FINAL REPORT TO
ONTARIO GRAPE & WINE RESEARCH INC.

1. Project Information

- Project Number: 000700
- Project Title: Sustainable practices for repelling MALB and seven spotted lady-beetles from Ontario vineyards.
- Applicant Name: Dr. Rebecca H. Hallett
  School of Environmental Sciences, University of Guelph
- Report Number: Final
- Reporting Period: Year 2
- Date of Submission: March 22, 2011
- Program Coordinator: Dr. Debra Inglis, CCOVI, Brock University

2. Executive Summary (in layman's terms – 1 page maximum)

The introduced biological control agent *Harmonia axyridis* (Coleoptera: Coccinellidae) has attained pest status in North America as its presence in vineyards during harvest may compromise the quality of the resulting wine. The objective of this research program was to identify both attractants and repellents for *H. axyridis*, in order to manipulate beetle behaviour and presence in vineyards. The antennal response of *H. axyridis* to grape (*Vitis vinifera* var. Riesling) volatile compounds was recorded by gas chromatographic-electroantennographic detection (GC-EAD). Compounds that consistently elicited antennal activity were hexanol, linalool, nonanal, and β-caryophyllene. In a four-arm olfactometer, *H. axyridis* were significantly attracted towards β-caryophyllene but displayed no behavioural response to hexanol, linalool, nor nonanal. In a vineyard, sticky traps baited with antennally active compounds, alone and in combination, failed to attract beetles. Sulphur dioxide, in the form of potassium metabisulfite (KMS), was determined to be an effective repellent against *H. axyridis*. In a Y-tube olfactometer, H. axyridis spent significantly less time in the KMS arm (2.5, 5, and 10 g/L) than in the control arm. When sprayed in a vineyard, KMS at 10 g/L consistently reduced the number of *H. axyridis* on grape vines 24 h after application. Future research should establish the duration of KMS repellency and the effect of environmental conditions on repellency. Future studies should

continue to examine the olfactory response of *H. axyridis* to grapes, as the composition of volatile compounds can vary greatly depending on the variety, environmental conditions, and cultural practices.

The intended outcome of this project was the identification of an attractive grape volatile that could be used to attract MALB, as well as identification of a repellent, which could be used together in developing a push-pull strategy to repel MALB from vineyards. As four antennally active compounds were identified, we expected to see behavioural activity to more than one of these compounds. However, none of the compounds proved sufficiently attractive for use in a push-pull program. On the other hand, our results with KMS as a repellent are very promising. As KMS is an acceptable additive during the wine making process, it represents a better choice than some other compounds to use as a repellent of MALB in vineyards.

We expected to be able to test attractive and repellent compounds against seven-spotted lady beetle as well in field trials, as they were reported to be present at significant numbers in the early part of the 2007 harvest, however no seven spotted lady beetles were found in our trials.

3. Detailed Description of the Project

a) Objectives and Project Input

Project Objectives

Project 1.2: To determine behavioral activity of vineyard volatiles to lady beetles chemicals that attract or repel ladybeetles identified using Y-tube olfactometer (Milestone 2).

- 1.2.1 - Olfactory assays of antenally-active grape berry compounds.
- 1.2.2 - Repellency assays with KMS (potassium metabisulfite).

Project 1.3: To develop a push-pull strategy to prevent lady-beetles from entering vineyards (Milestone 3).

- 1.3.1 - Field experiments to determine optimal trap design
- 1.3.2 - Determination of release rates for antennally active compounds.
- 1.3.3 - Field testing of antennally active compounds.
- 1.3.4 - Field evaluation of repellency of KMS.

Project Inputs Identify project inputs i.e. funding level, staff resources, cash and in-kind contributions and other resources utilized towards the completion of the project

- Graduate Student – Erik Glemser
- Research Assistant – Indrajith Wickramananda
- Insect Rearing Assistant - Cara McCreary

b) Project Activities and Outputs

Project Activities

Project 1.2: To determine behavioral activity of vineyard volatiles to lady beetles chemicals that attract or repel ladybeetles identified using Y-tube olfactometer (Milestone 2).

Research conducted under Project 1.1 (Milestone 1) of this project determined that antennae of the multicoloured Asian ladybeetle (MALB), *Harmonia axyridis*, respond to particular volatiles produced by grape berries (*Vitis vinifera* var. Riesling). During Project 1.2 (Milestone 2), olfactometer experiments were conducted with MALB to determine their response to grape volatiles and a putative repellent (potassium metabisulfite, KMS).

1.2.1 Olfactory assays of antenally-active grape berry compounds:

METHODS: A four-arm custom-made olfactometer was used to evaluate the behavioural response of MALB to antennally active compounds (**Fig. 1**). A hole (3.2 cm diameter) in the center of the floor was used to ventilate the arena. Connected to the ventilation hole was a removable tube which allowed introduction of beetles to the arena. A purified, humidified airstream was split into four airstreams using a series of glass Y-tubes connected to the four arms of the olfactometer with chemically resistant Tygon® tubing (4.8 mm I.D.). The arena was ventilated through the hole in the center of the floor using a vacuum pump at a flow rate of ca. 800 mL min-1. Thus, the total flow rate entering the arena (4 × 200 mL min-1) equalled the outflow. The olfactometer arena was placed inside a white tent to eliminate any visual distractions. A 60 W incandescent light was positioned 20 cm above the tent to provide diffuse lighting. Assays were conducted at 22 ± 2°C. Test compounds were randomly assigned for each repetition and were applied to No. 1 filter paper (0.5 x 3 cm, Whatman Inc., Piscataway, NJ, USA) inside a piece of chemically resistant tube which was subsequently connected to one arm of the olfactometer. The behavioural activity of hexanol (≥ 98%), linalool (≥ 97%), nonanal (≥ 95%), and β-caryophyllene (≥ 98%) was tested both individually and combined in a blend. Individually, all compounds were tested at 10 µL against three controls. In addition, β-caryophyllene was tested at 1.6 µL. The blend of compounds tested contained hexanol, linalool, nonanal, and β-caryophyllene in their naturally released ratio of 3 : 0.8 : 1 : 0.9, respectively, as determined by GC-MS. The blend was tested at 10 µL against three controls. In addition, the blend (57 µL) and β-caryophyllene (9 µL) were tested simultaneously through adjacent arms against two blank controls. Technical grade β-caryophyllene (≥ 80%) was also tested for the purpose of using it in field experiments.

Figure 1. Overhead view of the four arm olfactometer. The arena was divided into five areas, one central area (8 cm diameter) and four other areas corresponding to each arm of the olfactometer. Boundaries were superimposed onto the arena using a video monitor. Air was simultaneously pushed into the arena through 4 inflows and drawn through the central hole in the floor of the arena. Test compounds were introduced into one arm while the other three arms were controls. External dimensions: 32 × 32 × 2.5 cm. Internal height of arena: 1.8 cm. Central hole in the floor: 3.2 cm diameter.

Olfactory assays were conducted with laboratory raised adults less than 3 weeks old. Fifty males and fifty females (N=100) were assayed for each treatment; except technical grade β-caryophyllene(only 15 males and 15 females; N=30). MALB were selected at random from rearing cages and each individual was used only once per treatment. Beetles were gently placed into the ventilation tube which was then connected to the bottom of the arena. This design allowed beetles to climb into the arena. Once the beetle entered the arena, its movements were recorded for three min. A camera was placed above the arena and observations made from a monitor. Beetles that failed to enter the arena after two min and that remained motionless for more than two min in the arena were excluded from statistical analyses. The following observations were made: 1) initial direction of the insect upon entering the arena; 2) the first peripheral area entered (i.e. excluding the central area); 3) the total time spent in each area; and 4) the location of the insect at the end of the observation period.

RESULTS: In a preliminary experiment, the response of MALB to no odour was conducted to test for directional biases (N=25). Beetles entered all areas of the arena and showed no evidence that behaviour was influenced by either experimental design or external factors (data not shown).

