Identification and Field-testing of Sex Pheromone of Cranberry Tipworm, Dasineura oxyccana

Report to BC Cranberry Marketing Commission
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Assistants Jessica Iwanski (100% time; SFU Co-op student); Sneh Mathur (10% time; technician, AAFC-PARC).

Collaborators Dr. Gerhard Gries, Regine Gries and Grigori Khaskin, Department of Biological Sciences, Simon Fraser University; Dr. Bradley Sinclair, Canadian Food Inspection Agency, Ottawa; Dr. John Huber, Natural Resources Canada, c/o AAFC Eastern Cereals and Oilseeds Research Centre, Ottawa.

Start and end date of project April 1, 2011, to March 31, 2012.

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

Amount contributed by BCCMC
• $10,925 from BCCMC, matched by $30,000 from AAFC (per DIAP LMHIA agreement).
• Of this total, $12,639.50 was allocated to salary of student Jessika Iwanski; $7,782.75 to supplies and operating expenses; and $2,175 to AAFC administration cost.
• It was not possible to hire the second student requested in my 2010 grant proposal.
• As of Dec. 1, 2012, $16,152.75 remains uncommitted. This amount will likely be allocated to supplies for the Fitzpatrick and Gries labs. Unfortunately, no funding can be held over to support research in 2012. Government regulations require that all funds be spent by March 31, 2012, or returned to general government revenue.

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: use of fleet vehicle, technical support, gas chromatograph, electroantennograph, and related supplies.
Executive Summary

• My collaborators at SFU, namely G. and R. Gries and G. Khaskin, have identified and synthesized the pheromone of cranberry tipworm. Field tests conducted by J. Iwanski and myself confirmed the attractiveness of the blend of optical isomers shown in 2010 to be attractive and confirmed the identity of the major pheromone component. These results are a first in cranberry tipworm research.

• The identities of the major pheromone component and other attractive compounds are written in a scientific manuscript that is in preparation by S. Fitzpatrick, R. Gries, G. Khaskin, J. Iwanski, D. Peach and G. Gries. When the manuscript has been accepted for publication, the compounds’ identities will be in the public domain. Further information can be obtained from S. Fitzpatrick.

• Another season’s field research is required before an effective pheromone lure can be recommended and commercialized. The research would involve a comparison of the two most attractive lures on three cranberry farms throughout the growing season. The number of cranberry tipworm males (and females) caught in traps would be compared with the number of eggs and larvae detected by examining collected shoots under the microscope.

• The scientific manuscript, “Hymenopterous parasitoids of cranberry tipworm (Diptera: Cecidomyiidae) in British Columbia, Canada” by Daniel A. H. Peach, John T. Huber and Sheila M. Fitzpatrick, has been accepted for publication in The Canadian Entomologist.
Introduction and Original Objectives

Cranberry tipworm, Dasineura oxycoccana (Johnson) (Diptera: Cecidomyiidae), is an established pest in the United States where cranberry (Vaccinium macrocarpon Aiton) cultivation originated (Franklin 1948; Marucci 1954; Eck 1990) and in Quebec, Canada (Landry et al. 2002). In 1998, cranberry tipworm was observed for the first time on cranberry in British Columbia (BC), Canada (British Columbia Ministry of Agriculture and Lands 2009).

As cranberry plants come out of dormancy, adult tipworms begin emerging from the pupal stage. Mated females deposit eggs on the inner leaves of cranberry shoot tips that have just begun elongation (Cockfield and Mahr 1994; Cook 2011). First-generation larvae attack vegetative shoot tips that are above the flowers and, therefore, do not directly damage the current year’s crop (Marucci 1954). The apical meristem dies as a result of feeding by larvae and the shoot produces lower secondary branches with vegetative shoot tips that can be infested by subsequent generations (Mahr and Perry 2006). Ovipositing females usually avoid shoot tips containing flower buds (Mahr and Perry 2006). Indirect damage to the next year’s crop occurs in mid- to late summer of the current year, when larvae infest vegetative shoot tips that might otherwise set a fruit bud for the following year (Mahr and Perry 2006). In Maine and northern Wisconsin, USA (Mahr and Perry 2006), and in BC, the majority of damaged shoot tips do not flower in the next year, so growers consider tipworm a significant pest. During the growing season, many pupae remain within the cupped leaves of affected tips (Franklin 1948) but, in autumn, larvae descend to overwinter in the layer of detritus or soil beneath the plants (Voss 1996).

