

Agricultural Research Initiative

Development of lures and traps to monitor and control the cabbage maggot in Newfoundland and Labrador

Final Report

Agriculture Research Initiative

Submitted by

N. Kirk Hillier^{1*}, Peggy L. Dixon², Richard Hopkins³, Leah Madore⁴

*Contact: kirk.hillier@acadiau.ca
Phone: 902-585-1314
Fax: 902-585-1059

1. Department of Biology, Acadia University, 33 Westwood Ave., Wolfville, NS, B4P 2R6
2. Atlantic Cool Climate Crop Research Centre, Agriculture and Agri-Food Canada, 308 Brookfield Road, BLDG 25, St. John's, NL, A1E 0B2
3. Department of Ecology, Swedish University of Agricultural Sciences, Box 7044, 750 07, Uppsala, Sweden
4. Agrifoods Agency, Department of Natural Resources, Production & Market Development, Fortis Tower, Box 2006., Corner Brook, NL, A2H 6J8

Executive Summary

Description The cabbage maggot, *Delia radicum*, is a significant pest of vegetable brassicas such as cabbage, broccoli, turnip and rutabaga both nationally and in NL. Larvae (maggots) can cause stunting, yield loss and even death by feeding in and on roots. This study has investigated the potential for attractant lures to improve trapping technology for cabbage maggot flies by investigating chemical cues used for host choice and oviposition preference by the cabbage maggot. A range of behaviour-modifying compounds were investigated for their efficacy in attracting maggot flies in different, commercially-available trap types. Furthermore, experiments were conducted to determine the overall sensitivity of *D. radicum* to host plant volatiles, and to quantify volatiles being emitted from cabbage and other brassica plants. The overall objective is the development of an affordable, environmentally responsible and sustainable trapping system for this devastating pest.

Background and Rationale for Investigation

Vegetable Industry in Newfoundland and Labrador and Canada

Cole crops are extremely important to vegetable production in the Atlantic area, and are important fresh and processing crops (ref). Turnips and rutabagas also are important crops in Atlantic Canada, both for domestic markets and for export. Nationally, the farm gate value for rutabaga was \$13.6 million in 2002 (AAFC 2010); that for cabbage and broccoli were \$38 and \$32 million in 2005, respectively (AAFC 2005). The vegetable industry in NL was valued at over \$6.0 million in 2006 (NL Agrifoods 2012). The most important vegetable crops produced are potatoes, turnip, cabbage, carrot, and beets. As diets change to reflect more health-conscious consumers, NL producers are starting to change from a reliance on the traditional crops, to include kale, kohlrabi, cauliflower and Chinese vegetables. Whether traditional or new crops are grown, the cabbage maggot affects all vegetable brassicas. Rutabagas, for example, should produce marketable yields of 20,000 to 50,000 kg/ha under good growing conditions with adequate pest control (Anon 2013b); under severe cabbage maggot pressure, this can be reduced by 50-80%.

The cabbage maggot

The cabbage maggot overwinters as pupae in the soil in the field where they fed as maggots; timing of emergence of flies in the spring varies with temperature, soil type, moisture and other factors (Dixon et al. 2013). Newly emerged adults aggregate and mate at nectar sources; carbohydrates are essential for maturation of eggs, and protein is required for full fecundity (Finch and Coaker, 1969). Males stay at aggregation sites, but mated females leave these sites in low level flights (Bomford and Vernon, 2000) to oviposit in fields of host crops, where they are the prevalent sex. Females use chemical and visual cues to locate oviposition habitat. When they find suitable host plants, they deposit their eggs on or near the base, usually just below the soil surface (Dixon et al. 2002).

