Canola Agronomic Research Program

CARP 2006-01: "The development of a semiochemical monitoring and detection system for the diamondback moth on canola"

FINAL REPORT

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Table of Contents

Summary

The overall objective of this research was to develop a semiochemical-based monitoring system for diamondback moth on canola in western Canada. This research was designed to establish a system to help producers determine the spatial and temporal distribution of diamondback moth and predict population densities of the damaging larval stage. The original proposal was broken down into two phases of research: 1. Pheromone-based monitoring of diamondback moth males; and 2. Semiochemical-based monitoring of diamondback moth females and natural enemies; and a third phase of research dissemination: 3. Communication of research findings and applications to the canola industry. The majority of our research findings fall under the Phase I category, the scope of which has been greatly expanded since our initial application. There are limited findings in Phase 2 because we were unable to attract a significant number of female diamondback moths to semiochemicals. This report will serve as our initial dissemination to industrial stakeholders as part of Phase 3 and it comes with specific recommendations to canola producers and commercial producers of pheromone lures as well as recommended avenues of future research.

Phase 1: Pheromone-based monitoring of diamondback moth males

Two season-long, pheromone-based monitoring experiments were conducted at sites located in all major canola-growing regions of Alberta to evaluate five different commercially available pheromone lures. Traps baited with lures from APT (now APTIV) and PheroTech (now ConTech) consistently captured the greatest number of male diamondback moths. Attractiveness of lures was inversely related to pheromone release rate as determined in laboratory studies. Based on these, and other trapping experiments that directly compared the APT and PheroTech lures, it is recommended that the Prairie Pheromone Monitoring Network continue to use the PheroTech lures.

Three experiments tested the longevity of pheromone lures under field conditions. All three experiments demonstrated that older lures were more attractive than fresh lures. It is therefore recommended that the Prairie Pheromone Monitoring Network change the lure rotation schedule from the current three-week rotation to a six-week rotation. This will reduce the overall cost of the monitoring system.

Grey rubber septa lures baited with the commercially-available PheroTech blend were more attractive to male diamondback moths than red septa lures baited with the same blend. Laboratory studies indicated that the increased attractiveness of the grey lures may be the result of a difference in the ratio of the released components from the two lure types. It is therefore recommended that PheroTech (now ConTech) dispense pheromone into grey rather than the red lures that are currently sold. However, the current pheromone dose used in the commercial PheroTech lures (100 µg) was the most attractive of several doses tested in both lure types and should be retained in the commercially-available lures.

A season-long experiment tested the attractiveness of differently positioned traps baited with PheroTech lures. Traps positioned at 50 cm above ground level and those positioned at canopy height throughout the season caught a similar number of diamondback moths which was greater than the number captured in traps

positioned at 2 m above the ground. Therefore we suggest that the Prairie Pheromone Monitoring Network change the currently recommended trap position of 2 m to 50 cm above the ground.

Trap catch in traps baited with the commercially-available PheroTech lures was significantly related to densities of immatures sampled at the same sites when diamondback moth densities were moderate but not when they were low. This indicates that pheromone traps can be used to predict subsequent larval population densities but more research is required to establish an economic threshold based on pheromone-based trap capture.

Although APT and PheroTech lures were consistently the most attractive commercially-available pheromone lures tested, neither was as attractive as calling virgin female diamondback moths. Our laboratory studies demonstrated that male diamondback moths detected the three previously identified pheromone components from female pheromone gland extracts. However, our field trapping studies demonstrated that lures releasing pheromone blends with the acetate as the main component were more attractive than lures releasing pheromone blends with the aldehyde as the main component. Interestingly, all of the commercial lures we tested release the less attractive blends with the aldehyde as the main component. We therefore recommend that future research is warranted to commercialize of a new pheromone lure releasing a new pheromone blend in collaboration with ConTech.

Phase 2. Semiochemical-based monitoring of diamondback moth females and natural enemies

In three field experiments we incorporated host cues into the pheromone-baited traps in an effort to enhance male response to traps and to attract females and natural enemies to traps. We tested the effect of trap colour (yellow vs. white) and the incorporation of a greenleaf volatile, Z3-hexenyl acetate, into pheromone lures. This greenleaf volatile was chosen as it has previously been shown to enhance male diamondback moth response to pheromone and it is not as toxic of some of the crucifer-specific compounds that are implicated in host-finding behavior in this species. However, the addition of host cues had no effect on the attractiveness of traps to male moths and did not result in the attraction of a significant number of females or natural enemies to traps. These cues were tested at two times in the field season when competing cues from the crop would differ (pre-bloom and bloom). The addition of the greenleaf volatile to the pheromone blend did not increase the attractiveness of the pheromone to male diamondback moths at any of the doses tested. As a result of these conclusive findings, we did not further pursue the development of a semiochemical-based lure targeting female diamondback moths.

The findings of this study have provided several easy to implement recommendations to improve the efficacy of the pheromone-based monitoring system currently used to monitor diamondback moth activity in the prairie provinces. Our findings also demonstrate that pheromone-based monitoring is related to infestation levels, at least at moderate population densities. Therefore, pheromone-based monitoring should be encouraged and should be followed by sampling of immature stages. Further, we have made several recommendations to improve the pheromone formulation that may result in the development of a more attractive, commercially-available lure through future research and collaboration with ConTech.

Introduction

The diamondback moth, Plutella xylostella *is a world-wide pest of cruciferous crops (Talekar and Shelton, 1993). In the Canadian prairies, this species is considered a serious pest of canola (*Brassica napus *and* B. rapa*). diamondback moth was consistently ranked by canola producers in western Canada as one of their most significant insect problems (CCC 1999). Infestation of diamondback moth in the Canadian prairies is due primarily to migration of adult moths on wind currents from the south each spring (Phillip and Mengersen, 1989). If sufficient numbers of moths arrive in canola crops and lay eggs, the resulting larvae and subsequent generations can cause significant damage to canola as they feed on all plant parts at various stages within the growing season. Under outbreak conditions, insecticide application is necessary to control diamondback moth and prevent large yield loss. This has resulted in large acreages of canola treated with insecticide in western Canada. In 1985, approximately 467 860 ha were treated with insecticides to control diamondback moth in Alberta and Saskatchewan at a cost of $11.9 million (Madder and Stemeroff 1988). An outbreak in Saskatchewan in 1995 resulted in insecticidal treatment of 1.25 M ha that added $40-50 million to canola production costs (WCCP 1995). More recently, 1.8 M ha of canola were treated with insecticides to control a diamondback moth outbreak in 2001 (WCCP 2001). Another outbreak of diamondback moth affected the prairie provinces in 2005.*

Pheromone-baited traps can be used to monitor and detect male diamondback moth (Chisholm et al. 1983, Suckling et al. 2002) and a pheromone-trapping network is in place in the prairie provinces. However, female-baited traps routinely capture more male moths than synthetic traps (Suckling et al. 2002) indicating that key chemical constituents may be missing from the lures currently used. Further, the significance of male moth capture to population densities has not been determined for diamondback moth on canola. The focus of this study was to optimize pheromone-based monitoring of diamondback moth on canola and determine if trap catches can be indicative of population densities and crop damage. A second objective is to combine pheromone and host plant volatiles to attract both female and male moths to semiochemical-baited traps.

Chapter 1. Assessment of commercially-available pheromone monitoring tools for diamondback moth in canola

***Methods and Materials**:*

Field Experiments:

Experiment 1 (2006). Comparison of commercially-available diamondback moth sex pheromone lures.