In all areas where tipworm is a pest, monitoring involves examining shoot tips under a microscope to detect eggs and larvae, which 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. Monitoring by microscope is an excellent, accurate way of detecting eggs and larvae, but it is time-consuming. If adult tipworms could be detected, foreshadowing the appearance of eggs and larvae in the shoots, management of this pest would improve.

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. In 2010, the sex pheromone of cranberry tipworm was tentatively identified for the first time by Regine Gries and Grigori Khaskin at Simon Fraser University. A field test on a cranberry farm in Pitt Meadows BC showed that lures loaded with candidate pheromone were 6 times more attractive to cranberry tipworm males than were blank lures (Fitzpatrick 2010).
In 2011, components of the candidate pheromone were field–tested, individually and in combination. Here I present results of the field tests and discuss next steps in research and development of a tipworm pheromone monitoring lure.

**Objectives as written in the original proposal:**

**Primary Objective:** Conduct field tests of chemical compounds that are likely to be components of cranberry tipworm sex pheromone. These compounds were identified in 2010 by the Gries lab at SFU, as explained by Fitzpatrick (2010). Field tests will determine if single compounds or blends of compounds attract male tipworms to sticky traps.

**Subsequent Objectives:** 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.)

Gather supplementary data on behaviour of cranberry tipworm females in the afternoon and at night. Write a scientific manuscript reporting results on periodicity of calling behaviour.

Continue to study tipworm parasitoids and the role they play in reducing cranberry tipworm populations. Write a scientific manuscript on the discovery of two species of parasitoids attacking cranberry tipworm in BC (Fitzpatrick 2009).

**Methods**

Simon Fraser University (SFU) Co-op student Jessika Iwanski was hired to work from May through August, 2011, on this project. With assistance and supervision from the principal investigator and from collaborators Gerhard Gries, Regine Gries and Grigori Khaskin (examples of their work in Gries et al., 2002, 2005), Jessika carried out most of the field work described here.

Chemical compounds to be field–tested were synthesized by G. Khaskin, dissolved in hexane and pipetted onto grey rubber septa (= lures) by R. Gries. The identity of these compounds is written in a scientific manuscript that is in preparation by S. Fitzpatrick, R. Gries, G. Khaskin, J. Iwanski, D. Peach and G. Gries. When the manuscript has been accepted for publication, the compounds’ identities will be in the public domain. Further information can be obtained from S. Fitzpatrick.

Jessika Iwanski prepared delta–style traps (approx 15 cm long by 8.5 cm high by 10 cm wide) coated on two interior surfaces with sticky Tangletrap and baited with the grey lures (Fig. 1). Experimental design, tipworm identification and data analysis were done by S. Fitzpatrick. Field tests were conducted at a cranberry farm in Pitt Meadows, British Columbia.
in an area of vines that supported a population of cranberry tipworms. Traps were placed in the field, checked and retrieved by one or both of S. Fitzpatrick and J. Iwanski. As in 2010, traps were attached to wooden stakes (Fitzpatrick, 2010) and suspended on average 30 cm above the vine tips (Fig. 2). Insecticide was applied to the crop on July 25, 2011.

Figure 1. Delta–type trap coated on two interior surfaces with sticky Tangletrap and baited with a grey lure.
Figure 2. Traps were attached to wooden stakes and suspended on average 30 cm above the vine tips.

Four field tests were carried out. Each was laid out in a randomized block design having 10 blocks. Three tests had five treatments and one blank control. Spacing between traps was 10 metres (within blocks and between blocks). The fourth test had two treatments and one blank control. Spacing between traps in the fourth test was 10 metres within blocks and 14 or 20 metres between blocks. Traps remained in the field for 6 to 17 days until the number of tipworms trapped was judged to be sufficient to assess the treatments. Traps were then retrieved from the field and taken to PARC Agassiz, where they were stored in a cold room at 8°C for 1 to 27 days until they could be examined under a microscope at 16 times magnification. Tipworm adults were identified and counted. Counts were log-transformed to normalize their distribution, then analyzed by Analysis of Variance followed by Tukey’s test to assess differences between treatments.
Results

**Objective 1.** Conduct field tests of chemical compounds that are likely to be components of cranberry tipworm sex pheromone. These compounds were identified in 2010 by the Gries lab at SFU, as explained by Fitzpatrick (2010). Field tests will determine if single compounds or blends of compounds attract male tipworms to sticky traps.