Economic losses attributable to the cabbage maggot occur in turnip (*Brassica rapa* (L.)), rutabaga (*B. napus* var. *napobrassica* (L.)), and radish (*Raphanus sativus* L.), and in varieties of *B. oleracea* L. including cabbage, cauliflower, broccoli and Brussels sprouts. Among field crops, *D. radicum* attacks canola (*B. napus* L. and *B. rapa oleifera* (DeCandolle) Metzger) and mustards (*B. juncea* L. and *Sinapis alba* L.); of these, *B. rapa* is most and *S. alba* is least susceptible to maggot attack. Feeding can stunt or kill seedlings, or reduce the quality of mature rutabaga and turnip through unsightly scarring or disease entry (Holliday et al. 2013). Because the maggots are found in the soil and within the roots of plants, they are very difficult to target using insecticides, or detect using standard techniques. In conventional production, applications of insecticides at planting or as post-emergence high-volume drenches can protect brassicaceous vegetables from *D. radicum* damage. The only currently-registered active ingredient in Canada is chlorpyrifos (Health Canada, 2012), and resistance and regulatory issues cast doubt on its future.

Use of Insect Pheromones and Semiochemicals in Insect Pest Management

Awareness of the hazards of synthetic insecticide use has resulted in a demand for alternatives to and ways of reducing pesticide use, both publicly (through greater awareness), and in industry (through stricter regulation) (Carson 1962; Sachs 1993). Alternatives to insecticide treatment have evolved from an enhanced understanding of pest biology, and in the case of insect communication, this has led to the exploration of behaviour-modifying chemicals such as insect “semiochemicals” and pheromones. Semiochemicals are chemical substances that carry a message either between insects of the same species, between different species of insects or between insects and plants. An example of the latter is the chemical cues insects use to locate host plants (i.e. host plant volatiles), which can attract insect pests or repel them. The benefits of such products are that they are highly species-specific, non-toxic and rapidly biodegradable (Jutsum & Gordon 1989). Also, such ‘attractive’ or ‘repellent’ compounds may be used to modify behavior of pests and beneficial insects within a push–pull or stimulo-deterrent diversionary strategy (Cook et al., 2007). In such a strategy, pests may be diverted away from high value crops using attractants, while simultaneously being repelled from coincident high-value crops with repellents. Furthermore, natural enemies (predators and parasitoids) of insect pests may be simultaneously attracted, making the use of semiochemicals a much more viable integrated management strategy than broad-spectrum chemical insecticides.

The most widespread and widely documented types of pheromones are sex pheromones, which are used to increase the probability of successful mating (Jutsum & Gordon 1989). These are currently used in pest management for population monitoring, for example in NL sex pheromones are used to monitor the corn earworm and the European corn borer in corn, and the cranberry fruitworm in cranberry. Monitoring is an excellent tool but is not a direct pest control method. However, sex pheromones and other semiochemicals can also be used in “mass trapping” and “mating disruption” (Jones, 1998); in these instances, pest numbers are actually reduced by removal of large numbers from the population (mass trapping) or by making it difficult for males and females to locate one another (mating disruption). In mass trapping, host plant volatiles can be incorporated directly into a lure and placed in a trap or other agent to remove insects from the population (Trimble & Hagley 1988). Although most mass trapping research has focused on moths, there are some examples with flies. In the case of the oriental fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae), the use of baited traps led to its eradication from the Okinawa Islands in 1982 (Koyoma et al. 1984). In addition to direct control, traps can be used as a mechanism to distribute biological control agents, such as fungal pathogens, nematodes and viruses in a directed manner, minimizing their impact on non-target organisms.

Further, the use of attractant trapping technologies has been demonstrated as an effective tool for insect pest management in other wild berry crops in Atlantic Canada, such as lingonberries (partridgeberries) (*Vaccinium vitis-idaea*) in NL. The lingonberry

fruitworm, *Grapholita libertina*, is fruit-boring pest of lingonberries. By developing a synthetic attractant lure based upon female sex pheromones, it is now possible to forecast local infestation levels within a given year, and to monitor population trends over a wide area using pheromone-trapping technology (Hillier et al. 2002, 2003). This provided a simple and inexpensive means of monitoring pest thresholds throughout the province. These studies also provided good correlations between adult trap rates and subsequent larval densities and fruit damage (Hillier et al. 2004). Further research demonstrated potential for this technology in mass trapping techniques, depleting pest populations by removing males from the mating population.