The purpose of this experiment was to compare various commercial diamondback moth pheromone lures (from 5 different companies) for their attractiveness to male diamondback moth. This initial experiment was conducted in 2006 at twenty sites located across Alberta in all agricultural districts where canola is grown. We tested pheromone lures from five different pheromone companies located in Europe, Canada and the USA and a solvent control:

1. *Pherobank (The Netherlands)*
2. *PheroTech now ConTech (Canada)*
3. *APT now APTIV (USA)*
4. *Trécé (USA)*
5. *ISCA Technologies (USA)*
6. *Unbaited control*

As is conducted in the Prairie Pheromone Monitoring Network, canola fields were selected away from shelterbelts, steep ditches and greater than ½ kilometre away from a strong light source (such as a farm yard light). Traps consisted of plastic delta traps fitted with a removal sticky insert. Traps were placed in a linear transect at the edge of each field spaced 50 m apart and 1-1.5 m above the ground on an L-shaped hanger. One lure of each type was randomly positioned in a trap along the transect at each site. Traps were positioned at field sites during the first week of May 2006 and were monitored weekly until mid-August. Lures were changed at three-week intervals throughout the monitoring period, as is recommended in the Prairie Pheromone Monitoring Network. Male moth counts in pheromone-baited traps were normalized with a log x+1 transformation prior to analysis. The attractiveness of lures from the different companies was compared with a Randomized Block ANOVA on season-long moth counts. Multiple comparisons were made using the Student Newman-Keul's test (SigmaStat11). No moths were captured in 8 sites, so data from 12 of the 20 sites were used in the analyses.

Experiment 2 (2007). Comparison of commercially-available diamondback moth sex pheromone lures.

Experiment two was conducted in 2007 and compared the attractiveness of the same five commercial pheromone lures tested in 2006. Twenty field sites were established across Alberta and traps were erected in the beginning of May and monitored weekly until the middle of August. Sites were selected and traps were erected as in Experiment 1, but unlike Experiment 1 in 2006, lures used to bait traps in Experiment 2 were changed at 6-week intervals. Male moth counts in pheromone-baited traps were normalized with a log x+1 transformation prior to analysis. The attractiveness of lures from the different companies was compared with a Randomized Block ANOVA on season-long moth counts. Multiple comparisons were using the Student Newman-Keul's test (SigmaStat11). Data from 15 sites were used in the analyses.

Experiment 3 (2006). Lure longevity experiment of PheroTech (now ConTech) pheromone lures.

The purpose of this experiment was to determine the attractiveness of PheroTech (ConTech) monitoring lures after they have been aged under field conditions for various periods of time. PheroTech (ConTech) lures are the pheromone lures currently being used in the Prairie Pheromone Monitoring Network. We tested five differently aged lures at each of ten field sites in the Lethbridge area in July 2006. These treatments were as follows:

1. *Fresh lures (0 week), directly from the freezer*
2. *Lures aged for 2 weeks in outside enclosure at U of A*
3. *Lures aged for 4 weeks in outside enclosure at U of A*
4. *Lures aged for 6 weeks in outside enclosure at U of A*
5. *Lures aged for 8 weeks in outside enclosure at the U of A*

Canola fields in the Lethbridge area were selected and traps were erected as in Experiment 1 above. Traps were placed in the field at the beginning of July 2006 and were monitored weekly for three weeks. Male moth counts after three weeks were normalized with a log x+1 transformation prior to analysis. The attractiveness of differently aged PheroTech lures was compared with a Randomized Block ANOVA. Multiple comparisons were using the Student Newman-Keul's test (SigmaStat11).

Experiment 4 (2008). Lure longevity experiment of PheroTech (now ConTech) and APT (now APTIV) pheromone lures.

This experiment tested the attractiveness of the two most effective commercial lures after they had been aged under field conditions for various periods of time. The treatments tested were as follows:

1. *Fresh PheroTech (ConTech) lures (0 week), directly from the freezer*
2. *PheroTech (ConTech) lures aged for 4 weeks in outside enclosure at U of A*
3. *PheroTech (ConTech) lures aged for 8 weeks in outside enclosure at U of A*
4. *Fresh APT (APTIV) lures (0 week), directly from the freezer*
5. *APT (APTIV) lures aged for 4 weeks in outside enclosure at U of A*
6. *APT (APTIV) lures aged for 8 weeks in outside enclosure at U of A*
7. *Solvent control*

Aged lures were used to bait one trap at each of eight field sites in the Lethbridge area in July 2008. Traps were positioned in the field as in Experiments 1-3 and were monitored every 2 weeks for a four week period. Total male moth counts after four weeks were used in the analysis. The attractiveness of differently aged PheroTech and APT lures was compared initially with a Three-Way ANOVA to determine the effects of site, lure source (PheroTech vs. APT) and lure age (0,4, and 8 weeks). Further analysis with a Randomized Block ANOVA followed by the Student Newman-Keul's test (SigmaStat11) allowed for comparison of trap catch among individual treatments.

Experiment 5 (2007). Comparison of the attractiveness of PheroTech (now ConTech) and APT (now APTIV) pheromone lures with calling, virgin female diamondback moths.

The attractiveness of the two most effective commercial lures was compared to virgin female diamondback moths in a field cage study. Three field cages (1.8 m x 1.8 m x 1.8 m) were assembled at least 50 m apart on the University of Alberta South Campus Farm. In each cage two plastic delta traps (PheroTech International, Delta, BC) were positioned in the centre of the cage, 1 m above the floor and separated by 1 m diagonally across a potted canola plant in full flower. Test treatments were assigned randomly to one trap in each cage and consisted of: 1) one commercial pheromone lure from PheroTech (now ConTech); 2) one commercial pheromone lure from APT (now APTIV); 3) Three virgin female diamondback moths held individually in mesh bags. The other trap in each cage served as an unbaited control trap. Fifteen-25 male diamondback moths were placed in each cage and the proportion of released males that was captured in the trap was monitored for three nights. The experiment was replicated six times using fresh pheromone lures and traps in each replicate. The proportions of released males that were captured in the baited traps in each cage were compared with a Randomized Block ANOVA. Individual treatments were compared with a multiple comparison test using the Student Newman-Keul's test (SigmaStat11).

Laboratory Experiments:

Experiment 6 (2006). Release rate analysis of commercial pheromone lures used in field experiment #1.

Commercial lures used in field experiment 1 (above) were analyzed to determine the rate and ratio of release of pheromone components from lures under laboratory conditions. Five lures from each of the five commercial sources were aerated under laboratory conditions and volatile emissions were captured once per day for three days on an absorbent material (Porapak-Q) and extracted. Extracts were analyzed using mass spectrometry and the amount of Z11-16:Ald and Z11-16:Ac released from each lure was calculated. The total amount of Z11-16:Ald released over the three day collection period from each lure source was log transformed and compared using a Randomized Block ANOVA. The release rates of lures from each source were compared with a multiple comparison test using the Student Newman-Keul's test (SigmaStat 11).

Experiment 7 (2006). Release rate analysis of PheroTech (ConTech) pheromone lures used in field experiment #2.

Lures used in the lure longevity experiment (field experiment 3, above) were saved after the completion of the field experiment and analyzed to determine the rate and ratio of release of pheromone components under laboratory conditions. Five lures from each of the aging treatments (0, 2, 4, 6, 8 weeks) were aerated under laboratory conditions (22-23 ºC) and volatile emissions were captured once per day for three days on an absorbent material (Porapak-Q) and extracted. Extracts were analyzed using mass spectrometry. Because lures were aerated after the completion of the 3 week field experiment, actual field aging of each treatment at time of analysis was 3, 5, 7, 9, 11 weeks. The total amount of Z11-16:Ald released over the three day collection period from each lure source was log transformed and compared using a Randomized Block ANOVA. These values were visually compared to those from the fresh PheroTech (ConTech) lures aerated in chemical analyses experiment #1 above.

Results:

Field Experiments:

Experiment 1 (2006). Comparison of commercially-available diamondback moth sex pheromone lures.

Diamondback moth population densities were low in 2006 and trap capture in experiment 1 was low and variable (Figure 1). Numerically, traps baited with APT lures captured the most male diamondback moths, but the number of males captured was not significantly different than that captured in traps baited with the PheroTech or Trécé lures (Figure 1). Intermediate numbers of male moths were captured in traps baited with PheroTech, Trécé and ISCA lures but capture in traps baited with Pherobank lures was not significantly different from capture in traps baited with control lures (Figure 1).