The first field trial tested the compound found to be attractive in 2010 (designated ‘ABCD’) against its individual components (designated ‘A’, ‘B’, ‘C’, ‘D’) and a blank control (hexane only). ‘A’, ‘B’, ‘C’, ‘D’, and blank lures were loaded with 100 microlitres of treatment solution; ‘ABCD’ lures were loaded with 400 microlitres of solution.

Traps baited with compound ‘D’ caught significantly more male tipworms than traps baited with any of the other compounds (F = 34.7, P < 0.0001; Table 1). Traps baited with ‘ABCD’ caught more males than traps baited with ‘A’, ‘B’, ‘C’, or blank. **Compound ‘D’ is likely the major pheromone component of cranberry tipworm.**

Approximately 20 females were caught in each trap, regardless of treatment. Females were not attracted by any particular compound (F = 1.55, P = 0.19). They might have been attracted by the white colour of the traps. Blank traps caught an average of 18 females but only 6 males. Females may have been seeking a place to remain stationary and release pheromone, whereas males were seeking females. The fact that so few males were trapped in blank traps suggests that the females trapped there did not release much pheromone. Thus the presence of female tipworms in traps baited with test compounds did not adversely influence the results of field trials.

<table>
<thead>
<tr>
<th>Chemical Tested</th>
<th>Males (Mean ± SE)</th>
<th>Females (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>5.8 ± 1.4</td>
<td>17.9 ± 1.6</td>
</tr>
<tr>
<td>A</td>
<td>9.9 ± 1.4</td>
<td>19.6 ± 1.4</td>
</tr>
<tr>
<td>B</td>
<td>7.2 ± 2.1</td>
<td>21.9 ± 1.3</td>
</tr>
<tr>
<td>C</td>
<td>5.0 ± 1.3</td>
<td>18.6 ± 1.8</td>
</tr>
<tr>
<td>D</td>
<td>174.9 ± 29.5 **</td>
<td>16.4 ± 2.1</td>
</tr>
<tr>
<td>ABCD</td>
<td>35.1 ± 8.2 *</td>
<td>18.1 ± 2.5</td>
</tr>
</tbody>
</table>

The second field trial tested the major pheromone component ‘D’ against ‘D’ plus one of four structurally similar chemical components (designated ‘a’, ‘b’, ‘c’, ‘d’) and
a blank control. Traps baited with ‘D’, ‘D + a’, ‘D + b’, ‘D + c’ or ‘D + d’ caught similar numbers of males, and each of these treatments caught more males than did the blank traps (F = 12.6, P < 0.001; Table 2). **There is no additive or synergistic interaction between the major pheromone component ‘D’ and the compounds ‘a’, ‘b’, ‘c’ or ‘d’.** The number of females trapped did not differ among treatments or the blank (F = 0.67, P = 0.65).

**Table 2: Second Field Trial, July 27– Aug 3, 2011**

<table>
<thead>
<tr>
<th>Chemical tested</th>
<th>Males (Mean ± SE)</th>
<th>Females (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D only</td>
<td>5.0 ± 1.1 *</td>
<td>10.6 ± 1.0</td>
</tr>
<tr>
<td>D + a</td>
<td>9.2 ± 4.2 *</td>
<td>10.6 ± 0.9</td>
</tr>
<tr>
<td>D + b</td>
<td>3.7 ± 0.7 *</td>
<td>11.7 ± 0.8</td>
</tr>
<tr>
<td>D + c</td>
<td>10.6 ± 2.7 *</td>
<td>10.6 ± 1.1</td>
</tr>
<tr>
<td>D + d</td>
<td>6.6 ± 1.7 *</td>
<td>9.2 ± 0.6</td>
</tr>
<tr>
<td>Blank</td>
<td>0.3 ± 0.2</td>
<td>10.9 ± 1.3</td>
</tr>
</tbody>
</table>