In the current project, we tested the potential for chemically-attractive lures based on host volatiles to improve trapping technology for cabbage maggot flies. In particular, this is of interested to determine when, and how many, flies are moving into a vulnerable crop. Previous studies have documented chemical attractants based upon plant volatiles which are useful in trapping of these species. For example, Ishikawa et al. (1983 and 1984) demonstrated that 2-phenylethanol and valeric acid were effective attractants for the seed corn maggot. Finch and Skinner (1982) found allyl isothiocyanate (a volatile breakdown product of glucosinolates characteristic to Brassicaceae) to be effective in attracting and trapping the cabbage maggot. More recent studies have implicated dimethyl disulfide (DMDS) being released by infested roots as being a potent attractant for predators and parasitoids of the cabbage maggot, while simultaneously inhibiting oviposition by females (Ferry et al. 2009). We tested a range of previously described compounds to develop new, innovative attractants using state of the art technology at Acadia University.

Benefits to Industry and Economic Impacts

Results of this study will enable development of an enhanced trapping system the cabbage maggot based on plant volatiles. This will provide a range of direct benefits to industry including: increased precision in predicting infestation levels; better targeting of spray timing which will reduce the number of sprays required (reduced costs and less environmental damage, less impact on beneficial organisms, including pollinators, and predators and parasitoids which naturally lower cabbage maggot populations); documented information regarding which plant cultivars are most susceptible to infestation may inform planting decisions (i.e. cultivar choice). Over a longer term, this will enable identification of long-term trends (cycles, migration, invasion), which will again be beneficial in crop protection.

Funding and Partnerships

All partnerships and financial contributions must be identified in this section. If this is a multi-year study, project financial information from all years must be included.

Funding

Source (2013-2014)*	Cash	In-Kind	Confirmed
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Acadia University (\$9000) and ACOA-AIF fund (\$8500; MSc student salary)	\$17500		Y
Hillier Laboratory (analysis costs, depreciation of capital infrastructure)		\$5000	Y
Hillier (Time In-kind)		\$4000	Y
Dixon (Time In-kind)		\$2000	Y
Hopkins (Time In-kind)		\$4000	Y
	\$17500	\$15000	
Total Contributions: (39%)		\$33500	Y
Total Provided from AR Initiative: (61%)		\$49905	Y
TOTAL PROJECT VALUE:		\$82405	

Use of funds

Total requested: \$49905 61% of project costs requested from ARI
Cash and In-kind: \$32500 39% of project costs leveraged from other sources
Total Project Value: **\$82405**

*Note that this is a multi-year study which will be continuing during 2014-2015 via support from the ACOA-AIF fund, Acadia University and other future funding applications (i.e. a current funding proposal is under review for support under Newfoundland's current GF2 graduate student funding initiative).

Partnerships (and Key Personnel)

This project has initiated an interprovincial and international collaboration including Acadia University, Agriculture and AgriFood Canada (NL), the NL AgriFoods Agency, and the Swedish Agricultural University, Alnarp, Sweden.

Dr. N. Kirk Hillier, Acadia University (AU)

Dr. Hillier served as project lead, and supervising objectives 3-4 directly, and assisted on other objectives. His laboratory provided much of the infrastructure and technical support required for chemical analysis, volatile collection, and electrophysiological recording. He is director of CABL, one of the most comprehensive laboratory facilities for insect sensory physiology and chemical ecology in North America. He is presently leading a multi-institutional \$7.2M ACOA-AIF project to develop and commercialize insect pheromones and semiochemicals for pest management in forestry and agriculture. This funding is being leveraged, to provide salary for the MSc student for the duration of this project (this past year, and the coming year 2).

Dr. Peggy Dixon, AAFC

Dr. Dixon coordinated on-site field and laboratory assays in NL during the summer field season. This involved direct supervision of the grad student while in NL, as well as oversight of field studies, and infrastructure/technical support for insect rearing, plant propagation and lab/greenhouse bioassays. Her lab was also pivotal in raising and

shipping *Delia radicum* to Acadia for bioassays and chemical analyses. Her research experience is directly relevant to this study. In particular, she has focused on the management of cool climate crop pests, such as the cabbage maggot and various berry pests, which are of significant concern in NL horticulture.