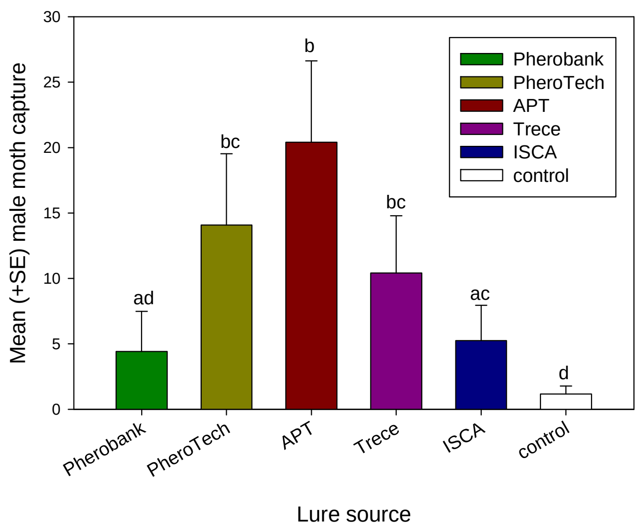


Figure 1. *Season-long capture of male diamondback moths in traps baited with pheromone lures from different commercial sources in 2006. Lures were changed at three-week intervals throughout the flight season. Bars labeled with the same letter are not significantly different (P>0.05, SNK test).*

Experiment 2 (2007). Comparison of commercially-available diamondback moth sex pheromone lures.

Diamondback moth population densities were moderate in 2007 and this was reflected in higher moth trap capture in all pheromone-baited traps in 2007 (Figure 2) as compared to 2006 (Figure 1). Male moth capture was less variable in 2007 and distinct differences were found among treatments (Figure 2). Season-long trap capture in traps baited with lures from all of the commercial sources was significantly higher than capture in traps baited with control lures (Figure 2). Traps baited with APT lures captured significantly more moths than any other trap. PheroTech lures, that are currently used in the Prairie Pheromone Monitoring Network, captured the second highest number of male diamondback moths in 2007 (Figure 2). As was the case in 2006 (Figure 1), Pherobank and ISCA lures attracted the fewest moths of the lures tested in 2007 (Figure 2).

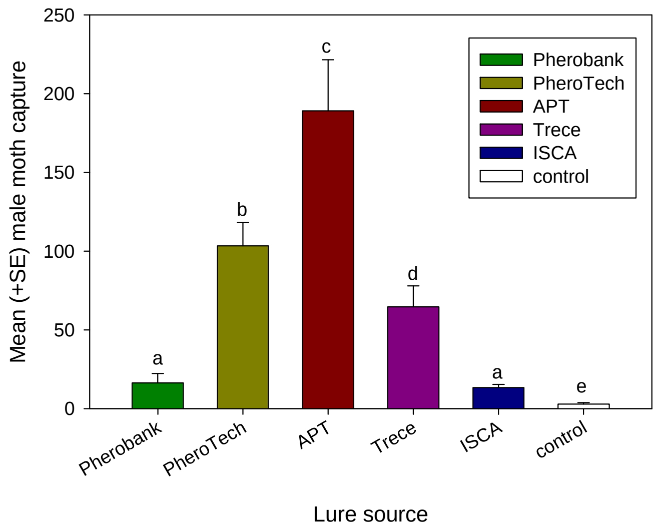


Figure 2. *Season-long capture of male diamondback moths in traps baited with pheromone lures from different commercial sources in 2007. Lures were changed at six-week intervals throughout the flight season. Bars labeled with the same letter are not significantly different (P>0.05, SNK test).*

Experiment 3 (2006). Lure longevity experiment of PheroTech (now ConTech) pheromone lures.

There was a significant effect of lure age (F=9.225, P<0.001) on the number of male moths captured in traps baited with PheroTech lures (Figure 3). Interestingly, traps baited with older lures that had been held outside for 8 weeks prior to deployment in the experiment, were more attractive to males than traps baited with fresh lures or those that had been held outside for two or 6 weeks (Figure 3). The results from this experiment indicate that the current 3-week lure change interval practiced in the Prairie Pheromone Monitoring Network is not necessary and a longer lure rotation interval should be adopted.

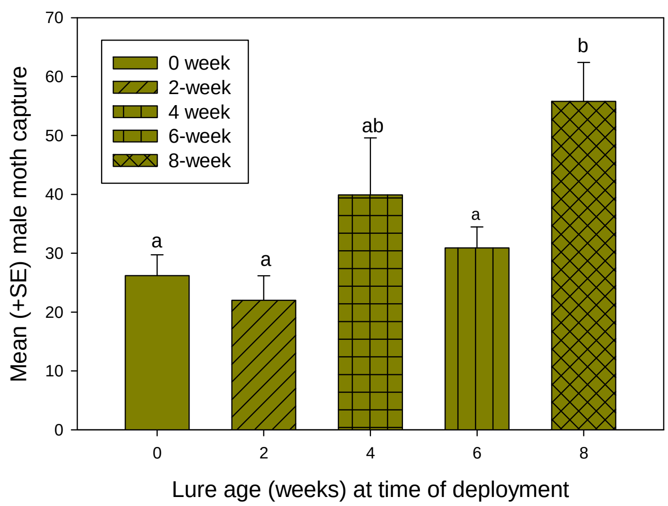


Figure 3. *Capture of male diamondback moths in traps baited with PheroTech pheromone lures aged for varying periods in 2006. Lures were aged outside prior to deployment in the experiment. Bars labeled with the same letter are not significantly different (P>0.05, SNK test).*

Experiment 4 (2008). Lure longevity experiment of PheroTech (now ConTech) and APT (now APTIV) pheromone lures.

There was a significant effect of lure age (F=4.905, P=0.024) and lure source (F=147.064, P<0.001) on the number of male moths captured in traps in Experiment 4. Again, traps baited with older lures captured more males, but this was not significant when multiple comparisons among individual treatments were conducted (Figure 4). Interestingly, in this experiment conducted at peak flight, PheroTech lures were more attractive to male diamondback moths than APT lures (Figure 4).

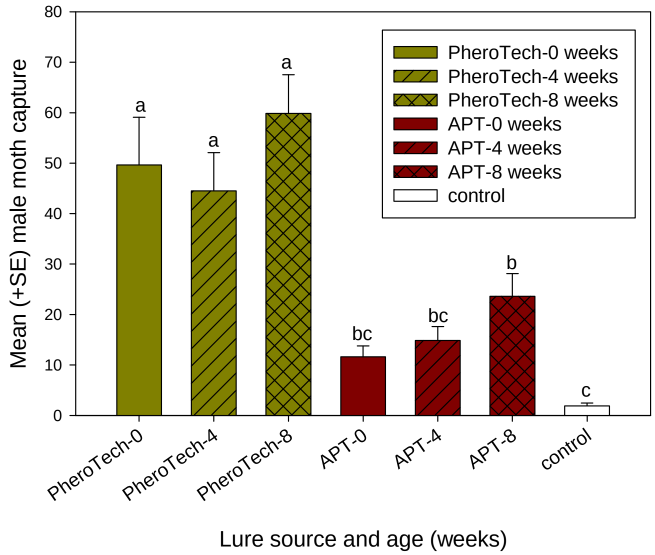


Figure 4. *Capture of male diamondback moths in traps baited with PheroTech and APT pheromone lures aged for varying periods in 2008. Lures were aged outside prior to deployment in the experiment. Bars labeled with the same letter are not significantly different (P>0.05, SNK test).*

Experiment 5 (2007). Comparison of the attractiveness of PheroTech (now ConTech) and APT (now APTIV) pheromone lures with calling, virgin female diamondback moths.

Experiment 5 was a field cage study that measured male diamondback moth response at a known population density to the two most attractive commercially-available pheromone lures as compared to the response to calling virgin females. In each cage, there was a baited trap and an unbaited control trap. No male moths were captured in the unbaited control traps over the course of the study (N=6 trapping periods). The proportion of released males that was captured in baited traps was low and variable (Figure 5). However, traps baited with virgin females captured a significantly greater proportion of released males than traps baited with either synthetic lure (Figure 5). There was no difference in the proportion of released males that were captured in baited with APT or PheroTech lures (Figure 5).