Harmonia axyridis spent significantly more time in β-caryophyllene (98% at 10 µL: $F_{1, 112}$ = 36.9, P < 0.001; 98% at 1.6 µL: $F_{1, 392}$ = 6.9, P = 0.012; 80% at 10 µL: $F_{1, 392}$ =27.1, P <0.001) than in control fields (**Fig. 2**). In contrast, the proportion of time spent in nonanal ($F_{1, 392}$ = 0.09, P = 0.763), linalool ($F_{1, 392}$ =0.01, P = 0.932), hexanol ($F_{1, 392}$ = 0.35, P = 0.557), and blend (10 µL) ($F_{1, 392}$ = 0.46, P = 0.498) were not significantly different than control fields. When presented with blend (57 µL) and β-caryophyllene (9 µL), *H. axyridis* showed no preference between the blend and β-caryophyllene ($F_{1, 392}$ = 0.87, P = 0.323), blend and control ($F_{1, 392}$ = 2.91, P = 0.089), nor β-caryophyllene and control ($F_{1, 392}$ = 0.40, P = 0.528).

CONCLUSIONS: In olfactory assays, *H. axyridis* was significantly attracted to β-caryophyllene, although a behavioural difference between females and males was detected. At the high dose (10 µL), both sexes were found in β-caryophyllene odour field significantly more than in control fields at the end of the observation period; however, at the low dose (1.6 µL), this difference was significant for males only. Similarly, Verheggen *et al.* (2007) found that *H. axyridis* females were less attracted to β-caryophyllene compared to males.

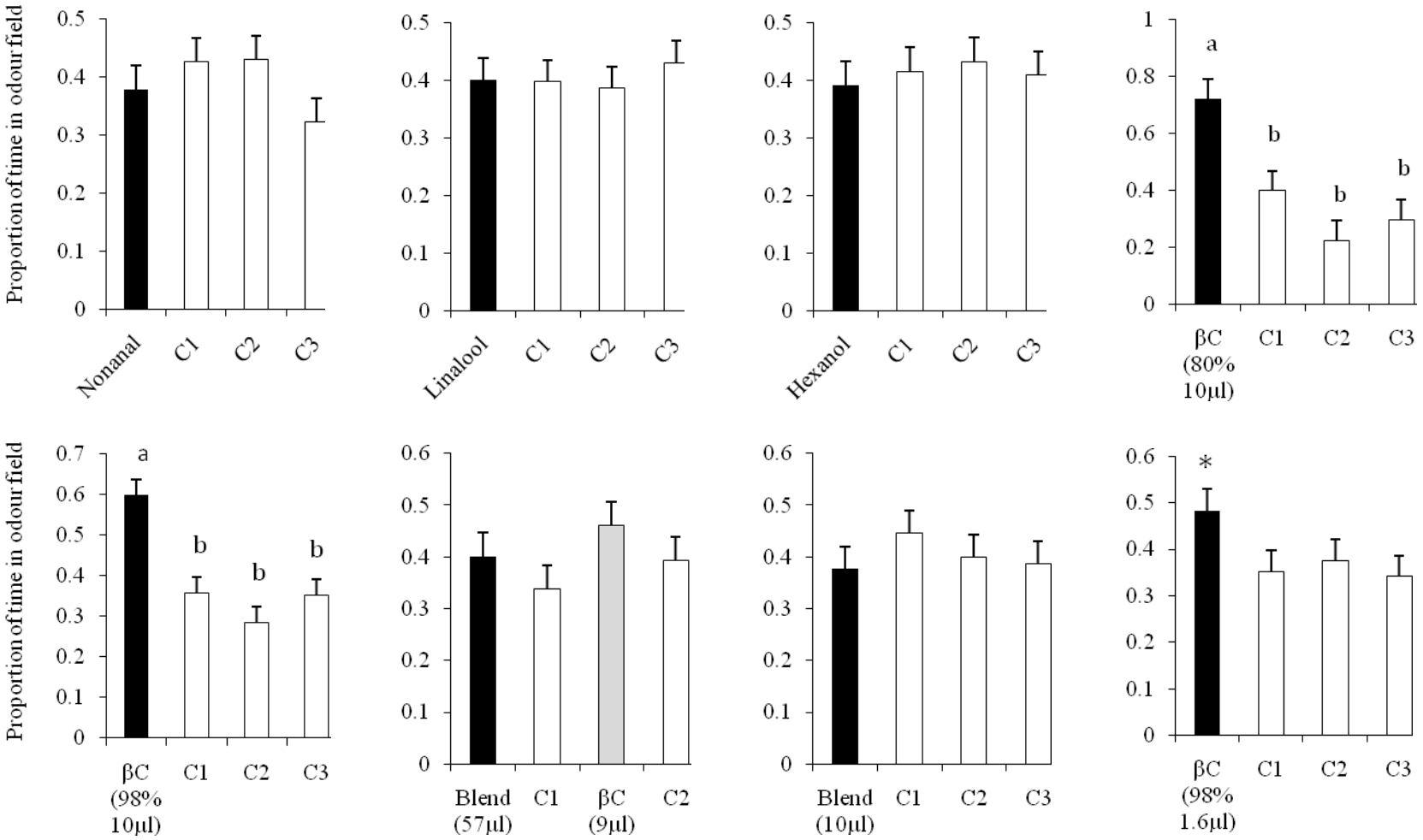


Figure 2. Proportion of time (mean ± SE) *Harmonia axyridis* spent within a four arm olfactometer (*N*=100), where C1-C3 are control arms and βC= β-caryophyllene. Means with different letters are significantly different (Tukey's HSD, α=0.05). * Significant difference was not detected using Tukey's HSD; however, a significant difference was detected when the control fields were pooled and compared to the treatment using ANOVA.

The response of *H. axyridis* to blends containing antennally active compounds is equivocal. *Harmonia axyridis* did not discern between the blend (10 µL) and control fields for any response. In the blend, β-caryophyllene represented 1.6 µL of 10 µL; however, when β-caryophyllene was tested alone, males were significantly attracted to β-caryophyllene at 1.6 µL. Since males were not attracted to the blend, it is possible that their response to β-caryophyllene was inhibited by the other three compounds. It is unlikely that the blend was repellent because *H. axyridis* did not avoid this odour field (Fig. 2). To resolve the response of *H. axyridis* to the blend, another assay was conducted with both the blend (57 µL) and β-caryophyllene alone (9 µL). The amount of blend was increased so that the concentration of β-caryophyllene, in the blend and alone, would presumably elicit a response in both sexes. However, when presented with both blend and β-caryophyllene, *H. axyridis* showed no preference for either blend, β-caryophyllene, or control fields. Based on previous experiments, *H. axyridis* should have preferred β-caryophyllene over control fields but this was not observed. Again, the blend did not appear to attract or repel *H. axyridis*. The overall lack of response may be the result of habituation to a high concentration of compounds within the olfactory arena.

As four antennally active compounds were identified, we expected to see behavioural activity to more than one of these compounds. Field testing should therefore be conducted with β-caryophyllene alone and in combination with the other antennally-active volatiles. As the other volatiles did not exhibit behavioural activity in olfactometer bioassays, it is not necessary to field test them individually for activity, however they may synergize MALB responses to β-caryophyllene when used in combination.

1.2.2 - Repellency assays with KMS (potassium metabisulfite).

METHODS: Potassium metabisulfite (KMS) is a common food and beverage additive that produces sulphur dioxide upon hydrolysis (Rose and Pilkington 1989, Ribéreau-Gayon *et al.* 2006). In the wine industry, sulphur is used as both an antimicrobial and an antioxidant (Baldwin 1951, Ough and Crowell 1987). Sulphur prevents the browning of wine caused by polyphenoloxidase and it inhibits the growth of unwanted lactic and acetic acid bacteria (Wedzicha 1984, Ough and Were 2005). KMS is not yet registered for use in vineyards in Ontario, but growers in Australia spray sulphur dioxide near harvest to control *Botrytis* (Wicks 2002). Use of KMS in vineyards has no negative effect on wine fermentation as residual sulphur dioxide is not detectable in the juice of grapes processed from KMS-sprayed vineyards one day after application (Dowling 2008). In addition, KMS is permitted for use in the wine-making process, and is thus more appropriate than some other chemicals for use in vineyards. There is anecdotal evidence that KMS has an irritant effect on fruit flies (Debra Inglis, Cool Climate Oenology and Viticulture Institute, Brock University, St. Catharines, ON, Canada, personal communication). In this study, the effectiveness of KMS as a repellent against *H. axyridis* was evaluated.