The third field trial tested the major pheromone component ‘D’ against ‘D’ plus one of the other three components (‘A’, ‘B’, ‘C’) of the compound found to be attractive in 2010 (‘ABCD’). ‘ABCD’ and a blank control were included in the trial. Traps baited with ‘DA’ caught the most males, and traps baited with ‘D’, ‘DB’, ‘DC’ or ‘ABCD’ caught fewer than ‘DA’ traps but more than blank traps (F = 19.4, P < 0.001; Table 3). **Compound ‘A’ added to major pheromone compound ‘D’ increased attractiveness of the lure.**

In the third field trial, compound ‘D’ alone did not catch significantly more males than did compounds ‘ABCD’, although there was a trend for ‘D’ to catch more than ‘ABCD’. This result differs from the first field trial. During the third field trial, there were many fewer tipworms caught per day in the traps than in the first field trial, therefore the third field trial lasted 18 days, whereas the first field trial lasted 9 days. In the third trial, the number of males trapped was unevenly distributed among blocks (F = 2.8, P = 0.012). The first field trial was a perhaps a better test of the relative attractiveness of compounds ‘D’ and ‘ABCD’. However, results of the third field test suggest that, when populations are low, the ‘ABCD’ lure and the ‘D’ lure catch statistically similar numbers of male tipworms. The number of females trapped did not differ among treatments or the blank (F = 1.8, P = 0.13).
Table 3: Third Field Trial, Aug 8–25, 2011

<table>
<thead>
<tr>
<th>Chemical tested</th>
<th>Males (Mean ± SE)</th>
<th>Females (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D only</td>
<td>10.3 ± 3.7 *</td>
<td>13.9 ± 1.4</td>
</tr>
<tr>
<td>DA</td>
<td>24.2 ± 4.2 **</td>
<td>12.1 ± 1.1</td>
</tr>
<tr>
<td>DB</td>
<td>3.9 ± 1.0 *</td>
<td>13.2 ± 1.5</td>
</tr>
<tr>
<td>DC</td>
<td>9.7 ± 3.0 *</td>
<td>14.1 ± 2.2</td>
</tr>
<tr>
<td>ABCD</td>
<td>3.5 ± 0.6 *</td>
<td>9.2 ± 1.1</td>
</tr>
<tr>
<td>Blank</td>
<td>1.3 ± 0.4</td>
<td>11.6 ± 0.3</td>
</tr>
</tbody>
</table>

The fourth field trial tested major pheromone component ‘D’ against ‘D + a’ and a blank control. Traps baited with ‘D’ caught a statistically similar number of males as traps baited with ‘D + a’, and both baits caught more males than the blank control (F = 191.9, P < 0.0001; Table 4). This result was also seen in the second field trial. Chemical compound ‘a’ is the major component of blueberry midge pheromone. **Results of the fourth field trial show that blueberry midge pheromone is not antagonistic to cranberry tipworm.** A separate experiment in blueberry showed that cranberry tipworm pheromone was not antagonistic to blueberry gall midge. Theoretically, the combination ‘D + a’ could be used to monitor cranberry tipworm in cranberry and blueberry gall midge in blueberry. The number of females trapped did not differ among treatments or the blank (F = 0.4, P = 0.68).

Table 4: Fourth Field Trial, Aug 30 – Sept 9, 2011

<table>
<thead>
<tr>
<th>Chemical tested</th>
<th>Males (Mean ± SE)</th>
<th>Females (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>74.8 ± 11.8 *</td>
<td>8.5 ± 1.4</td>
</tr>
<tr>
<td>D + a</td>
<td>92.2 ± 13.9 *</td>
<td>8.5 ± 1.0</td>
</tr>
<tr>
<td>Blank</td>
<td>0.6 ± 0.2</td>
<td>9.9 ± 1.2</td>
</tr>
</tbody>
</table>
**Objective 2.** 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.)

The most attractive compound is ‘D’, which is the major component of cranberry tipworm pheromone (Table 1). It is possible that attractiveness might be increased by adding ‘A’ (Table 3).

Unfortunately, compound ‘D’ is very difficult and time-consuming to synthesize. Dr. Khaskin attempted for several months to synthesize this compound before succeeding. Therefore, ‘D’ by itself is too expensive to use as a commercial pheromone lure.