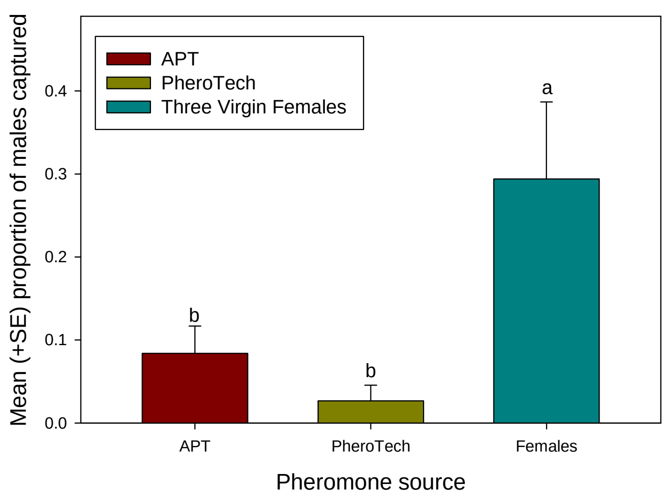


Figure 5. *Proportion of diamondback moth males captured in baited traps positioned in field cages in Experiment 5 in 2007. Bars labeled with the same letter are not significantly different (P>0.05, SNK test).*

Laboratory Experiments:

Experiment 6 (2006). Release rate analysis of commercial pheromone lures used in field experiment #1.

Aeration studies of lures from the different commercial sources indicated that in all cases the major compound released from lures was Z11-16:Ald. Z11-16:Ac was released as a minor component and Z11-16:OH was not detected. Pheromone release rate from the lures differed widely among lures from the different commercial sources (Figure 6). Pherobank and ISCA lures released the most *pheromone and PheroTech, Trécé and APT lures released the least pheromone (Figure 6). Interestingly, lures that released the most pheromone were the least attractive to diamondback moths in field experiment 1 (Figure 1).*

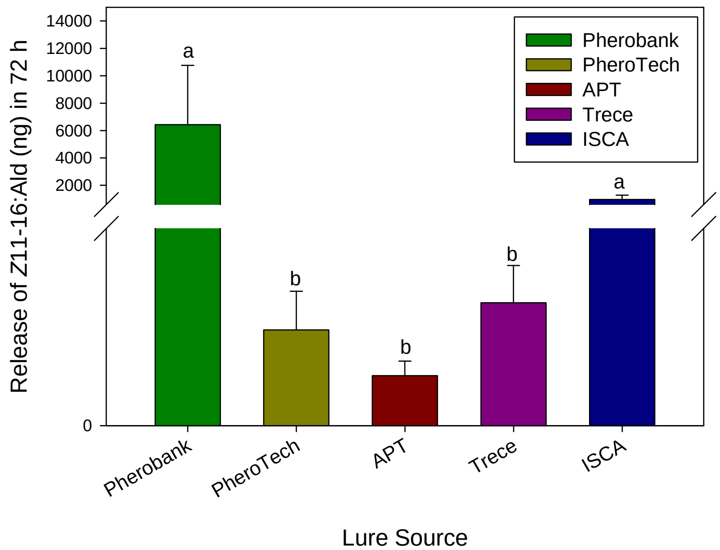


Figure 6. *Release rate (ng) of Z11-16:Ald over a 3 day period from commercially-available pheromone lures. Bars labeled with the same letter are not significantly different (P>0.05, SNK test).*

<u>*Experiment 7 (2006). Release rate analysis of PheroTech (ConTech) pheromone lures used in field experiment #2.*</u>
Release rate of the major aldehyde component (Figure 7) was low and steady after lures were field aged for 3 to 11 weeks. There was no significant difference in the amount of Z11-16:Ald released from lures aged 3-11 weeks (Figure 7). When the release rate of fresh PheroTech lures is visually compared to aged lures, it appears that there was an initial burst of release that diminishes by three weeks of release under field conditions (Figure 7). These findings indicate that the initial burst of pheromone release is above the threshold for male diamondback moth pheromone response, as males were less attracted to traps baited with fresh and two-week old lures in Field Experiment #2.

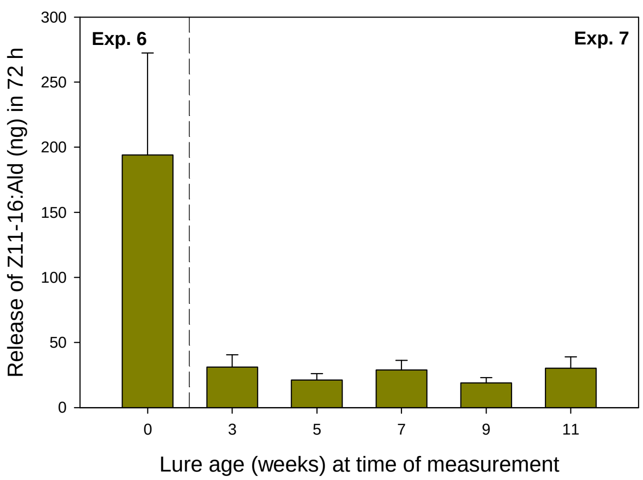


Figure 7. *Release rate (ng) of Z11-16:Ald over a 3 day period from PheroTech lures aged for different periods of time in the field prior to analysis. Release rate did not vary among lures aged 3-11 weeks, Randomized Block Design (F=0.900, P=0.487). Release rates are visually compared to those from fresh PheroTech lures collected in Experiment 6.*

<u>Conclusions</u>:

1. *Lures from APT (now APTIV) and PheroTech (now Contech) were consistently the most attractive of the five commercial lures tested in field experiments in canola.*
2. *Attractiveness of the commercial pheromone lures is correlated with the inverse of pheromone release rate from the lures. Lures releasing low rates of pheromone are the most attractive (APT, PheroTech, Trécé) and lures releasing high rates of pheromone are the least attractive (Pherobank, ISCA).*

3. *In the three experiments in which APT lures and PheroTech lures were directly compared, APT lures were more attractive than PheroTech lures in Experiment 2, PheroTech lures were more attractive than APT lures in Experiment 4 and both lure types attracted a statistically similar number of moths in Experiments 2 and 5. Therefore continued use of the Canadian-produced PheroTech lures is recommended in the Prairie Pheromone Monitoring Network.*

4. *Older lures (both PheroTech and APT) are more attractive to male diamondback moths. A longer lure change rotation (every 6 weeks) should replace the 3 week lure change rotation currently used in the Prairie Pheromone Monitoring Network.*

5. *Pheromone release from the lures occurs in an initial burst and then remains constant at very low release rates.*

6. *Although traps baited with lures from PheroTech and APT captured the most male diamondback moths of the commercially available lures tested, they were not as attractive as calling virgin female diamondback moths.*

Chapter 2. Factors influencing attractiveness of pheromone-baited traps to diamondback moth in canola

Methods and Materials:

Effect of pheromone blend on the attractiveness of pheromone-baited traps to male diamondback moths:

1. *Experiment 1 (2006). Analysis of female diamondback moth gland content using gas chromatographic-electronantennographic detection (GC-EAD).*

To determine what compounds calling female diamondback moth emit and males respond to, we conducted GC-EAD analysis on female gland extracts. This analysis couples a recorded neurological response from the male moth antenna with response from the gas chromatograph to identify the compounds in the extract. Female diamondback moth pheromone glands were dissected during the first three hours of the scotophase. Glands were extracted in HPLC-grade hexane at room temperature for 30 minutes. Aliquots of 1 female equivalent of pheromone gland extract were analyzed by GC-EAD with a Hewlett Packard 5890A gas chromatograph equipped with a fused silica column (30 m x 0.32 mm ID) coated with DB-5 (J&W Scientific, Folsom, CA, USA).

2. *Experiment 2(2007). Attractiveness of various pheromone blends to male diamondback moths.*

Six different pheromone blends consisting of different ratios of the three known pheromone components were tested at 8 field sites in southern Alberta in July 2007. Blends were chosen to encompass the pheromone blends that have shown to be attractive to male diamondback moths in cropping systems around the world (Table 1). Traps were monitored at two-week intervals over a four-week trapping experiment. The attractiveness of lures containing the different pheromone blends was compared with a Randomized Block ANOVA on total moth counts over the four week trapping period. Multiple comparisons were made using the Student Newman-Keul's test (SigmaStat11).