Dr. Richard Hopkins, SLU

Dr. Hopkins has been primarily engaged for intellectual contributions to this project, given his expertise with *Brassica* pest host-plant interactions. He visited the Dixon lab to assist in project design and trap selection for remaining studies. Dr. Hopkins' research interests focus on insect-plant interactions with a focus on insect-host plant selection and induced changes in plants. His research particularly focuses on the *Brassica* system, working on pests such as root maggots on cabbage, cauliflower and canola.

Ms. Leah Madore, NL Agrifoods Agency

Ms. Madore has assisted in project development will act as liaison for future work with the cooperating growers. She will also be involved in project development and technology transfer at its conclusion (Year 2 and beyond).

Mr. Loay Jabre, AU

Mr. Jabre is a Master's student with Acadia University, and will be responsible for most technical aspects of this study. He has an will continue to integrate results from this study into his thesis to be defended in April 2015.

OTHERS

Dr. Carolyn Parsons and Mr. Todd Power, AAFC. Dr. Parsons and Mr. Power provided technical support in Dr. Dixon's entomology lab. Mr. Ryan Oram was also recruited as the summer undergraduate technician at AAFC during the summer months.

Methods and Implementation

Please note that it is not be possible to identify and test novel compounds in a single year study. The actual project extends over two years, and although we were only able to request ARI funding for year 1, objectives were set for both years, with the year clearly indicated (Other funds will be used to cover continuing research in year 2).

The Objectives of this study are as follows:

- (i) Examine factors which influence cabbage maggot host choice;
- (ii) Identify biologically active compounds to which these flies respond. (particularly host plant odours which may be attractive or repellent);
- (iii) Use electroantennography to test the sensitivity of *Delia* spp. to the candidate attractant compounds from objective ii;

- (iv) Compare, in the field, efficiency and accuracy of currently used traps (eg sticky traps, Brassica-eye traps) with and without lures containing attractant compounds

Location All field research was conducted in NL (AAFC and a local farm) and lab assays were conducted at Acadia during the fall and winter.

Objective i. Examine factors which influence cabbage maggot host choice in the field and laboratory

We are examining behavioural host choice by the cabbage maggot to a range of previously described synthetic compounds (eg. allyl isothiocyanate, dimethyl sulphate) and actual host plants, to develop a hierarchy of host preference. This is being conducted using a large two-choice arena and artificial host plants (plates with sticky tanglefoot paste on them and lures dosed with selected compounds). *STILL UNDERWAY.*

Objective ii. Identify biologically active compounds to which these flies respond (particularly host plant odours which may be attractive or repellent):

This objective was focused on identification of volatiles which may influence cabbage maggot oviposition behaviour and host choice. Volatile sampling was conducted in two ways. The first was "Solid Phase Microextraction" (SPME), in which a small absorbent fibre is introduced to a sealed air system surrounding the plant, and which samples the volatiles being emitted. The second was via a push-pull volatile collection system in which a plant is contained in a sealed container (bag, glass jar). Charcoal filtered air is drawn into the system and a pump pulls the air through a solid phase volatile organic compound (VOC) trap filled with an adsorbent such as Porapak Q. This adsorbent trapped and concentrated volatile compounds over the sampling timeframe. Traps were removed and stored at low temp until analysis.

Different VOCs produced by each treatment were analysed . At CABL (Hillier Lab) at Acadia University, VOC samples were rinsed in solvents to produce extracts. These extracts, along with SPME fibre collections, have been analysed by linked Gas Chromatography-Electroantennography identify key compounds (peaks) which are of interest. Gas Chromatography-Mass Spectrometry was used to identify unknown compounds within these extracts (*STILL UNDERWAY*).

Objective iii Use electroantennography to test the sensitivity of *Delia* spp. to the candidate attractant compounds from objective ii.

