FINAL PROJECT REPORT
Canola Agronomic Research Program (CARP)

The Final Report should fully describe the work completed for the year and note the personnel involved. It should also note any deviations from the original plan and next and/or corrective steps as may be required if deviations are noted. The report should also provide an update on the status of the Project including forecasted date of completion. A complete statement of expenses should be included. In the event major changes are anticipated within the budget supporting notes along with a proposed budget should also be included. The report should also capture a complete summary of activity for the year.

Project Title: Development of a pheromone-based monitoring system for a newly identified *Contarinia* midge on the Canadian prairies

Research Team Information

Lead Researcher:		
Name	***Institution***	***Project Role***
Boyd Mori	University of Alberta (Formerly AAFC-Saskatoon)	Co-lead
Meghan Vankosky	AAFC-Saskatoon	Co-lead
Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
Daniel Bray	University of Greenwich	Pheromone collection and identification
David Hall	University of Greenwich	Pheromone identification and synthesis

Project Start Date: 1 April 2018 **Project Completion Date:** 31 March 2020

Reporting Period: April 1, 2019 to March 31, 2020

CARP Project Number: 2017.13

Instructions: This Final Project Report shall be completed and submitted on or about March 31st of the fiscal year that the agreement is in effect (upon completion of the project). The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a detailed account upon completion of the project. Final project financial reporting should be provided at this time.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to the CCC contact listed below.

In addition to the Final Project Report, a one-page Research Abstract including rationale, objective, methodology, summary and conclusions (with a summary graph/table or supporting image for the project), acknowledgement and references is due upon completion. The Research Abstract is intended for use in publications such as the *Canola Digest* and the CCC Research Hub and is intended to support messaging to all audiences.

Please include the funding acknowledgements outlined in your research agreement in all deliverables (publications, presentations, etc.) from this project.

1. Date of Completion:

31 March 2020

2. Status of Activity: (please check one)

_____ Ahead of Schedule _____ On Schedule _____ Behind Schedule X Completed

Comment: The project is now complete.

3. Completed actions, deliverables and results; any major issues or variance between planned and actual activities.

The pheromone produced by female canola flower midge (CFM), *Contarinia brassicola* (Diperta: Cecidomyiidae), was identified through a combination of gas chromatography-electroantennographic detection (GC-EAD), gas chromatography-mass spectrometry (GC-MS) and field experiments from 2017-2019.

Summary of Results:

Objective 1: Identify and synthesize pheromone components from *Contarinia brassicola*.

In 2017, midge larvae were collected from several sites in Northeastern Saskatchewan throughout the summer, brought back to the laboratory for rearing, and shipped to Dr. Bray and Prof. Hall at the University of Greenwich. Dr. Bray entrained volatiles produced by both virgin males and females and confirmed male-specific electrophysiological responses to two compounds with GC-EAD (Fig. 1). Prof. Hall then identified the compounds via GC-MS and a coupled gas chromatograph-flame ionizing detector (GC-FID) and synthesized the compounds, 2,7-diacetoxynonane (major component) and 2-acetoxynonane (minor component) (Fig. 2 & 3).