3. *Experiment 3(2008). Attractiveness of various pheromone blends to male diamondback moths.*

Six different pheromone blends (Table 1) consisting of different ratios of the three known pheromone components were tested at 8 field sites in southern Alberta in July 2008. This experiment was designed to determine the importance of Z11-16:OH to the attractiveness of the various blends to male diamondback moths. Traps were monitored at two-week intervals over a four-week trapping experiment. The attractiveness of lures containing the different pheromone blends was compared with a Randomized Block ANOVA on total moth counts over the four week trapping period. Multiple comparisons were made using the Student Newman-Keul's test (SigmaStat11).

4. *Experiment 4(2008). Attractiveness of various pheromone blends to male diamondback moths.*

Nine different pheromone blends (Table 1) consisting of different ratios of the two main pheromone components (Z11-16:Ac and Z11-16:Ald) were tested at 8 field sites in southern Alberta in July 2008. Traps were monitored at two-week intervals over a four-week trapping experiment. The attractiveness of lures containing the different pheromone blends was compared with a Randomized Block

ANOVA on total moth counts over the four week trapping period. Multiple comparisons were made using the Student Newman-Keul's test (SigmaStat11).

Table 1. Pheromone blends tested in field trapping experiments conducted in 2007 and 2008

Experiment	Treatment	Z11-16:Ac (µg)	Z11-16:Ald (µg)	Z11-16:OH(µg)
2 (2007)	Control	0	0	0
	Blend 1	90	10	1
	Blend 2	70	30	1
	Blend 3	50	50	1
	Blend 4	30	70	1
	Blend 5	30	70	10
	Blend 6	10	90	1
3 (2008)	Control	0	0	0
	Blend 1	70	30	0
	Blend 2	70	30	1
	Blend 3	70	30	10
	Blend 4	30	70	0
	Blend 5	30	70	1
	Blend 6	30	70	10
4 (2008)	Control	0	0	0
	Blend 1	5	50	0
	Blend 2	15	50	0
	Blend 3	25	50	0
	Blend 4	35	50	0
	Blend 5	50	50	0
	Blend 6	50	5	0
	Blend 7	50	15	0
	Blend 8	50	25	0
	Blend 9	50	35	0

Effect of pheromone dose, lure type and lure age on the attractiveness of pheromone-baited traps to male diamondback moths:

1. *Experiment 5 (2007). Attractiveness of different pheromone doses released from different lure types (red and grey septa).*

The effect of pheromone dose and lure type on male diamondback moth attraction were tested at 8 field sites in southern Alberta in July, 2007. The diamondback moth pheromone blend used commercially by PheroTech (now ConTech) was formulated at four different doses (0, 0.01X, 0.1X, X, 10X), where X refers to the commercially used dosage. Each pheromone dose was dispensed in 100 µl of hexane onto each lure type (pre-extracted red and grey septa) and compared to lures dosed with 100 µl of hexane alone. Lure type has been shown to affect the attraction of diamondback moth males in cabbage ecosystems (Mayer and Mitchell 1999) by altering the release rate of the pheromone components. Traps were monitored at 2-week intervals and a total trap catch over the 4-week period was used in analyses. Data were analyzed using a three-way ANOVA with site, pheromone dose and lure type as the three main factors in the model.

2. *Experiment 6 (2008). Effect of pheromone lure age and type (red and grey septa) on attraction of male diamondback moth.*

The effect of pheromone lure age and type on male diamondback moth attraction were tested at 8 field sites in southern Alberta in July, 2008. The diamondback moth pheromone blend used commercially by PheroTech (now ConTech) was formulated at the commercially used dosage and dispensed in 100 µl of hexane onto each lure type (pre-extracted red and grey septa). Lures were *aged outside for 0, 2, 4, 6, and 8 weeks prior to deployment in the field. Traps were monitored at 2-week intervals and a total trap catch over the 4-week period was used in analyses. Data were log transformed and analyzed using a three-way ANOVA with site, lure age and lure type as the three main factors in the model.*

3. *Experiment 7 (2008). Release rate analysis of lures tested in field experiment 6, above.*

   Grey and red lures aged for 0,2,4,6,8 weeks in an outdoor enclosure at the University of Alberta were aerated in the laboratory to determine the release rate of the two major pheromone components (Z11-16:Ald and Z11-16:Ac). Three lures of each treatment were aerated together in one chamber for 6 days at 24-28°C. Because lures were aerated together, treatments were not replicated and could not be compared statistically.

Effect of host cues in combination with pheromone on the attractiveness of baited traps to diamondback moths

1. *Experiment 8 (2007). Effect of trap colour and green leaf volatile on attraction of diamondback moths to pheromone lures during full bloom.*

   The effect of trap colour and the addition of a green leaf volatile to pheromone lures on male diamondback moth attraction were tested at 8 field sites in southern Alberta in July, 2007 when the canola crop was in full bloom. Three trap colours were tested (painted yellow, painted white and unpainted white). Each type of trap was baited with a lure releasing pheromone alone, a green leaf volatile alone (Z3-hexenyl acetate), or a 1:1 ratio of pheromone and green leaf volatile. Trap catch was compared to capture in traps baited with solvent alone. Traps were monitored at 2-week intervals and catch over the 4 weeks was used in analyses. Data were log x+1 transformed prior to analysis using a Three-Way ANOVA with trap colour, pheromone and greenleaf volatile specified as the main factors.

2. *Experiment 9 (2008). Effect of trap colour and greenleaf volatile on attraction of diamondback moths to pheromone lures before full bloom.*

   This experiment tested the same treatments as Experiment 8 in 2007 but was established earlier in the season (June rather than July) to determine if green leaf volatiles in combination with synthetic sex pheromone and trap colour would be more attractive to diamondback moth adults without the competition of a crop in full bloom. Eight sites near Lethbridge were established and sampled at 2 week intervals for the 1 month duration of the experiment. Male and female moths were counted on the sticky traps at each sampling date. Traps were analyzed for reflectance levels for each of the colours. Data were square root ransformed prior to analysis using a Three-Way ANOVA with trap colour, pheromone and greenleaf volatile specified as the main factors.

3. *Experiment 10 (2008). Effect of greenleaf volatile dose on attraction of diamondback moths to pheromone lures.*

   This experiment investigated varying doses of green leaf volatile (Z3-hexenyl acetate) in combination with commercial pheromone blend in an attempt to increase captures of adult diamondback moth. The trial was established during peak flight in southern Alberta (July, 2008). There were eight sites near Lethbridge and moths were counted at 2 week intervals for the 1. The treatment schedule is listed in Table 2. Data were square root transformed prior to analysis with a Two-Way ANOVA, followed by a Student Newman Keul's multiple comparison test to compare individual treatments.

Table 2. Treatments tested in Experiment 10, 2008.

Treatment	*Ratio of Pheromone (µg): Greenleaf Volatile (µg)*	
Control	*0*	*0*
Pheromone	*100*	*0*
Pheromone + 0.1x GLV	*100*	*10*
Pheromone + 1.0x GLV	*100*	*100*
Pheromone+ 10.0x GLV	*100*	*1000*
Pheromone + 100.0x GLV	*100*	*10000*

Effect of pheromone trap height on season-long trap capture of diamondback moths

4. *Experiment 11 (2008). Effect of trap height on season-long trap capture of male diamondback moths in pheromone-baited traps.*

This season-long experiment investigated the effect of trap height on the attraction of male diamondback moths to pheromone-baited traps. The treatments were delta traps baited with the commercially available PheroTech (now ConTech) lures positioned at one of three heights: 1) the industry standard of 2 m above the ground; 2) a low height of 50 cm above the ground; and 3) crop height. Traps positioned at crop height were moved up over time so as to remain at canopy level throughout the trapping period. Traps were set up at eight different sites in southern Alberta with 50 m spacing between traps. Traps were checked, adults counted and sticky inserts changed at 2 week intervals throughout the flight season from May-August (2008). Lures were changed at the industry recommended three-week interval.