A series of volatiles identified from volatile extractions and a literature review of a range of Brassica cultivars were screened further using Gas Chromatography-Electroantennography (GC-EAD; a technique which uses an excised insect antenna as a

simultaneous ‘detector’, so when a chromatograph produces a ‘peak’, we may determine how sensitive the insect is to this compound). By comparing peaks between the GC-EAD and GC-MS, we can identify which compounds are of behavioural relevance to *D. radicum* and *D. platura* for future study under lab bioassay or field conditions.

Objective iv Compare in the field, efficiency and selectivity of currently used traps (eg. sticky traps, Brassica-eye traps), with and without lures containing attractant compounds

We compared three different commercial traps (sticky yellow traps, Brassicaeyes and Ecospys) using both commercially-available lures, and 3 mixtures of novel compounds (allyl isothiocyanate, ethyl isothiocyanate, and a combination of Z3-hexenol/Z3-hexenyl acetate). Trap capture was analysed to compare *D. radicum* capture and bycatch between trapping methods.

Timelines, Milestones and Deliverables

ACTIVITY	Timeline	Milestone	Status
Field trapping studies and colony development:	May-Oct 2013	Cabbage maggot will be collected in NL and breeding colonies established (May-June) and maintained through the project (May 2013-Feb. 2014)	Completed (with some challenges)
Chemical analyses:	July 2013- February 2014	Samples of volatiles collected from each treatment in the ‘Behavioural Assays’ will be analyzed by GCMS and contents identified.	Completed
Electrophysiological recording:	August 2013- February 2014	Key compounds of interest (from previous 2 milestones) will be assayed by electroantennography and GC-linked electroantennography and key compounds of interest isolated for each <i>Delia</i> species studied.	Partially completed (underway)
Behavioral assays:	July 2013 – January 2014	Laboratory and greenhouse bioassays for plant oviposition choice completed, and the effects of putative behavior-modifying chemicals documented (NL and NS)	Underway (delayed due to colony challenges)
Data analysis and reporting:	January 2014- March 2014	Summary reporting of data and statistical analyses for year-end reporting.	Completed

Deliverables: A number of tangible and intangible deliverables will be gained from this project. First and foremost, a better understanding of *Delia* spp. host plant preference will be developed which may influence cultivar choice and planting strategies. In addition, this study will specifically examine the chemical ecology of *Delia* spp. to identify attractant which when integrated into a lure, can enable a range of monitoring and control techniques, or repellent compounds which might be useful in preventing attack. This development will provide a direct tangible product for direct application in IPM. Communication and grower education regarding these new technologies will also

be a direct deliverable. Finally, training of highly qualified personnel is another deliverable to the industry and region in general.

Results and Discussion

This section must include all research findings (i.e. tables, graphs) and is an opportunity to validate exactly what was found from your research as well as interpretation of your findings and what they mean. At the end of your discussion you should have discussed all of the results and provided an explanation for your findings complete with references.

(i) Examine factors which influence cabbage maggot host choice;

Progress on this objective has been relatively slow to date. We had significant issues with maintaining a viable colony of *D. radicum* at Acadia University. This meant relying on shipments of flies from AAFC in Newfoundland. Furthermore, females have very low fertility until recently – we suspect this may have been due to exposure to low temps during shipping, or very low humidity conditions (<10%) in the animal care facility at Acadia University.

(ii) Identify biologically active compounds to which these flies respond. (particularly host plant odours which may be attractive or repellent);

To date, the most significant finding has been that isothiocyanate compounds frequently associated with *Brassica* plants (and *Delia* spp. attraction) are produced in very low concentrations from undamaged plants. This has led to two hypotheses which are being examined at present: 1. *D. radicum* are more important as 'secondary' pests, being more attracted to plants which are already under attack or stressed from herbivory from other species; and/or 2. *D. radicum* use volatiles other than isothiocyanates to localize undamaged *Brassica* plants. Both hypotheses are being tested in continuing research during 2015.

A series of behaviourally-relevant compounds have been isolated (Table 1) for further study. We have a compliment of 52 compounds which are present in variable concentrations from *Brassic*as.

