Cook et al. 2010; Peach et al. 2010; Mathur et al. 2010. Scientific manuscripts based on these deliverables are in progress.

Anticipated funding request in 2011

In 2011, research on identification of *D. oxycoccana* pheromone will continue. Collaborators R. and G. Gries at SFU will require some funding for materials related to gas chromatography, electrophysiology and pheromone synthesis. A student will be required to assist R. Gries and to carry out field tests of candidate pheromone components and blends. (Dan Peach would be the first choice for student assistant but, in summer 2011, he may be studying entomology in Europe.)

Melissa Cook has completed research for her Master of Science (originally Master of Pest Management) thesis. She may begin a Ph.D. program and may continue research on *D. oxycoccana*; if so, she would benefit from half-time assistance from a student.

The studies of genetic characteristics of *D. oxycoccana* on cranberry and blueberry, and search for parasitoids of *D. oxycoccana*, might be expanded to include cranberry- and blueberry-growing regions in North America. Initial discussions with César Rodriguez have opened the door to a very interesting project. Deliverables for growers would include estimates of likelihood that *D. oxycoccana* and its parasitoids move between cranberry and blueberry. If we can better understand *D. oxycoccana* and its guild of parasitoids, we will be better able to decide what action threshold should be used for cranberry tipworm management.

I thank the BC Cranberry Marketing Commission and BC Cranberry Growers Association for funding this research in 2010.

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