Olfactory assays were conducted in 2009 and 2010 using laboratory-reared and feral-collected beetles, respectively. The laboratory colony was established with beetles collected from ornamental plants around the University of Guelph, Guelph, ON, in 2008, and subsequently maintained in the laboratory at 22 ± 2°C and a photoperiod of L16:D8. Adults and larvae were held in mesh cages and provided *ad libitum* with bird-cherry oat aphids

(*Rhopalosiphum padi* L.) and corn leaf aphids (*R. maidis* (Fitch)) cultured on barley (*Hordeum vulgare* L.). In the autumn of 2010, feral beetles were collected from plants around the University of Guelph, held in mesh cages outside under natural temperature and photoperiod conditions, and provided with a limited supply of aphids. Adults were sexed using external morphological characteristics as described by McCornack et al. (2007). Beetles were selected at random from cages and individuals were tested once per treatment. Olfactory assays were conducted with laboratory-reared adults less than three weeks old, whereas the age of adults collected from the field was unknown. Ten males and 10 females (N=20) were assayed for each treatment in both 2009 and 2010. Individual beetles were gently placed into the end of the Y-tube, which was then covered with nylon mesh to prevent beetles from escaping. Beetle movements were then recorded for 10 min. Beetles that did not move from the base of the olfactometer into one of the arms were considered non-responders and were excluded from analyses. Each assay was conducted until results with 10 responsive beetles of each sex were obtained.

RESULTS: Laboratory-reared beetles were significantly repelled by KMS at all concentrations as they spent significantly more time in control than in KMS arms (2.5 g/L: F = 5.59; df = 1,36; P = 0.024; 5 g/L: F = 41.98; df= 1,36; P < 0.001; 10 g/L: F = 39.32; df = 1,36; P < 0.001) (**Fig. 3**). Similarly, KMS was significantly repellent to feral beetles at all concentrations (2.5 g/L: F = 15.40; df = 1,36; P = 0.004; 5 g/L: F = 16.84; df= 1,36; P = 0.0002; 10 g/L: F = 19.87; df = 1,36; P < 0.001) (**Fig. 3**). For all treatments with both beetle types, neither sex nor the sex*treatment interactions were significant sources of variation. There were 1, 8, and 7 non-responsive lab-reared beetles and 3, 2, and 5 non-responsive feral beetles in assays with 2.5, 5, and 10 g KMS/L, respectively.

Numerically fewer beetles occurred in the KMS odour field than in the control odour field after the first minute of the assay, and this effect was generally more pronounced with increasing concentration of KMS for both lab-reared (2.5 g/L: P= 0.227; 5 g/L: P= 0.031; 10 g/L: P= 0.039) and feral beetles (2.5 g/L: P= 0.070; 5 g/L: P= 0.453; 10 g/L: P= 0.023) (**Fig. 4**). At the end of the assay (i.e. 10 min), more beetles occurred in the control odour field than in the KMS odour field, and this effect was more pronounced with increasing concentration of KMS for both lab-reared (2.5 g/L: P= 0.791; 5 g/L: P= 0.092; 10 g/L: P= 0.002) and feral beetles (2.5 g/L: P= 0.549; 5 g/L: P= 0.227; 10 g/L: P= 0.077) (**Fig. 4**).

CONCLUSIONS: *Harmonia axyridis* was repelled by KMS in lab experiments. Sulphur dioxide is responsible for this repellency as beetles did not make physical contact with KMS in olfactory assays. Sulphur dioxide is highly volatile, and thus is very effective in the lab. In laboratory assays, beetles were significantly repelled by KMS at all three concentrations.

Figure 3. Proportion of time (arcsine square-root transformed; mean ± SE) spent by *Harmonia axyridis* adults in potassium metabisulfite (KMS) (g/L) or control arms of a Y-tube olfactometer. Laboratory-reared and feral beetles were placed individually into the olfactometer and their movements recorded for 10 min. For each experiment, *N*=20. Significant differences between treatments and the control are denoted by an asterisk (*) (Tukey's HSD, α=0.05).

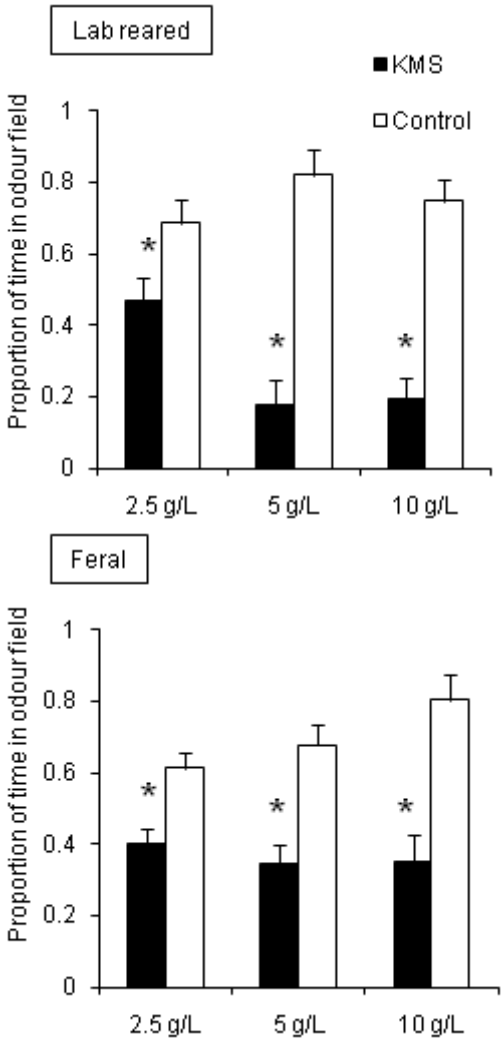
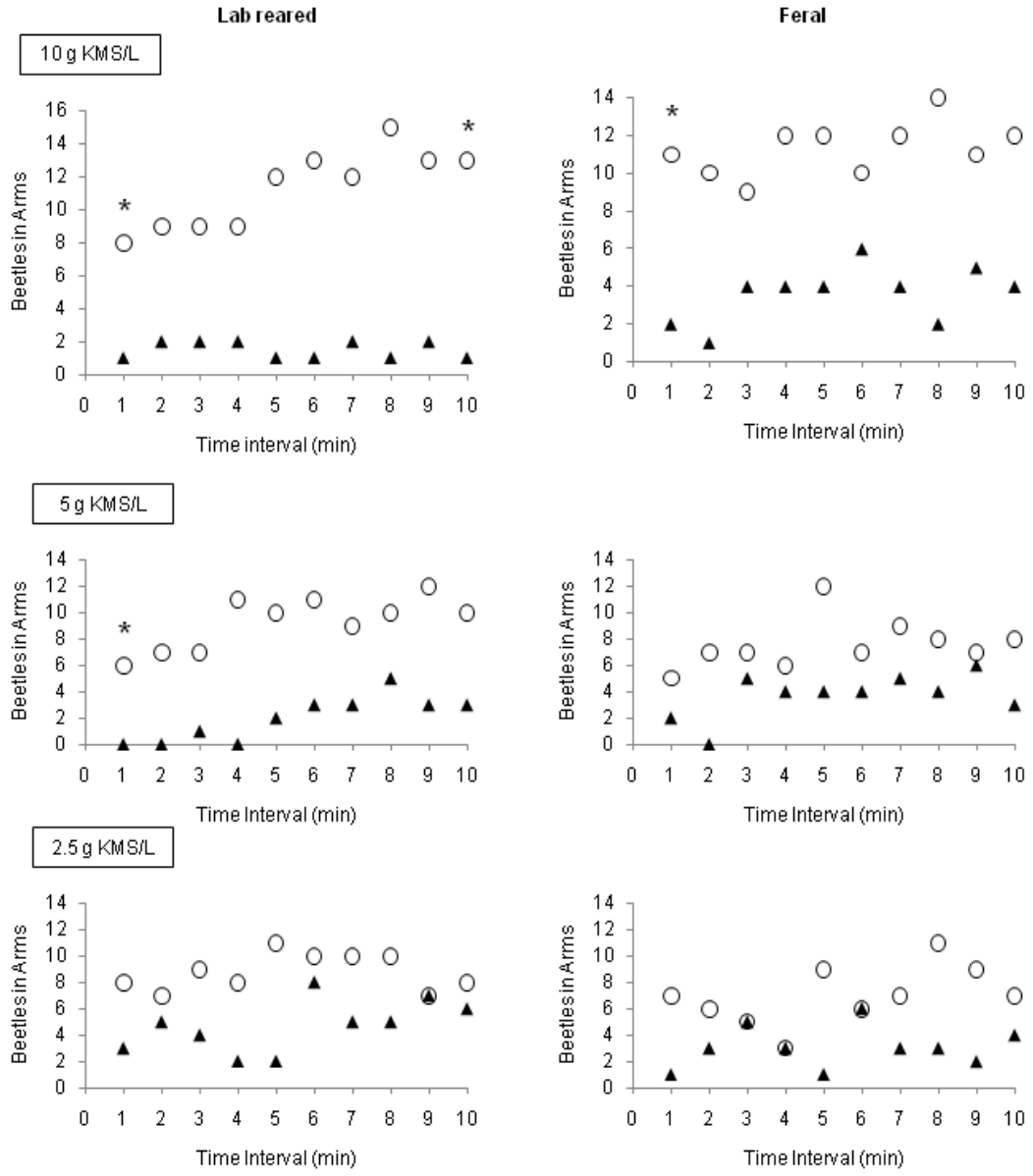