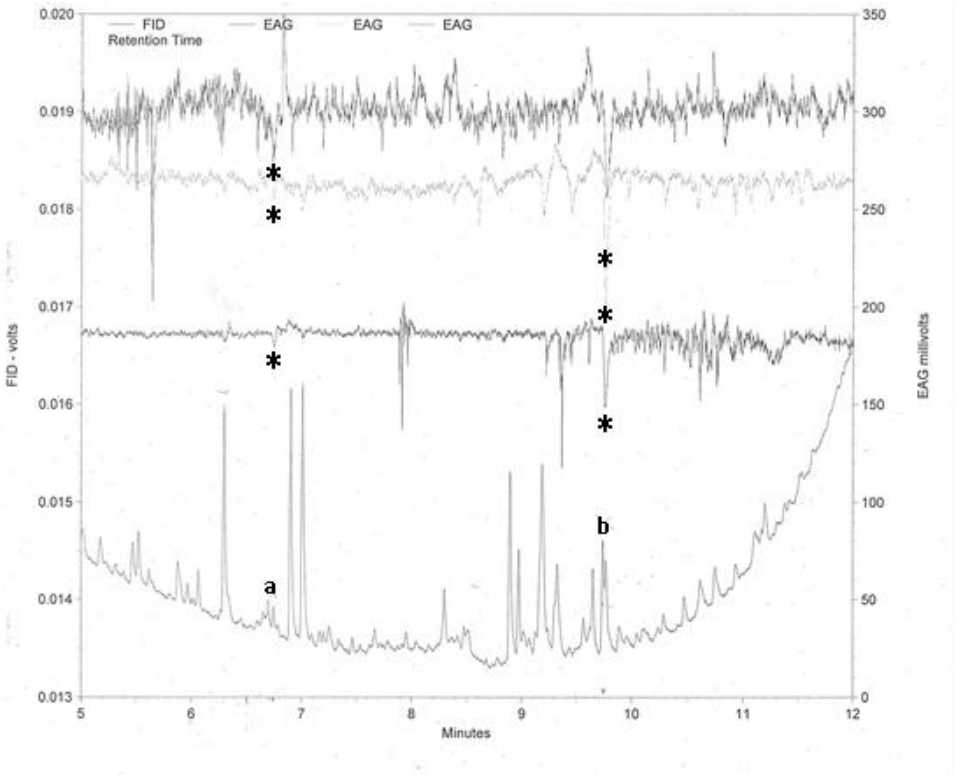

Figure 1. EAD responses (*) of three male *C. brassicola* antennae to peaks in female volatile entrainments initially

identified as: 2-acetoxynonane (minor component, a) and 2,7-diacetoxynonane (major component, b).