Results:

Effect of pheromone blend on the attractiveness of pheromone-baited traps to male diamondback moths:

1. *Experiment 1 (2006). Analysis of female diamondback moth gland content using gas chromatographic-electronantennographic detection (GC-EAD).*
   GC-EAD analysis of female diamondback moth pheromone gland extracts revealed three antennally-active components that correspond to the previously identified pheromone components of this species (Figure 8). However, the amount of each compound in the gland extract was so small that we were unable to determine the ratio of each component as it occurs in the pheromone gland.

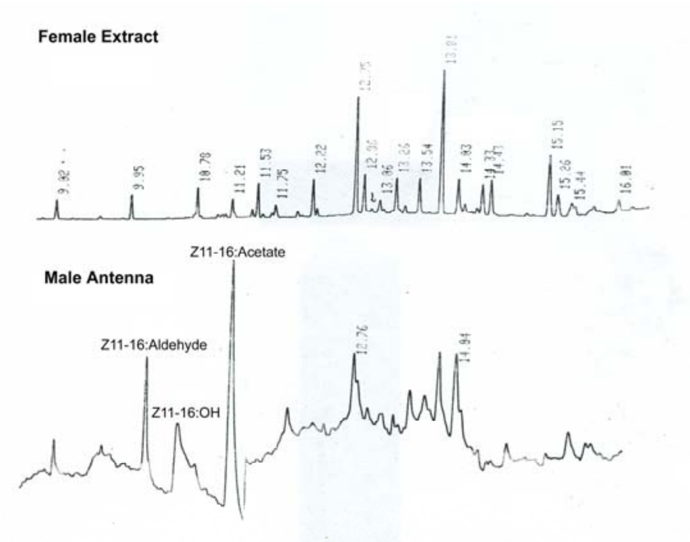


Figure 8. *Flame ionization detector and electroantennographic detector (EAD: male DBM antenna) responses to an aliquot of 1 female equivalent of pheromone gland extract of female DBM.*

2. *Experiment 2(2007). Attractiveness of various pheromone blends to male diamondback moths.*

The most attractive blend tested contained 70:30:1 ratio of Z11-16:Acetate: Aldehyde: Alcohol (Figure 9). This is in contrast to previous studies (Chisholm et al. 1979) in the Canadian prairies in which baits with the aldehyde as the main component have been most attractive. However, in studies in which the proportion of pheromone gland components in female gland extracts have been measured, the acetate has been the main component in moths from New Zealand (Suckling et al. 2002) and Korea (Yang et al. 2007) but not in Texas (He et al. 2003). Most commercially available pheromone lures release the aldehyde as the main component.

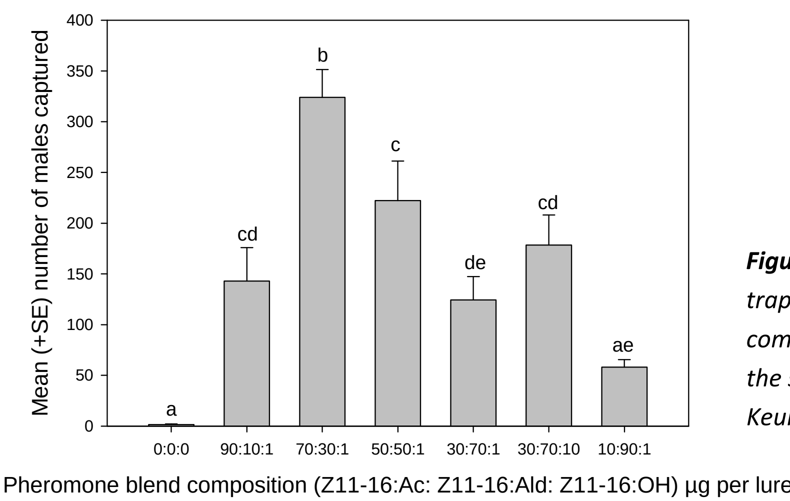


***Figure 9**. Mean number of male diamondback moths captured in traps baited with various ratios of the three known pheromone components over a four-week trapping period in 2007. Bars with the same letters are not significantly different (Student Newman Keul's test, P>0.05)*

3. *Experiment 3(2008). Attractiveness of various pheromone blends to male diamondback moths.*

The most attractive blend tested in 2008 was also the 70:30:1 ratio of Z11-16:Acetate: Aldehyde: Alcohol (Figure 10). This supports our findings from 2007 and suggests that blends containing the acetate component as the major component are more attractive to male diamondback moths. Trap catch was improved, but not significantly, by the addition of 1 µg of the minor component Z116-OH to the 70:30 blend of the acetate and aldehyde.

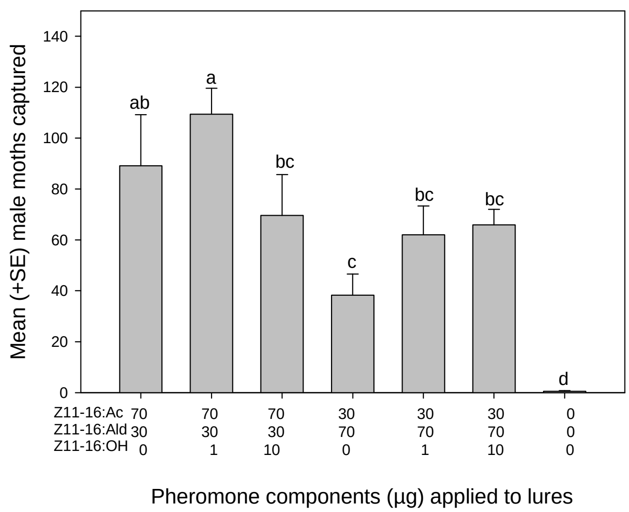


Figure 10. *Mean number of male diamondback moths captured in traps baited with various ratios of the three known pheromone components over a four-week trapping period in 2008. Bars with the same letters are not significantly different (Student Newman Keul's test, P>0.05)*

4. *Experiment 4(2008). Attractiveness of various pheromone blends to male diamondback moths.*

There was wide variation in the number of male diamondback moths captured in traps baited with varying ratios of the two major pheromone components (Figure 11) in 2008. However, the results of experiment 4 do support our previous findings that blends with the acetate as the major pheromone component are more attractive to male diamondback moths than blends with the aldehyde as the major component.

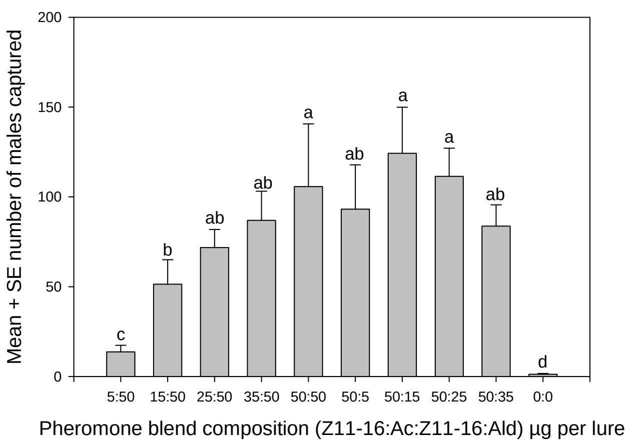


Figure 11. *Mean number of male diamondback moths captured in traps baited with various ratios of the two major pheromone components over a four-week trapping period in 2008. Bars with the same letters are not significantly different (Student Newman Keul's test, P>0.05)*

Effect of pheromone dose, lure type and lure age on the attractiveness of pheromone-baited traps to male diamondback moths:

1. *Experiment 5 (2007). Attractiveness of different pheromone doses released from different lure types (red and grey septa).*

There was a significant effect of pheromone dose (F= 24.213, P<0.001) and lure type (F= 18.950, P<0.001) on the number of male diamondback moths that were captured in experiment 5, conducted in 2007. Trap capture peaked at the commercially used dosage (Figure 12) and decreased at the highest dose tested. Pheromone released from grey rubber septa was more attractive to male diamondback moths than pheromone released from red rubber septa (Figure 12). Red rubber septa lures are currently used in the Prairie Pheromone Monitoring Network.