Table 1: Compounds of interest isolated from Brassica cultivars	
cis-hex-3-enyl acetate	(E,E)-a-farnesene
cis-hex-3-en-1-ol	β -myrcene
4-pentenyl isothiocyanate	(Z)-3-hexen-1-yl 2 methylbutanoate
2-methylbutylisothiocyanate	dimethyl disulphide
methylthiocyanate	trans-2-hexen-1-ol
5-methylthiopentyl isothiocyanate	undecanal
allyl isothiocyanate	hexyl acetate
4-methylthiobutyl isothiocyanate	3-methylbutanenitrile
prop-1-enyl isothiocyanate	trans-3-hexanal
ethyl isothiocyanate	hexanol-1
nonanal	hexanal
R limonen	2-methylbutanenitrile
S limonene	methallylcyanide
dodecanal	4-(methylthio) butanenitrile
β -citronellol	benzenepropanenitrile
prop-1-enyl isothiocyanate	methanol
α -thujene	ethanol
α + pinene	acetone
α - pinene	pentan-3-one
racemic A pinene	1-Octanol
β -pinene	3-Pentanone
1,8-cineole (aka eucalyptol)	2-methyl anisole
α -terpinene	hexadecanol
sabinene	benzyl benzoate
decanal	(Z)-3-hexen-1-yl 3-methylbutanoate
benzonitrile	β -elemene

- (iii) Use electroantennography to test the sensitivity of *Delia* spp. to the candidate attractant compounds from objective ii;

GC-EAD analysis is being conducted using a 'Master-mix' of components (Table 1) to assay all of these components on a single antennal prep and compare relative sensitivity to each. This aspect is *STILL UNDERWAY*. Furthermore, we are comparing 3 species for relative sensitivity – *D. radicum*, *Delia platura* and *Delia floralis*. Each of these species exhibits differential agricultural importance, and differential preference to various *Brassica* cultivars (and in some instances, other plant species). By using this comparative approach, we will dissect elements of host preference which are dictated by the olfactory system.

An option for this part of the study is to compare *D. radicum* responses with those of a related root maggot species (seed corn maggot, *Delia platura*); this is a generalist which

we would expect to respond differently to specific brassica volatiles, than the cabbage maggot, which is a much more specialised feeder.

- (iv) Compare, in the field, efficiency and accuracy of currently used traps (eg sticky traps, Brassica-eye traps) with and without lures containing attractant compounds.

A series of putative compounds identified from the literature were tested in field trapping in 2013. Several different attractants and 3 different trap designs were tested for their ability to detect *D. radicum* in the field. All *Delia radicum* have been identified and counted from these traps. Key findings:

- 1) Brassic-eye traps, which are touted as the industry standard for trapping flies, were entirely ineffective in trapping *Delia radicum* whatsoever (Figure 1). A single male fly was captured from all sites. Furthermore, it is apparent that these traps may have problems with bycatch of beneficial insects, in that at one site, hundreds of bees were trapped and killed.