Figure 4. Distribution of laboratory-reared and feral *Harmonia axyridis* adults between potassium metabisulfite (▲) (KMS) (g/L) and control (○) arms of a Y-tube olfactometer over the 10 minute assay period. For each experiment, *N*=20. At specified time intervals, beetles that were located neither in the control or treatment arms but rather in the base of the Y-tube are not illustrated. Fisher's exact binomial test was used to determine if the observed frequency of beetle location in treatment areas deviated significantly from random after 1 and 10 minutes of assay. Significant differences between treatments and the control are denoted by an asterisk(*),(α=0.05).



<u>Project 1.3:</u> To develop a push-pull strategy to prevent lady-beetles from entering vineyards (Milestone 3).

In order to develop a push-pull strategy to prevent presence of lady beetles in vineyards during harvest, we conducted a number of experiments as activities to determine the best type of trap to use for lady beetles, the rates at which attractant volatiles should be released in the field, the attractiveness of β-caryophyllene on its own and in combination with other antennally active volatiles, and evaluation of the repellence of KMS under field conditions.

<u>1.3.1 - Field experiments to determine optimal trap design</u>

METHODS: *Experiment 1: Soap and water traps.* Candidate soap and water traps were evaluated for their ability to capture *H. axyridis*. The experiment was conducted in field cages (2 x 1 x 1 m) on three sampling dates. Five traps were replicated two times on each sampling date in a randomized complete block design. Lindgren funnel, Fruit Fly, Outdoor Fly, and Universal traps (all from Contech, Victoria, BC, Canada) and Rescue!® WHY traps (Sterling, Spokane, WA, USA) were evaluated (**Fig. 5**). Traps were attached to a rope traversing the length of the cage. Traps were separated by ca. 20 cm and suspended 10 cm from the top of the cage. All traps were baited with 1 mL of β-caryophyllene (≥ 80%) held in 2 mL centrifuge vials, except for the WHY traps in which the top and bottom chambers were each baited with 0.5 mL of β-caryophyllene. Traps contained a water and soap (unscented, Seventh Generation, Burlington, VT, USA) solution to drown trapped insects. *Harmonia axyridis* were obtained from the laboratory colony. One hundred *H. axyridis* (unsexed) were released into each cage. The number of beetles captured was recorded after 24 h.

Experiment 2: Sticky traps. Candidate sticky traps were evaluated for their ability to capture *H. axyridis*. Experiments were conducted in field cages (2 x 1 x 1 m), with four traps each replicated five times in a randomized complete block design. Sticky traps included: double sided card (10 x 22 cm), milk carton (7 x 19.5 cm), flower pot (15.2 cm), and mailing tube (7.6 x 20 cm). Traps were painted with Tremclad® sunbeam yellow (Tremco, Toronto, ON, Canada) and subsequently covered with adhesive glue (The Tanglefoot Company, Grand Rapids, MI, USA). Traps were attached to a rope traversing the length of the cage. Traps were separated by ca. 20 cm and suspended 10 cm from the top of the cage. Each trap was baited with 1 mL of β-caryophyllene (≥ 80%) in a 2 mL centrifuge vial attached to the top of the trap. *Harmonia axyridis* were obtained from a laboratory colony as previously described. One hundred *H. axyridis* (unsexed) were released into each cage. The number of beetles captured was recorded after 24 h.

Experiments 3 and 4: Sticky trap vs. soap and water trap. Traps that captured the greatest number of *H. axyridis* in the previous two experiments were compared. The sticky tube and WHY traps (unaltered) were compared in Exp. 3 , and a further experiment (Exp. 4) was performed with the WHY trap painted yellow so that both traps were the same colour. Two traps were replicated six times in a randomized complete block design. Both experiments were conducted as previously described; however, traps were separated by ca. 30 cm. *Harmonia axyridis* were obtained from the laboratory colony as previously described. One hundred *H. axyridis* (unsexed) were released into each cage. The number of beetles captured was recorded after 24 h.

Figure 5. Candidate soap and water traps that were evaluated for their ability to capture *Harmonia axyridis*. The follow traps were tested: (A) Contech® Fruit Fly trap; (B) Rescue!® WHY trap; (C) Contech® Outdoor Fly trap; (D) Lindgren funnel trap; (E) Universal trap.

RESULTS. In Exp. 1, the WHY trap captured significantly more *H. axyridis* compared with the Fruit Fly, Outdoor Fly, and Universal traps, but not significantly more than the Lindgren trap (**Table 1**). In Exp. 2, the number of beetles captured was not significantly affected by trap type; however, the mailing tube had the highest mean captures. Therefore, both the mailing tube and WHY trap were selected for further testing. In Exps. 3 and 4, the mailing tube captured more beetles than the WHY trap; however, the difference was significant only in Exp. 4 (Table 1).

CONCLUSIONS. As the yellow mailing tube was superior to the yellow WHY trap, which in turn was superior to all other trap types tested, we concluded that yellow mailing tube traps are best for use in capturing MALB. Yellow mailing tube traps were used in all further experiments.

Table 1. Number (mean ± SE) of *Harmonia axyridis* captured in candidate traps baited with β-caryophyllene (Exp. 1, 3, 4: *N*=6; Exp. 2: *N*=5). In Exp. 2, means were standardized based on trap surface area (cm^2). One hundred beetles (unsexed) were release into each cage and capture rates were evaluated after 24 h. Means within the same experiment followed by the same letter are not significantly different, Tukey's HSD, α=0.05.

Trap	Trap colour	Mean (± SE) No. of beetles captured	
Exp. 1			
Fruit fly	Clear	0.0 ± 0.0	b
Lindgren funnel	Black	2.3 ± 0.4	ab
Outdoor fly	Clear	0.2 ± 0.2	b
Universal	Green	1.7 ± 0.6	b
WHY Rescue	Clear	6.3 ± 2.3	a
Exp. 2			
Card	Yellow	0.9 ± 0.2	
Flower pot	Yellow	1.0 ± 0.2	
Mailing tube	Yellow	1.3 ± 0.3	
Milk carton	Yellow	0.6 ± 0.2	
Exp. 3			
Mailing tube	Yellow	9.2 ± 3.3	
WHY Rescue	Clear	4.5 ± 1.7	
Exp. 4			
Mailing tube	Yellow	7.3 ± 1.5	a
WHY Rescue	Yellow	2.8 ± 0.8	b

1.3.2 - Determination of release rates for antennally active compounds.