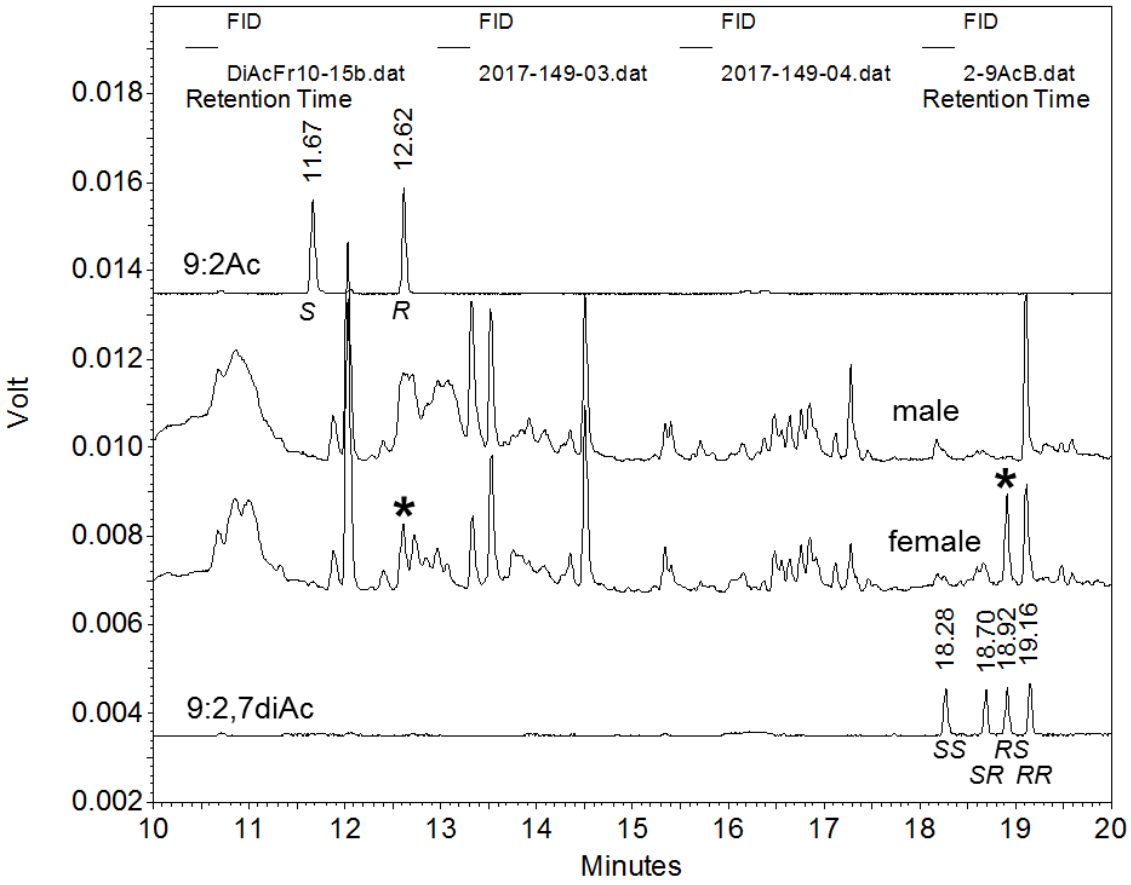

Figure 2. GC-FID on β-Cyclodextrin column of (from top) 2-acetoxynonane, collection of volatiles from male *Contarinia brassicola*, collection of volatiles from female *Contarinia brassicola*, 2,7-diacetoxynonane; * female-specific peaks; assignment of configuration of 2,7-diacetoxynonane according to Hooper et al. (2007).

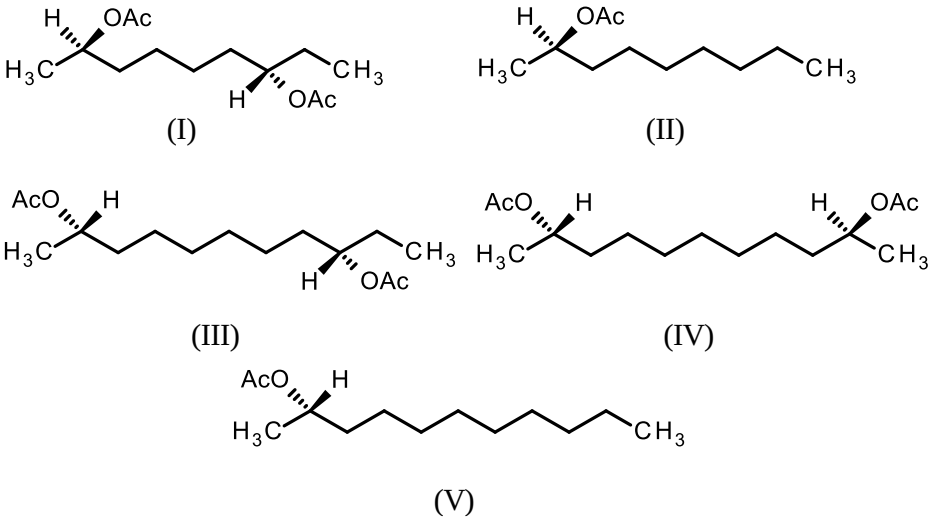

Figure 3. Pheromone components produced by female *Contarinia brassicola* (I: (2*R*,7*S*)-2,7,-diacetoxynonane; II: (2*R*)-acetoxynonane) compared with the known pheromone components of swede midge, *Contarinia nasturtii* (III(2*S*,9*S*)-diacetoxyundecane; IV: (2*S*,10*S*)-diacetoxyundecane; V: (2*S*)-acetoxyundecane).

Objective 2: Optimize the pheromone lure for response of male *Contarina brassicola*.

In 2018, pheromone lures were prepared at the University of Greenwich and shipped to AAFC-Saskatoon for field testing.