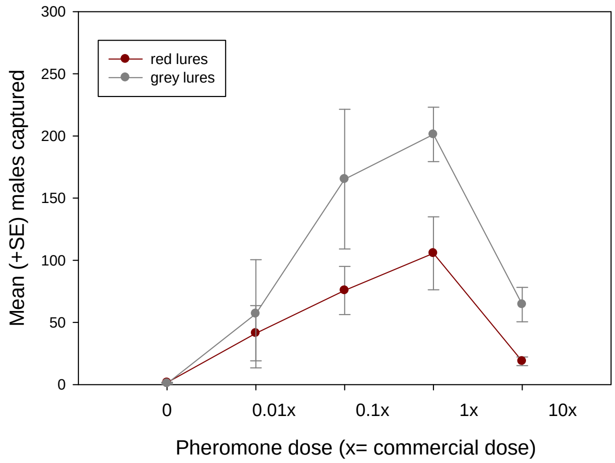


Figure 12*. Mean number of male diamondback moths captured in traps baited with red or grey rubber septa lures loaded with various doses of the commercially available pheromone blend.*

2. *Experiment 6 (2008). Effect of pheromone lure age and type (red and grey septa) on attraction of male diamondback moth.*

In 2008, both red and grey septa were tested again as release devices for the commercially available pheromone blend and dose. In this experiment, lures were aged for varying periods of time in an outdoor enclosure at the University of Alberta prior to use in the experiment. Similar to our findings in 2007, pheromone released from grey septa was more attractive than that released from red septa (F=116.331, P<0.001) (Figure 13). There was also a significant effect of lure age (F= 4.512, P<0.001) on the number of male moths attracted (Figure 13). Older lures were more attractive to male moths than fresh lures (Figure 13) which supports the findings of our earlier studies using commercially-available lures (Figures 3 and 4).

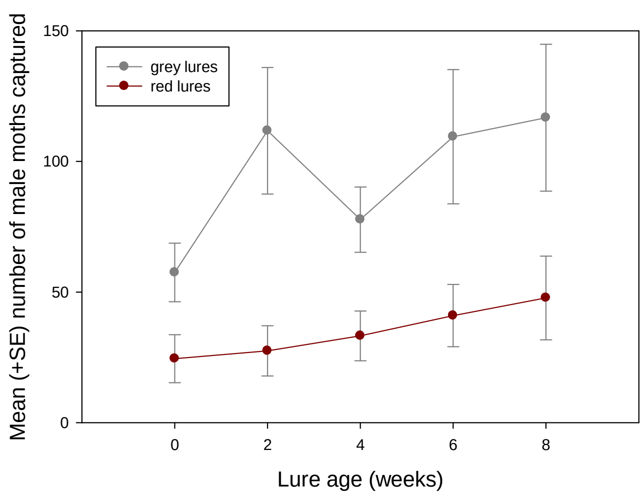


Figure 13*. Mean number of male diamondback moths captured in traps baited with red or grey rubber septa lures loaded with the commercially available pheromone blend at the commercial dose. Lures were aged outdoors for varying periods of time prior to use in the experiment.*

3. *Experiment 7 (2008). Release rate analysis of lures tested in field experiment 6, above.*

Release rate data for the lures tested in experiment 6 was low and variable (Figure 14). Although the fresh lures released approximately the same rate of pheromone as the fresh lures tested in Chapter 1, we did not see the initial burst of pheromone release followed by a drop in release rate in subsequent weeks (Figure 7). This might be because of the manner in which they were aged. In Chapter 1 (experiment 7), lures were aged in groups in a mesh bag followed by use in the field in individually-baited traps prior to release rate measurement. In Chapter 2 (experiment 7), lure release rate was measured after the aging process in mesh bags. Interestingly the spike in release rate of pheromone at week 4 (Figure 14) corresponds to a decreased trap capture in the grey septa-baited traps (Figure 13). The release rate data also demonstrate that grey lures may be more attractive to male moths because they release a lower aldehyde:acetate ratio than the red lures (Figure 14). This supports our *earlier findings that blends with a greater concentration of acetate are more attractive to male diamondback moths (Figures 9, 10 and 11).*

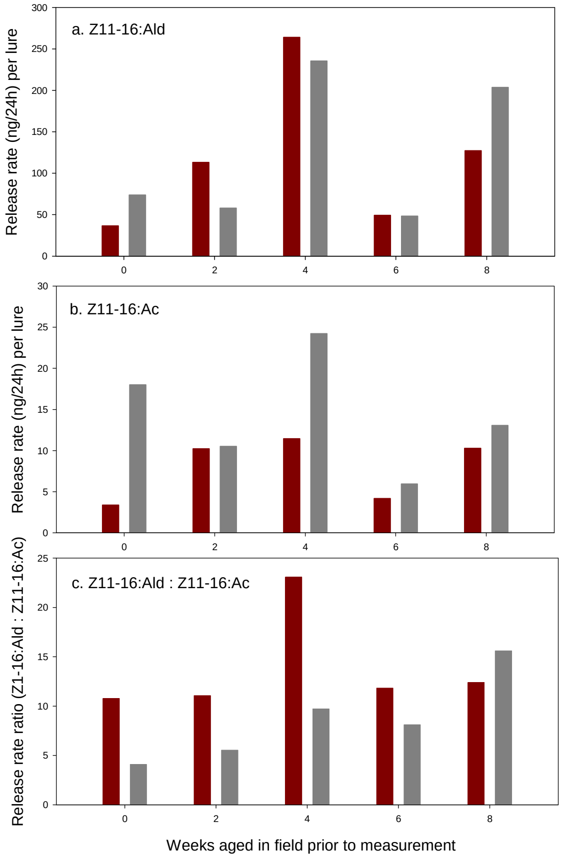


***Figure 14**. Release rate (ng) of a) Z11-16:Ald and b) Z11-16:Ac over 24 h from lures aged for different periods of time in an outdoor enclosure prior to analysis. c) The ratio of release of Z11-16:Ald to Z11-16:Ac from grey and red septa.*

Effect of host cues in combination with pheromone on the attractiveness of baited traps to diamondback moths:

1. *Experiment 8 (2007). Effect of trap colour and green leaf volatile on attraction of diamondback moths to pheromone lures during full bloom.*

Significant moth trap capture occurred only in traps baited with pheromone (Figure 15). There was no increase in trap capture as a result of the incorporation of the green leaf volatile to the pheromone lure. Trap colour did not affect the number of diamondback moths captured (Figure 15) but there was a trend towards the painted traps being less attractive, regardless of colour.

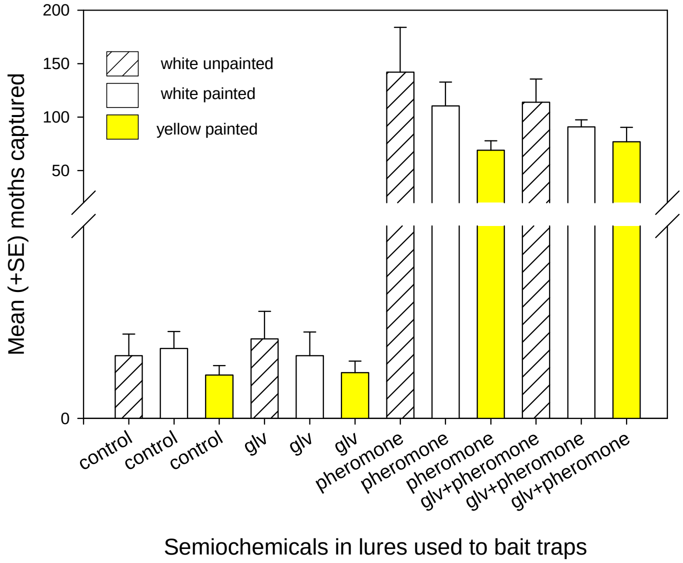


Figure 15. *Mean number of male diamondback moth captured in variously coloured traps baited with semiochemical treatments in 2007 at full bloom. The green leaf volatile (GLV) was Z3-hexenyl acetate and it was incorporated into lures in a 1:1 ratio with pheromone.*

2. *<u>Experiment 9 (2008). Effect of trap colour and greenleaf volatile on attraction of diamondback moths to pheromone lures before full bloom.</u>*

In 2008, the effect of host cues (trap colour and greenleaf volatile) and pheromone on diamondback moth response to semiochemical lures was tested early in the flight season (June). This experiment was designed to determine if host cues had a role in orientation behaviour early in the season when canola is not yet in bloom. However, the response to the semiochemical-baited traps early in the season mirrored what we saw at peak flight when canola is in full bloom (Figure 15). There was no significant effect of trap colour or the presence of greenleaf volatiles on the number of males captured in traps (Figure 16). The combination of greenleaf volatiles with pheromone did not enhance trap capture over pheromone alone (Figure 16). Moth trap catch in 2008 (Figure 16) was lower than 2007 (Figure 15) due to lower population densities in 2008 and because the experiment was conducted at the initiation rather than the peak of flight activity in 2008.