METHODS. The evaporation rate of all four compounds was determined using four chemical dispensers. Chemical dispensers consisted of a cotton wick and a reservoir vial (2 mL centrifuge vial [Fisherbrand, Ottawa, ON, Canada] or 4 mL culture vial [Simport, Beloeil, QC, Canada]). Four mL vials contained 3.5 mL of test compound, while 2 mL vials contained 1.5 mL. Cotton wicks (9 mm diameter, Paterson Dental, Montreal, QC, Canada) were inserted into the vials so that it touched the bottom of the vial and extended 1 and 2 cm above the top of the vial. Dispensers were replicated four times for each test compound. The initial weight of the dispenser was recorded. Dispensers were held inside a fume hood at 22 ± 2°C and were weighed every 24 h until their weights were approximately constant.

RESULTS. The evaporation rate of β-caryophyllene was relatively stable and decreased slowly over time (**Fig. 6**). In contrast, evaporation rates from dispensers containing linalool, hexanol, and nonanal generally followed first-order kinetics where evaporation is dependent on the amount of chemical remaining (see **Fig. 7**) (Leonhardt *et al.* 1990). Following these dynamics, evaporation is initially high but greatly decreases over time as the amount of chemical in the dispenser is reduced.

CONCLUSIONS. These results were used to determine which release devices should be used in field experiments with potential attractants (see Section 1.3.3. below) in order to achieve desired release rates and ratios of test compounds.

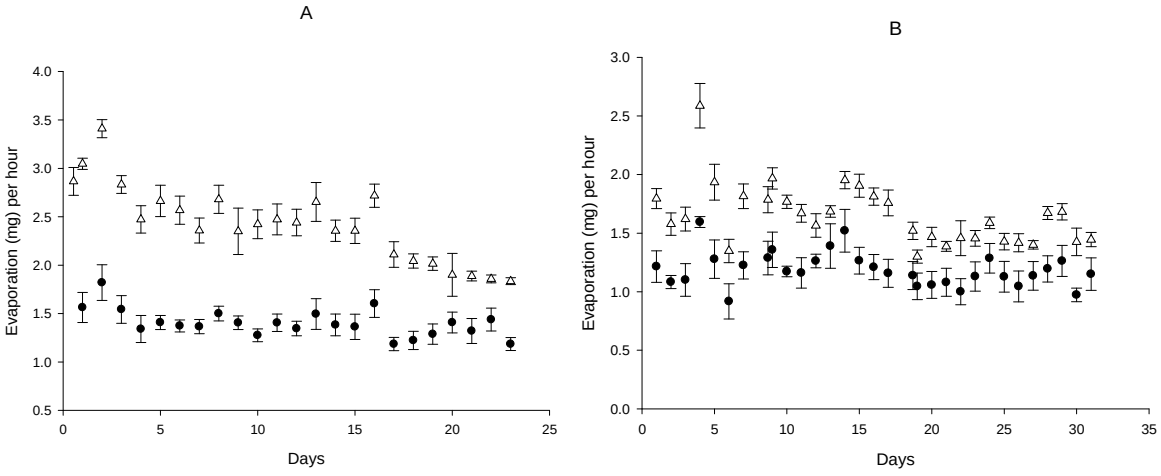


Figure 6. Evaporation (mean ± SE, *N*=3) of β-caryophyllene from chemical dispensers, A: 2 mL vial; B: 4 mL vial. • = 1 cm cotton wick; Δ = 2 cm cotton wick.

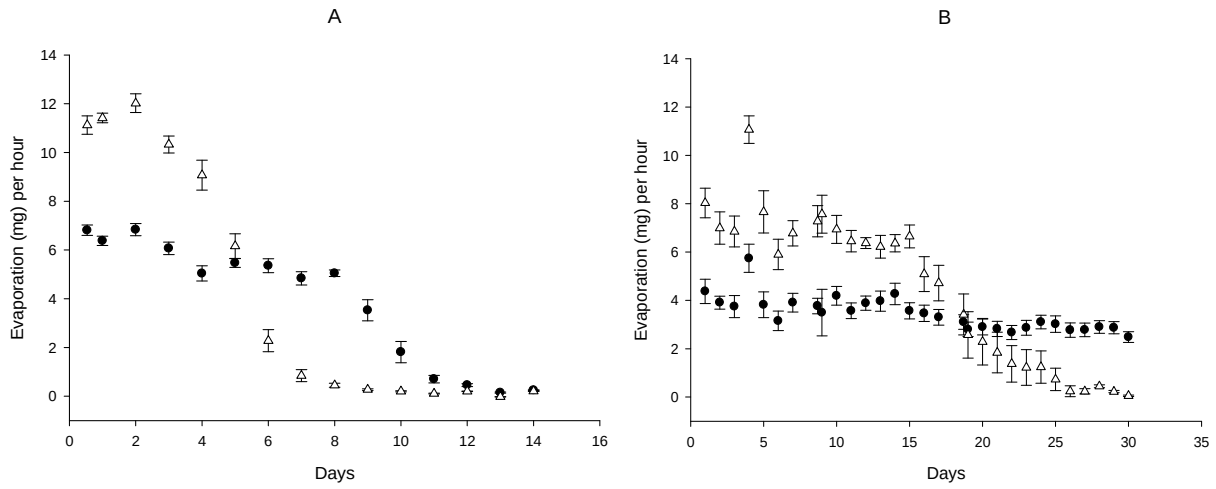


Figure 7. Evaporation (mean ± SE, *N*=3) of linalool from chemical dispensers. A: 2 mL vial; B: 4 mL vial. • = 1 cm cotton wick; Δ = 2 cm cotton wick.

1.3.3 - Field testing of antennally active compounds.

β-*Caryophyllene Dose-Response Field Test*.

METHODS. A preliminary experiment was conducted in a soybean field (43°22'N, 80°23'W) near Cambridge, ON, from Aug. 22 – 26, 2009. The field had not been sprayed with insecticides for several months. Three treatments (i.e. release rates of β-caryophyllene) and a control were replicated five times in a randomized complete block design. Treatments and replicates were separated by 5 and 10 m, respectively. Traps were at least 20 m from the edge of the field. Traps (7.6 x 25 cm diam. x length) were constructed from acrylonitrile butadiene styrene (ABS) tubes. Traps were painted sunbeam yellow and subsequently covered with adhesive glue. Traps were attached to wooden posts and positioned ca. 10 cm above the soybean canopy. β-Caryophyllene (≥ 80%) was released from cotton dispensers at three rates, ca. 1, 10, and 100 mg/h. Dispensers were attached above the trap, so that the cotton wick was exposed. To protect the dispensers from rain, a Petri dish (15 cm diameter) was positioned 2 cm above the cotton wick. Traps were replaced every two days. Harmonia axyridis were sexed and counted in the laboratory.

RESULTS. The release rate of β-caryophyllene did not significantly affect the number of H. axyridis captured using either yellow or black coloured traps (**Table 2**).

CONCLUSIONS. MALB was not attracted to β-caryophyllene in field experiments. In dose-response experiments, the rate of β-caryophyllene (1, 10, or 100 mg/h) did not affect the number of H. axyridis captured on sticky traps. In the first experiment sticky traps were coloured yellow which is attractive to many coccinellids (Mensah 1997; Dowell and Cherry 1981). Thus, traps utilized both visual (yellow) and olfactory cues (β-caryophyllene). Since control and β-caryophyllene-baited traps captured an equal number of H. axyridis, we hypothesized that the visual cue was more attractive than the olfactory cue. To test this hypothesis, the experiment was repeated and the yellow traps were substituted with black traps. Nevertheless, results from this experiment were similar to those in the first experiment:

Table 2. Number (mean ± SE) of *Harmonia axyridis* captured trap^{-1} day^{-1} (Exp. 1: *N*=5; Exp. 2: *N*=4). Sticky traps were baited with β-caryophyllene. Both experiments were conducted in a soybean field (43°22'N, 80°23'W) near Cambridge, ON.