Lures consisted of 1: Blank control, 2: Racemic mixture of the 4 isomers of the major component (2,7,-diacetoxynonane), 3-6: each of the 4 isomers presented separately were compared in a field experiment starting on the 28 June 2018 (Experiment 1). The experiment was conducted at 10 sites in north-eastern Saskatchewan, and after one week, the traps, inserts and lures were replaced and trap position randomized. The trap baited with a lure containing the RS isomer caught numerically more male CFM; however, this was not significantly greater than other tested lures (log-transformed data: GLMM $\chi^2 = 15.93$, d.f. = 5, $p = 0.15$) and the total number of midges captured was extremely low (Fig. 4).

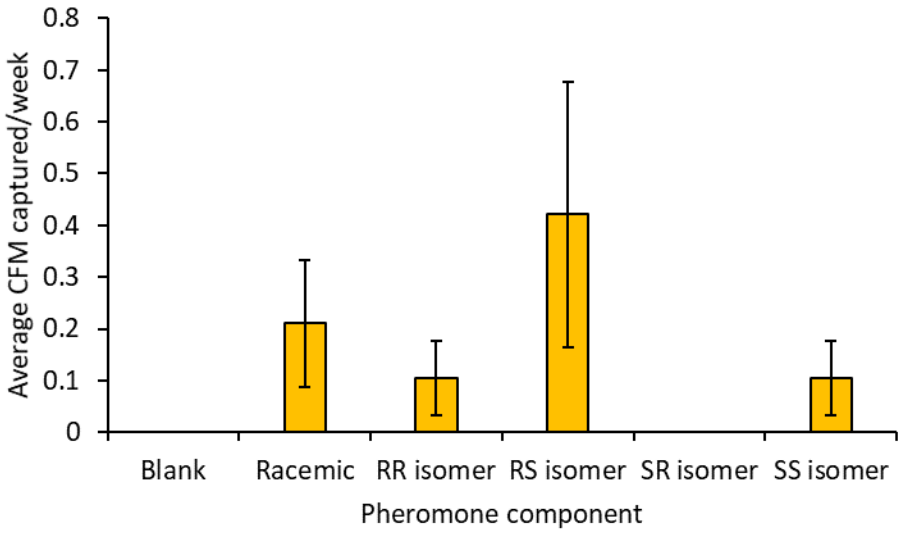

Figure 4. Average male *Contarinia brassicola* (CFM) (± SE) captured per week with traps baited with lures containing different isomers of the major pheromone component (2,7,-diacetoxynonane) or a racemic mixture containing all 4 isomers. There were no significant differences in the number of males captured between any of the treatments.

Further experiments were conducted in 2018 to compare lure formulations and to test the lures at other times during the season, but very few CFM (7 males over 3 weeks) were captured.

In 2019, additional pheromone lures were prepared at the University of Greenwich and shipped to AAFC-Saskatoon for field testing (Experiment 2). Due to the inability of single components of the pheromone blend to attract males, lures containing the RS ((2*R*,7*S*)-2,7,-diacetoxynonane) isomer and varying amounts of the RR isomer ((2*R*,7*R*)-2,7,-diacetoxynonane) of the major pheromone components were tested. In addition, during the first experiment in 2019 a lure containing the two major component isomers (RS and RR), plus the minor component ((2*R*)-2-acetoxynonane) was tested. There were significant differences in the number of male CFM captured by the various lures (log-transformed data: GLMM $\chi^2 = 45{,}275$, d.f. = 6, $p < 0.00001$). The lure containing a 10:1 ratio of the RS and RR isomers captured significantly more CFM males than all other lures tested (Fig. 5). There was no significant difference in the number of males captured with the 10:5 ratio of the major component isomers (RR and RS) and 10:10:1 ratio of RR, RS (major component) and R (minor component); however, both lures captured significantly more males than the blank, RS alone, or 1:1 ratio RS:RR (major components) (Fig. 5).