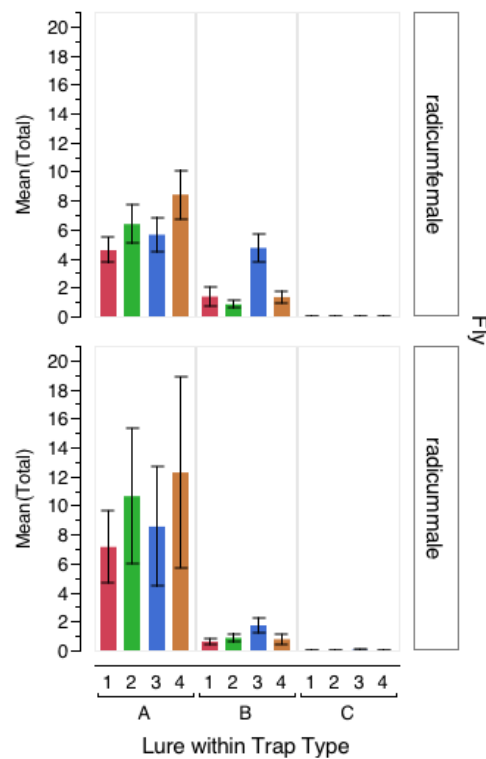


Figure 1: Trap capture of male and female *D. radicum* in A) Yellow sticky card, B) Ecospy, and C) Brassicaeye traps. Lures are labeled as: 1) control, 2) Allyl isothiocyanate, 3) Ethyl isothiocyanate, 4) Z3-hexenol/Z3-hexenyl acetate. Trap capture by sex, and lure were not significant; Trap design was significant ($P < 0.05$).

- 2) Yellow card traps were most effective in trapping large densities of *Delia radicum*, however, these were also confounded by massive amounts of bycatch of other fly species (Figure 2). From an application standpoint, the difficulty in delineating *D. radicum* from other flies makes this undesirable, as it requires expert training and a microscope to identify the correct species.
- 3) EcoSpy traps captured less flies than the yellow card traps, but these traps were much more selective in only trapping *Delia* species of flies. Therefore, year 1 of this study results in the recommendation that this trap type would provide the most reliable estimates of fly densities, without significant confounding bycatch.

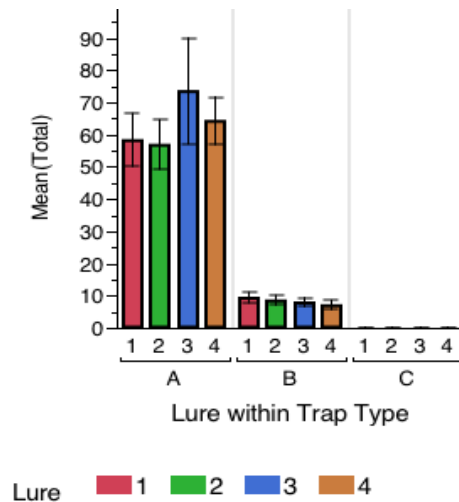


Figure 2: Bycatch of other confounding fly species in A) Yellow sticky card, B) Ecospy, and C) Brassicaeye traps. Lures are labeled as: 1) control, 2) Allyl isothiocyanate, 3) Ethyl isothiocyanate, 4) Z3-hexenol/Z3-hexenyl acetate. Trap capture by lure was not significant; Trap design was significant ($P < 0.05$).

Plans are to replicate this field experiment in 2014, and add a second treatment with new putative attractants identified from electrophysiological studies. Furthermore, we hope to advance additional testing of Ecospy traps and sticky traps, with variable visual cues (green sticky cards). After analyzing the traps in 2014, we hope to have identified a trap-lure combination that is attractive, species specific and practical to use in the fields.

Communications and Outreach

To date, results have not been published in the scientific literature. We do anticipate 2 publications in 2014-2015 based on these results, once we have a second field season completed along with interspecies comparisons of electrophysiological response.

This research has been presented at a regional conference:

Jabre, L.*, Dixon, P.L., and Hillier, N.K. 2013. Using *Brassica* volatiles to develop lures and traps to control the cabbage root maggot. Acadian Entomological Society Meeting, Charlottetown, PEI, August 15th, 2013.

Additional conference presentations will be made at a regional and national scientific meetings in August and September of 2014.

Conclusion and Future Recommendations

In summary, this work has identified a range of volatile compounds which may influence cabbage maggot fly behavior, particularly those which attract females from overwintering sites to new fields. Trapping trials have concluded that commercially available Ecospy traps provide a reasonable balance between detection of *Delia* maggots, and minimizing bycatch, in relation to yellow sticky traps and Brassicaeye traps.

Continuing research in 2014 will further refine the compliment of odorants which are most effective to modify behavior under field conditions. In the future, this research will enhance monitoring strategies, and may validate semiochemical components of brassicas as a means of controlling *Delia radicum* and other cruciferous pests using mass trapping or cutting-edge attract-and-kill technology in the future by integrating an entomopathogenic fungus in traps.

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Appendices

N/A