	Mean ± SE	
Release rate (mg/h)	Exp. 1: Yellow traps	Exp. 2: Black traps
Control	4.2 ± 0.6	0.7 ± 0.2
1	3.7 ± 0.8	0.7 ± 0.3
10	3.9 ± 0.6	0.9 ± 0.2
100	4.3 ± 1.1	0.9 ± 0.3

H. axyridis was not attracted to traps containing β-caryophyllene. The total number of H. axyridis captured with yellow traps was greater than with black traps, suggesting that ladybeetles are more attracted to yellow than to a silhouette. This suggests that *H. axyridis* was responding positively to the visual cue in the first experiment. Other studies involving β-caryophyllene have reported similar results (Nalepa et al. 2000). β-Caryophyllene elicits significant antennal activity in the coccinellid *C. maculata*; however, in field experiments, *C. maculata* was not attracted to traps containing β-caryophyllene (Zhu et al. 1999).

There are a several explanations as to why *H. axyridis* did not respond to β-caryophyllene in the dose-response field experiments. It is possible that β-caryophyllene is one component in a blend of compounds that are attractive to *H. axyridis*. There are numerous examples where the compounds in a pheromone or host odour are not attractive individually, but when combined in a specific ratio are attractive. In addition, behavioural responses of insects may be influenced by a number of factors including physiological conditions and time of year. Since *H. axyridis* was not attracted to β-caryophyllene in this dose-response experiment, we were unable to identify the most attractive concentration. Thus, for further field experiments, we selected a concentration of β-caryophyllene that was ecologically and biologically reasonable.

Field-Testing of Antennally Active Compounds in a Vineyard

METHODS. The experiment was conducted at a commercial vineyard (43°39'N, 79°26'W) in Beamsville, ON, for five weeks from Sep. 20 to Oct. 25. The 2.3 ha block of Gewürztraminer grapes was located below the Niagara escarpment. Rows were oriented in a south-north direction and separated by ca. 2.5 m. Vines were planted in 1999 and were separated by ca. 1.2 m within rows.

This experiment compared attraction of β-caryophyllene alone or in combination with hexanol, nonanal, and/or linalool in all possible combinations as follows: (i) β-caryophyllene, (ii) β-caryophyllene + hexanol, (iii) β-caryophyllene + nonanal, (iv) β-caryophyllene + linalool, (v) β-caryophyllene + hexanol + nonanal, (vi) β-caryophyllene + hexanol + linalool, (vii) β-caryophyllene + nonanal + linalool, and (viii) β-caryophyllene + all compounds. Nine treatments were replicated three times in a randomized complete block design. Treatments and replicates were separated by ca. 10 and 21 m, respectively. Traps were at least 10 m from the edge of the vineyard.

Chemicals were released from dispensers with a cotton wick (1 cm) and a reservoir vial. The vial size and length of the cotton wick were adjusted in order to obtain the desired release rate. Nonanal (3.4 mg/h), linalool (4.1 mg/h), and hexanol (11.4 mg/h) were dispensed from 4 mL vials, while β-caryophyllene (3.0 mg/h) was released from two 2 mL vials. Dispensers containing nonanal, linalool, and hexanol were replaced every week, while those containing β-caryophyllene were replaced biweekly. Dispensers were attached to the top of the trap, such that the cotton wick was fully exposed. To protect the dispensers from rain, a Petri dish (15 cm diameter) was positioned 2 cm above the cotton wick. Yellow sticky traps (see Exp. 5) were attached to the top of row posts, with the bottom of each trap ca. 1.6 m above the ground. Sticky traps were replaced twice per week. Coccinellids were counted and identified to species in the laboratory; *H. axyridis* were also sexed.

RESULTS. *Harmonia axyridis* accounted for nearly 99% of all captured coccinellids; consequently, all other species were excluded from statistical analyses.

The number of *H. axyridis* captured by sticky traps was significantly influenced by date ($F_{9, 178}$ = 33.88, P = < 0.001); however, neither treatment ($F_{8, 178}$ = 0.33, P = 0.953) nor date*treatment interactions ($F_{72, 178}$ = 0.83, P = 0.811) were significant (**Fig. 8**). Similar results were obtained when sexes were analyzed separately (results not shown). The total number of *H. axyridis* captured per day was highly variable from day to day.

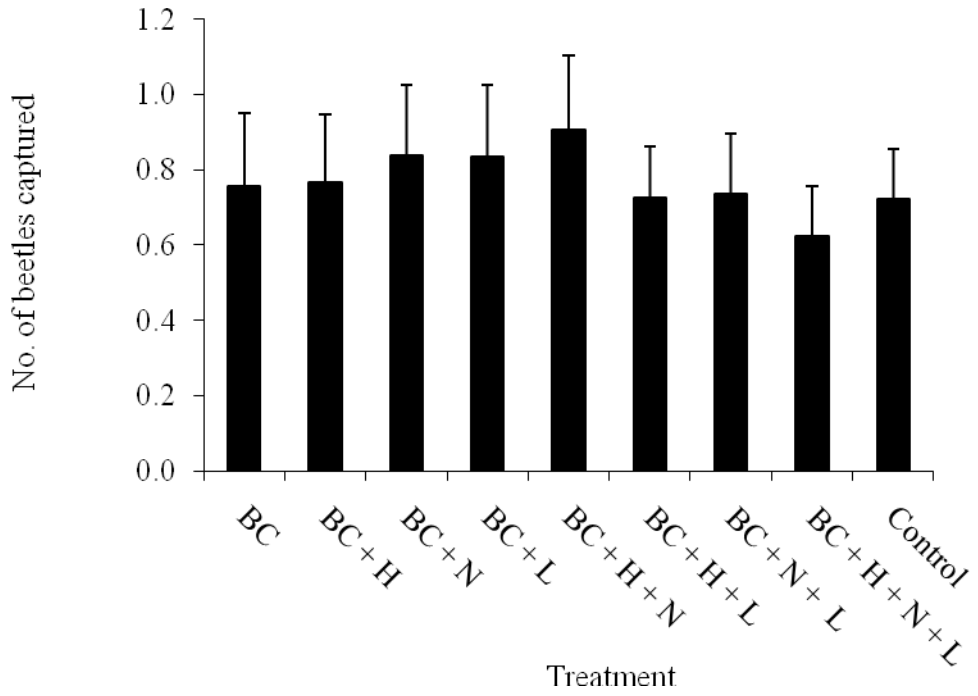


Figure 8. Number (mean ± SE, *N*=10) of *Harmonia axyridis* captured $trap^{-1}$ day^{-1}. Sticky traps were baited with antennally active wine grape volatiles, where BC=β-caryophyllene (3.0 mg/h), H=hexanol (11.4 mg/h), N=nonanal (3.4 mg/h), and L=linalool (4.1 mg/h). The experiment was conducted at a vineyard (43°39'N, 79°26'W) in Beamsville, ON.

CONCLUSIONS. From this study there is no evidence to suggest that *H. axyridis* aggregate in vineyards in response to olfactory cues associated with grape volatiles. Instead, this occurrence may result from other factors or mechanisms. It is possible that *H. axyridis* orient towards visual cues associated with vineyards. During their dispersal flight, the landing behaviour of *H. axyridis* is influenced by visual cues in a stepwise sequence (Nalepa et al. 2000). The dispersal flight is initially guided by orientation towards large geographic features (macrosites) that form contrasting silhouettes on the horizon (Hagen 1962, Nalepa et al. 2000). As the macrosite is progressively resolved, *H. axyridis* orient towards and subsequently land on linear, dark contrasts located within the macrosite (Nalepa et al. 2005). In the Niagara wine region the dominant landscape feature is the Niagara escarpment, with vineyards predominantly located below the Niagara escarpment while soybean fields are found above the escarpment. Large populations of *H. axyridis* develop above the escarpment during the summer. In the autumn, *H. axyridis* are observed flying off the top of the escarpment. As beetles disperse from the top of the escarpment they pass over vineyards below. Vineyard rows form linear, dark contrasting patterns and may provide visual stimuli for *H. axyridis* and promote landing.