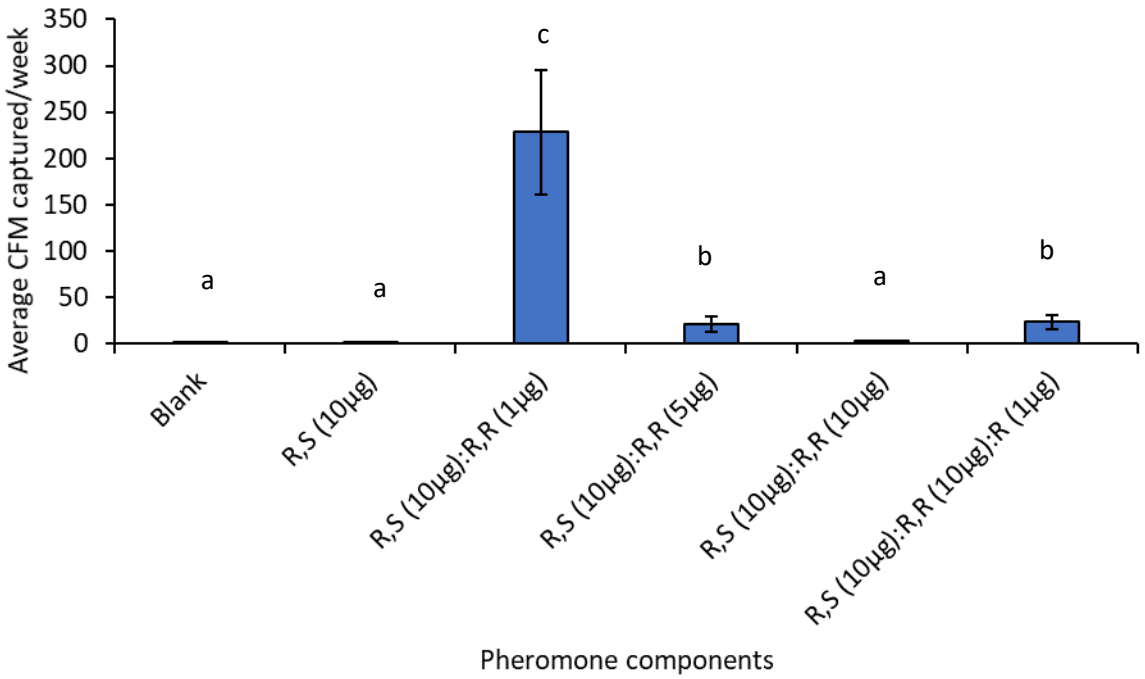

Figure 5. Average number of male *Contarinia brassicola* (CFM) captured (± SE) per week with traps baited with lures containing different amounts of isomers of the major pheromone component (2,7,-diacetoxynonane) or the major component and minor ((2*R*)-acetoxynonane) (Experiment 1). Different letters above the bars indicates significant differences ($P < 0.05$).

Another experiment (Experiment 3), further investigated the difference in CFM male capture while holding the amount of the RS isomer constant and varying the amounts of the RR major component, and R minor component. A 10:1:0.5 ratio of RS and RR major components, and R minor component captured significantly more male CFM than all other tested treatments (Fig. 5) (log-transformed data: GLMM $\chi^2 = 305.1$, d.f. = 6, $p < 0.00001$), including the 10:1 RS:RR lure which was the most attractive in the previous experiment (Fig. 4). All traps baited with lures captured more CFM than the blank control. The 10:1 and 10:2 RS:RR lures captured similar number of males and more than 10:0.5 RS:RR, and 10:0.1 and 10:5 RS:RR (Fig. 6). The 10:0.5 RS:RR lure capture more than the 10:0.1 and 10:5 RS:RR, which captured similar numbers of males (Fig. 6).