The blend ‘ABCD’, which is a compound composed of four optical isomers, can be synthesized and might be cost-effective as a commercial pheromone. ‘ABCD’ is better than a blank lure and better than ‘A’, ‘B’ or ‘C’ alone (Tables 3 and 1). When tipworm populations decreased in late August, ‘ABCD’ was as effective as ‘D’.

**Objective 3.** Gather supplementary data on behaviour of cranberry tipworm females in the afternoon and at night. Write a scientific manuscript reporting results on periodicity of calling behaviour.

Work on this objective was postponed. Available data on calling behaviour will be included in the pheromone manuscript. See Fitzpatrick (2009).

**Objective 4.** Continue to study tipworm parasitoids and the role they play in reducing cranberry tipworm populations. Write a scientific manuscript on the discovery of two species of parasitoids attacking cranberry tipworm in BC (Fitzpatrick 2009).

The scientific manuscript, “Hymenopterous parasitoids of cranberry tipworm (Diptera: Cecidomyiidae) in British Columbia, Canada” by Daniel A. H. Peach, John T. Huber and Sheila M. Fitzpatrick, has been accepted for publication in The Canadian Entomologist (Peach et al., in press).

**Discussion, Deliverables, and Future Work**

My collaborators at SFU, namely G. and R. Gries and G. Khaskin, have identified and synthesized the pheromone of cranberry tipworm. Field tests conducted by J. Iwanski and myself confirmed the attractiveness of the blend of optical isomers shown in 2010 to be attractive (Fitzpatrick, 2010) and confirmed the identity of the major pheromone component. These results are a first in cranberry tipworm research.

Another season’s field research is required before an effective pheromone lure can be recommended and commercialized. The research would involve a comparison of the lure ‘ABCD’ against ‘D’ on three cranberry farms throughout the growing season. The number of cranberry tipworm males (and females) caught in traps would be...
compared with the number of eggs and larvae detected by examining collected shoots under the microscope.

Accompanying this report are two posters and one published manuscript. These give an overview of the new knowledge about cranberry tipworm generated by the Fitzpatrick lab. These works are:


• “DNA barcodes suggest cryptic speciation in Dasineura oxycoccana (Diptera: Cecidomyiidae) on cranberry and blueberry in British Columbia” by S. Mathur, M.A. Cook, B.J. Sinclair and S.M. Fitzpatrick. Poster presented at North American Cranberry Workers Research and Extension Workers Conference (NACREW) 2011. Scientific manuscript of this research has been submitted to Florida Entomologist.


The work in press, “Hymenopterous parasitoids of cranberry tipworm (Diptera: Cecidomyiidae) in British Columbia, Canada” by Daniel A. H. Peach, John T. Huber and Sheila M. Fitzpatrick, to be published in The Canadian Entomologist, will be circulated when the publication process is complete.

**Anticipated funding request in 2012**

In 2012, a student will be required to assist with the field research described above. I intend to request funding for the student and some funding for lab supplies. The DIAP LMHIA budget for 2012–2013 shows a contribution of **$8,050 from the cranberry industry** and a matching contribution of $15,270 from AAFC, for a total of $23,320, less $3,500 overhead to AAFC, leaving $19,820 for the student and supplies. This amount should be adequate.
Acknowledgements

I thank Darsh Banns for hosting the pheromone research trials. I am grateful to the BC Cranberry Marketing Commission and BC Cranberry Growers Association for funding this research in partnership with Agriculture and Agri-Food Canada in 2011.

This study was funded in part by the Investment Agriculture Foundation of B.C. through programs it delivers on behalf of Agriculture and Agri–Food Canada and the B.C. Ministry of Agriculture.

Agriculture and Agri–Food Canada, the B.C. Ministry of Agriculture and the Investment Agriculture Foundation of B.C. are pleased to participate in the delivery of this project. We are committed to working with our industry partners to address issues of importance to the agriculture and agri–food industry in British Columbia. Opinions expressed in this report are those of the author and not necessarily those of the Investment Agriculture Foundation of B.C., the B.C. Ministry of Agriculture or Agriculture and Agri–Food Canada.
References

Voss, K.K. 1996. Studies on the cranberry tipworm (Dasineura oxygenocca (Johnson)) and a predator, Toxomerus marginatus (Say) in Wisconsin. M. Sc. Thesis, University of Wisconsin, Madison, WI.