Alternatively, *H. axyridis* may enter vineyards randomly as they are highly mobile and travel long distances during dispersal flight (Obata et al. 1986). After entering vineyards, *H. axyridis* may encounter damaged grapes which are rich in sugars (Plocher and Parke 2001). Contact with grape sugars would presumably function as an arrestant to searching behaviour, similar to honeydew. When aphids feed, they excrete honeydew, a substance that is rich in sugars (Klingauf 1987). Honeydew is a food source for hundreds of insect species, including predatory coccinellids (Hagen 1962). Numerous studies have shown that the abundance of coccinellids significantly increases in the presence of honeydew, natural or artificial (Evans and Richards 1997, Wade et al. 2008). There have been conflicting reports on the role of honeydew; some studies have found that honeydew functions as an arrestant (Carter and Dixon 1984, Hagen 1986, McEwen et al. 1993), while others have suggested that it is attractive to beneficial insects (Saad and Bisshop 1976, Dean and Satasook 1983). Regardless of the mechanism, sugars from damaged grapes would likely contribute to an increased the abundance of *H. axyridis*. Furthermore, since *H. axyridis* are unable to break through the outer cuticle of grapes, their distribution would be influenced by the occurrence of damaged grapes.

It has been suggested that coccinellids utilize pheromones in long range detection of overwintering sites (Yakhontov 1938, Edwards 1957). This assumption is based on observations that *H. axyridis* return to overwintering sites from previous years. There is evidence to suggest that β-caryophyllene functions as an aggregation pheromone for *H. axyridis*. β-Caryophyllene is a sex-specific compound released by aggregating and overwintering *H. axyridis* females (Brown et al. 2006). In Coleoptera, the sex that produces and releases a pheromone is less sensitive to it than the responding sex is (Dickens 1986). Correspondingly, the antennal and behavioural response of males to β-caryophyllene is significantly greater than those of females, as found in this study. Despite these results, studies have yet to produce convincing evidence that pheromones such as β-caryophyllene facilitate either long-distance orientation or aggregation in *H. axyridis* (Nalepa et al. 2000). Instead, orientation to aggregation sites appears to be influenced by visual cues, and may explain the return of *H. axyridis* to overwintering sites from previous years. Provided that the landscape does not change, coccinellids will continue orienting to the same macrosites year after year (Obata et al. 1986).

Ladybug taint is not limited to *H. axyridis* as other species of coccinellids contain pyrazines (Cudjoe et al. 2005). The sensory profile of Vidal and Cabernet Sauvignon wines tainted with *Coccinella septempunctata* is consistent with *H. axyridis* ladybug taint (Pickering et al. 2010). Though the presence of other coccinellids in vineyards is a concern, results from this study indicate that the threat posed by other species of coccinellids is negligible. In total, sticky traps captured 700 *H. axyridis*, five *Hippodamia varigata*, and three *C. septempunctata*. Furthermore, during a survey of grape vines (100 vines on both Oct. 17 and 29) a total of 1,254 *H. axyridis* were observed and no other species of coccinellid was found. The abundance of *H. axyridis* in vineyards is the result of earlier season population dynamics in soybean fields. Early in the growing season the most common coccinellid is *C. septempunctata*, but as the season progresses *H. axyridis* becomes the dominant coccinellid in soybean fields (Zhu and Park 2005).

Olfactory cues associated with Riesling grapes were generally not attractive to *H. axyridis*; however, grape varieties are distinct and produce a wide range of aromatics (Schreier et al. 1976). Future work should continue to examine the olfactory response of *H. axyridis* to grapes as the composition of volatile compounds can vary greatly depending on the variety, environmental conditions, and cultural practices (Sànchez-Palomo et al. 2005, Ribéreau-Gayon et al. 2006). In addition, future studies should consider testing a combination of olfactory and visual stimuli since *H. axyridis* utilize both during prey searching, and dispersal to overwintering sites is influenced predominantly by visual cues.

1.3.4 - Field evaluation of repellency of KMS.

METHODS. Two field experiments were conducted to evaluate repellency of KMS in a 2.3 ha wine grape (*Vitis vinifera* var. Gewürztraminer) vineyard located below the Niagara escarpment in Beamsville, ON. The Niagara escarpment runs along the south side of the vineyard (ca. 20 m). Rows were oriented in a south-north direction and separated by ca. 2.5 m. Vines were planted in 1999 and separated by ca. 1.2 m. Experiments were located on the south side of the vineyard between the second and third posts to avoid potential edge effects. Treatment plots consisted of a 7 m row length (i.e. post to post) and included 5-6 vines. Treatments were arranged in a randomized complete block design and in each experiment treatments were replicated five times.

In the first experiment, three KMS treatments were applied at 2.5 (½x field rate), 5 (field rate), and 10 g/L (2x field rate) on 17 October. In the second experiment, two KMS treatments were applied at 5 and 10 g/L on 29 October. Control vines were sprayed with water alone. KMS or water alone was applied as a foliar spray using a carbon dioxide–pressurized precision plot sprayer (R & D Sprayers, Opelousas, LA) at 350 kPa in water equivalent to 1000 L/ha. A four nozzle (Tee Jet 8002VS) boom spray was used which allowed for uniform coverage of the entire vine (i.e. cane/trunk, fruit, and foliage). Plots were sprayed on both sides and were separated by ca. 5 m (i.e. one buffer row). Treatment and buffer rows were alternated between application dates; treatment rows from 17 October were buffer rows on 29 October, while buffer rows from 17 October were sprayed on 29 October.

Observations on the presence of *H. axyridis* were made 24 h after application. Five vines from each plot were examined; when a plot included six vines, one vine was randomly excluded from counting. For reliability of data collection, two field technicians examined the vines for presence of *H. axyridis*. The technicians worked in tandem looking at the same vine, one on either side. This system increased accuracy and efficiency by limiting the potential for double counting or overlooking beetles.

RESULTS. *Harmonia axyridis* accounted for all coccinellids observed on grape vines. The number of beetles on vines was significantly reduced by the application of KMS on both trial dates (17 October: $F = 3.39$; df= 3, 90; $P = 0.0215$; 29 October: $F = 13.44$; df = 2, 68; $P < 0.001$) (**Table 3**). On 17 October, vines sprayed with KMS at 10 g/L had significantly fewer beetles than control vines. On 29 October, the number of *H. axyridis* on control vines was significantly higher than on vines sprayed with KMS at either 5 or 10 g/L.

CONCLUSIONS. The discovery that KMS is an effective repellent against beetles suggests that it is a promising control method for use in vineyards, but more research is needed to develop comprehensive pest management recommendations. Future studies should determine the duration of repellency and the effect of environmental conditions, such as wind speed and temperature, on the efficacy of KMS. The behavior of beetles following KMS applications should also be investigated to determine optimal use patterns of KMS in vineyards.

Table 3. Mean number of *Harmonia axyridis* present on grape vines (*Vitis vinifera* var. Gewürztraminer) 24 h after application of potassium metabisulfite (at 2.5, 5, or 10 g/L), and percent reduction in beetles relative to the untreated control. Treatments were replicated five times (*N*=20) in each trial. Means within the same trial followed by the same letter are not significantly different (Tukey's HSD; α=0.05).

Date and treatment	Mean (SE)	% reduction of beetles
17 Oct.		
Control	2.72 ± 0.50a	-
2.5	2.60 ± 0.48a	4%
5	1.92 ± 0.46ab	29%
10	0.92 ± 0.21b	66%
29 Oct.		
Control	18.84 ± 1.77a	-
5	13.76 ± 1.25b	30%
10	9.40 ± 1.04b	50%

c) Reach and Communication

Primary target audience/beneficiaries

The primary targets of this research are Ontario grape producers, vineyard crop consultants and provincial extension specialists. Additional beneficiaries include representatives of agrochemical companies, members of the broader pest management community, and the scientific community.