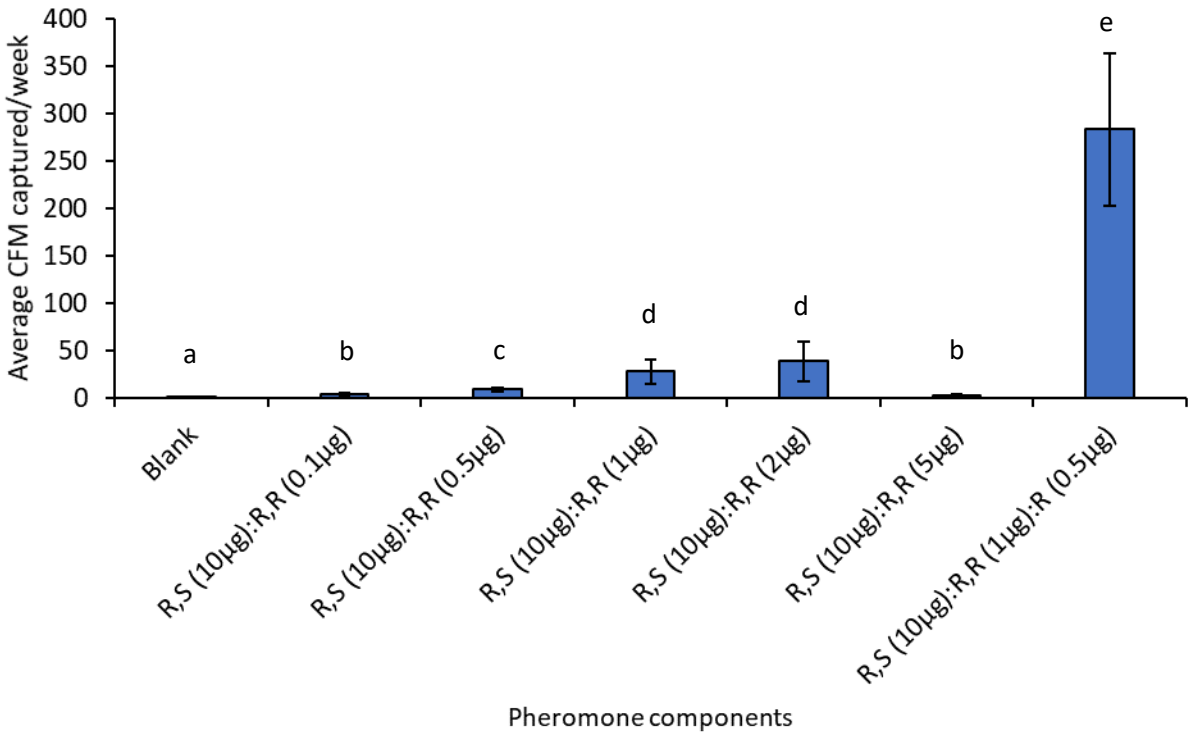

Figure 6. Average male *Contarinia brassicola* (CFM) captured (± SE) per week with traps baited with lures containing different amounts of isomers of the major pheromone component (2,7,-diacetoxynonane) or the major component and minor ((2*R*)-acetoxynonane) (Experiment 2). Different letters above the bars indicates significant differences ($P < 0.05$).

Due to additional time needed to refine the pheromone blend and additional field studies undertaken to refine the blend in 2019, the lure and trapping system for CFM still needs further optimization (Objective 3 – incomplete).

4. Significant Accomplishments

The pheromone components released by calling female C. brassicola have been identified and synthesized. A lure containing a 10:1:0.5 blend of (2*R*,7*S*)-2,7,-diacetoxynonane, (2*R*,7*R*)-2,7,-diacetoxynonane and (2R)-acetoxyundecane is highly attractive to male CFM in field conditions.

Acknowledgements: This research is part of the Canola Agronomic Research Program (CARP Grant 2017.13) with project funding provided by the Alberta Canola Producers Commission (Alberta Canola) and the Saskatchewan Canola Development Commission (SaskCanola). We acknowledge the assistance of S. Hladun, A. Hamilton, J. Smith, J. Kim, and K. Saita in the completion of the fieldwork associated with this project.

5. Research and Action Plans

A second proposal has been submitted to CARP to further optimize the pheromone trapping for CFM, and to determine if pheromone-monitoring traps can be used to monitor population levels in the field.

6. Final Project Budget and Financial Reporting

Please see attached report (to be submitted in March 2020).

Please forward an electronic copy of this completed document to:

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