Total number of people reached

- OMAFRA Annual Grape Tailgate Tour, ~20 attendees.
- Ontario Pest Management Conference, ~100-150 attendees.
- Ontario Fruit and Vegetable Conference, ~50-100 attendees reached.
- International Organization for Biological Control, Nearctic Regional Section Conference, ~30-70.

Outreach Activities to the targeted audience/beneficiaries

Presentations:

Glemser, E., R.H. Hallett, M.K. Sears, G. Pickering, and D. Inglis. 2010. A novel method for controlling multicoloured Asian ladybeetles in vineyards. International Organization for Biological Control, Nearctic Regional Section. May 11-13, 2010, Niagara Falls, Ontario. (Poster).

Glemser, E.J., R.H. Hallett, D. Inglis, G.J. Pickering, and M.K. Sears. 2010. A Novel Method for Controlling Multicoloured Asian Lady Beetles in Vineyards. Ontario Fruit and Vegetable Conference, February 24-25, 2010, St. Catharines, ON. (Poster).

Glemser, E.J., Sears, M.K., and R.H. Hallett. 2009. Orientation of *Harmonia axyridis* to volatiles associated with ripe or damaged wine grapes, *Vitis vinifera*. Ontario Pest Management Conference, November 12, 2009, Guelph, ON. (Paper).

Glemser, E.J., Sears, M.K., and R.H. Hallett. 2009. *Harmonia axyridis* in Niagara vineyards: An update on current research projects. OMAFRA Annual Grape Tailgate Tour, August 11, 2009, Vineland, ON.

MSc. Thesis:

Glemser, Erik. Olfactory responses of the multicoloured Asian ladybeetle (*Harmonia axyridis*) to vineyard volatiles. **2010**. M.Sc. Thesis, Univ. of Guelph. 130 pp. May 2008 to April 2010. (Co-Advisor with M.K. Sears).

Scientific Publication:

Glemser, E.J., R.H. Hallett, D. Inglis, G.J. Pickering, W. McFadden-Smith, and M.K. Sears. A novel method for controlling Multicolored Asian lady beetle (Coleoptera: Coccinellidae) in vineyards. Submitted to *Environmental Entomology*, 9 April 2010. 16 pp. MS# EN10-086. *In revision, to be resubmitted before end of March 2011*.

Acknowledgement of OGWRI throughout the project

OGWRI was acknowledged as a supporter of this project orally and on acknowledgement slides at presentations and in the acknowledgments sections of conference posters and scientific publications.

4. Project Outcomes (actual vs. expected) at short and long-term

a) Short-term

Short-term outcomes

The intended outcome of this project was the identification of one or more attractive grape volatiles that could be used to attract MALB, as well as identification of a repellent, which could be used together in developing a push-pull strategy to repel MALB from vineyards.

As four antennally active compounds were identified in Project 1.1 (Milestone 1), we expected to see behavioural activity to more than one of these compounds. However, none of the compounds proved sufficiently attractive for use in a push-pull program.

We developed an attractive yellow trap (yellow mailing tube trap) which could prove useful in monitoring ladybeetles in and around vineyards, on its own or in combination with an attractant lure identified in the future.

Our results with potassium metabisulfite (KMS) as a repellent are very promising as

MALB were strongly repelled by KMS in both lab and field tests. As KMS is an acceptable additive during the wine making process, it represents a better choice than some other compounds to use as a repellent of MALB in vineyards.

Public good/benefit of the project

KMS may prove to be useful in developing management programs for MALB in vineyards, and potentially in urban settings where MALB can be a nuisance pest.

b) Long Term

Key indicators of long-term project success

Further trials to confirm efficacy of KMS as a repellent of ladybeetles will be necessary before it can be registered for this purpose. If such trials are undertaken, as well as further trials to determine the length of time over which KMS remains repellent, then use of KMS as a repellent could become part of an integrated pest management program for ladybeetles in vineyards, resulting in reduced losses of grapes due to presence of ladybeetles and a significant increase in profits for Ontario grape producers.

5. Final Comments and Conclusions

Previous studies have reported the use of sulfur dioxide for fumigation of stored food (Friendship 1990), but, to the best of our knowledge, the use of sulfur dioxide for repelling insects has not been reported prior to this study. In laboratory assays, repellency can be attributed to the presence of volatile sulfur dioxide as beetles did not make physical contact with the KMS solution, while repellency in the vineyard may occur from both volatile sulfur dioxide and physical contact with the mildly-acidic KMS solution.

The behavioral response of insects to chemical stimuli may be influenced by a number of factors including physiological condition and time of year. The behavioral response of the ladybeetle *Stethorus punctum picipes* (Casey) to herbivore-induced plant volatiles is influenced by the time of year (James and Price 2004, James 2005). However, the behavioral response of *H. axyridis* to KMS was not affected by their physiological condition, as KMS was significantly repellent to beetles reared under reproductive conditions and to pre-diapause beetles collected during the autumn.

Attempts to quantify the release of sulfur dioxide from our KMS treatments was not possible as the concentration of sulfur dioxide produced from 2.5 g KMS/L (lowest rate evaluated in this study) greatly exceeded the range of detection (0-1000 ppm) of the Pulsed Fluorescence SO2 Analyzer (Model 43i High Level; Thermo Scientific, Waltham, MA). However; as KMS, when added to wine, produces approximately half its weight in sulfur dioxide (Conde et al. 2007), the amount of sulfur dioxide released can be assumed to be approximately half the treatment amount of KMS. KMS was repellent to beetles within one minute of the start of olfactory assays, indicating that KMS rapidly volatilizes to sulfur dioxide.

The repellency of KMS in vineyards may be affected by environmental factors, including wind speed and temperature. Therefore, it is recommended that applications of KMS in vineyards be made on calm days.

The application of KMS did not eliminate the presence of *H. axyridis* on grape vines, but that may not be necessary to achieve from a wine sensory (i.e. aroma and taste) perspective. Based on sensory thresholds for beetle taint, the tolerable numbers of H. axyridis are 1530 and 1260 beetles/t in white and red grapes, respectively (Pickering et al. 2007a). For Concord juice, the sensory detection threshold for beetle taint is 1800 beetles/t (Ross et al. 2007). The sensory detection threshold for beetle taint is variable (Pickering et al. 2007b), because different grape varieties have unique physical and chemical characteristics (Galvan et al. 2007). If the number of beetles in a vineyard exceeds the established sensory detection threshold, KMS could be used to reduce the number of beetles below the threshold level.

From a management perspective, repellents should generally have long-term activity to avoid frequent applications. Persistence is most desirable with building treatments where aggregations of *H. axyridis* are always unwanted. In vineyards, however, the presence of beetles is only a problem during grape harvest. Attempting to repel beetles from vineyards at any other time is futile as beetles would likely return. To repel *H. axyridis* from vineyards, a candidate repellent should be applied immediately prior to harvest without the risk that residues will compromise wine quality. The use of KMS in vineyards has no negative effect on wine fermentation as residual sulfur dioxide is not detectable in the juice of wine grapes sprayed with KMS 1 day earlier (Debra Inglis, unpublished data), and thus KMS is a suitable repellent for use in vineyards.

A potential issue with the use of KMS in vineyards is environmental pollution. In the atmosphere, sulfur dioxide is oxidized and produces sulfate, a major air pollutant and contributor to acid rain (Penkett et al. 2007). The fate of sulfur in vineyards has not been examined, but presumably sulfur is lost to the environment (Balasubramaniam and Poole 1995). Sulfur dioxide is not considered highly phytotoxic (Balasubramaniam and Poole 1995) and there was no visual evidence that KMS caused phytotoxicity to grape vines in this study.

The discovery that KMS is an effective repellent against beetles suggests that it is a promising control method for use in vineyards, but more research is needed to develop comprehensive pest management recommendations. Future studies should determine the duration of repellency and the effect of environmental conditions, such as wind speed and temperature, on the efficacy of KMS. The behavior of beetles following KMS applications should also be investigated to determine optimal use patterns of KMS in vineyards.