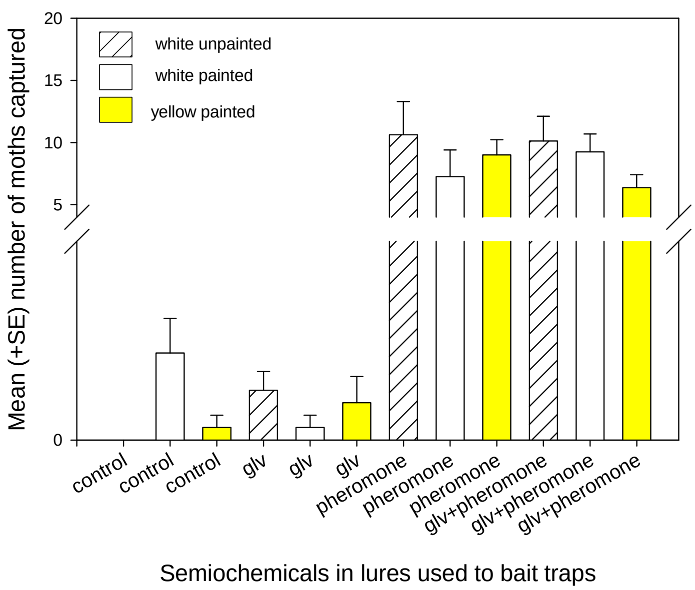


Figure 16. *Mean number of male DBM captured in variously coloured traps baited with semiochemical treatments in 2008 before bloom. The green leaf volatile (GLV) was Z3-hexenyl acetate and it was incorporated into lures in a 1:1 ratio with pheromone.*

3. <u>*Experiment 10 (2008). Effect of greenleaf volatile dose on attraction of diamondback moths to pheromone lures.*</u>

There was no effect of the addition of the greenleaf volatile Z3-hexenyl acetate at doses ranging from 10-10000µg on the attractiveness of pheromone lures to diamondback moths (Figure 17). All semiochemical-baited lures were significantly more attractive than the solvent control but there was no difference in attractiveness among semiochemical treatments (Figure 17).

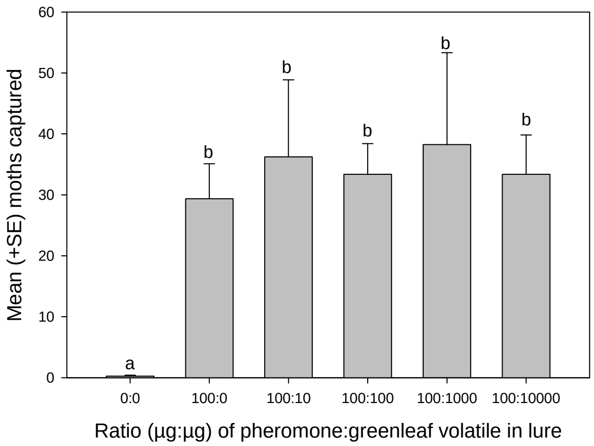


Figure 17. *Mean number of male diamondback moths captured in traps baited with semiochemical treatments in 2008. The green leaf volatile (GLV) was Z3-hexenyl acetate and it was incorporated into lures at various ratios with pheromone. . Bars with the same letters are not significantly different (Student Newman Keul's test, P>0.05)*

Effect of pheromone trap height on season-long trap capture of diamondback moths

1. <u>*Experiment 11 (2008). Effect of trap height on season-long trap capture of male diamondback moths in pheromone-baited traps.*</u>

There was a significant effect of trap height on the number of male diamondback moths captured in pheromone-baited traps (F=8.994, P<0.001). Traps positioned at the industry standard of 2 m off the ground captured significantly fewer diamondback moths than traps positioned at 50 cm or traps that were moved over time to remain at the same height as the canopy, which caught a statistically similar number of moths (Figure 18). Not surprisingly, there was a significant effect of time of season on moth trap catch (F=26.510, P<0.001).

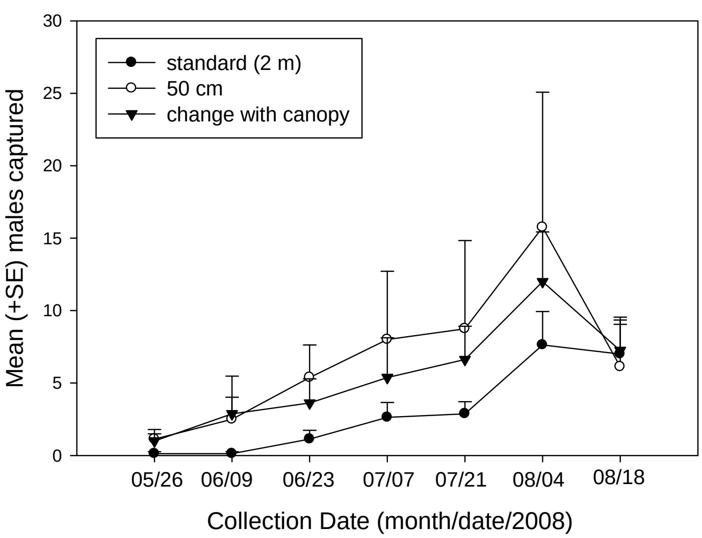


Figure 18. *Mean number of male diamondback moths captured in pheromone-baited traps positioned at different heights over the flight season.*

Conclusions:

1. *Pheromone blends with the Z11-16:Acetate component as the major pheromone component are more attractive than pheromone blends with the Z11-16:Aldehyde component as the major pheromone component. All the commercial lures we have tested release Z11-16:Aldehyde as the major component.*

2. *The pheromone blend used commercially in PheroTech lures is more attractive to male moths when it is released from grey rubber septa, as compared to red rubber septa. This may be because a lower aldehyde:acetate ratio is released from grey rubber septa.*

3. *As we saw in Chapter 1, pheromone lures that have been aged in the field are more attractive to diamondback moths than fresh lures.*

4. *The pheromone dose currently used in PheroTech lures (100 µg) is the most attractive dose to male diamondback moths.*

5. *The addition of host cues (trap colour and greenleaf volatile) did not enhance the attractiveness of pheromone-baited traps when tested early in the season or during full bloom.*

6. *The greenleaf volatile tested, Z3-hexenyl acetate, did not enhance the attractiveness of the pheromone lure at any of the tested doses.*

7. *Traps positioned at 50 cm above the ground or moved with the crop canopy height captured more male diamondback moths than traps positioned at 2 m above the ground. The 2 m height is currently the industry standard in the Prairie Pheromone Monitoring Network.*

Chapter 3. Development of a pheromone-based monitoring system to predict larval densities of diamondback moth in canola

Methods and Materials:

The capability of pheromone-baited traps to predict larval densities was tested at 15 field sites in southern-central Alberta in 2007 and 2008 (Figure 19). The commercially available lure from PheroTech (now ConTech) that is currently used in the Prairie Pheromone Monitoring Network was used to bait traps. Three and six traps were erected at each site in the beginning of May in 2007 and 2008. Traps were monitored every 2 weeks until the middle of August. Throughout the monitoring period lures were changed at six-week intervals in 2007 and at three and six week intervals (n=3 each per site) in 2008. After adult diamondback moth presence was detected in pheromone-baited traps, larvae were sampled at each site every two weeks. Larval samples were obtained by harvesting 50 canola plants per site per sampling date. Plants were transported from the field to the laboratory and dipped in ethanol to remove insects. Larval number and development stage were noted.

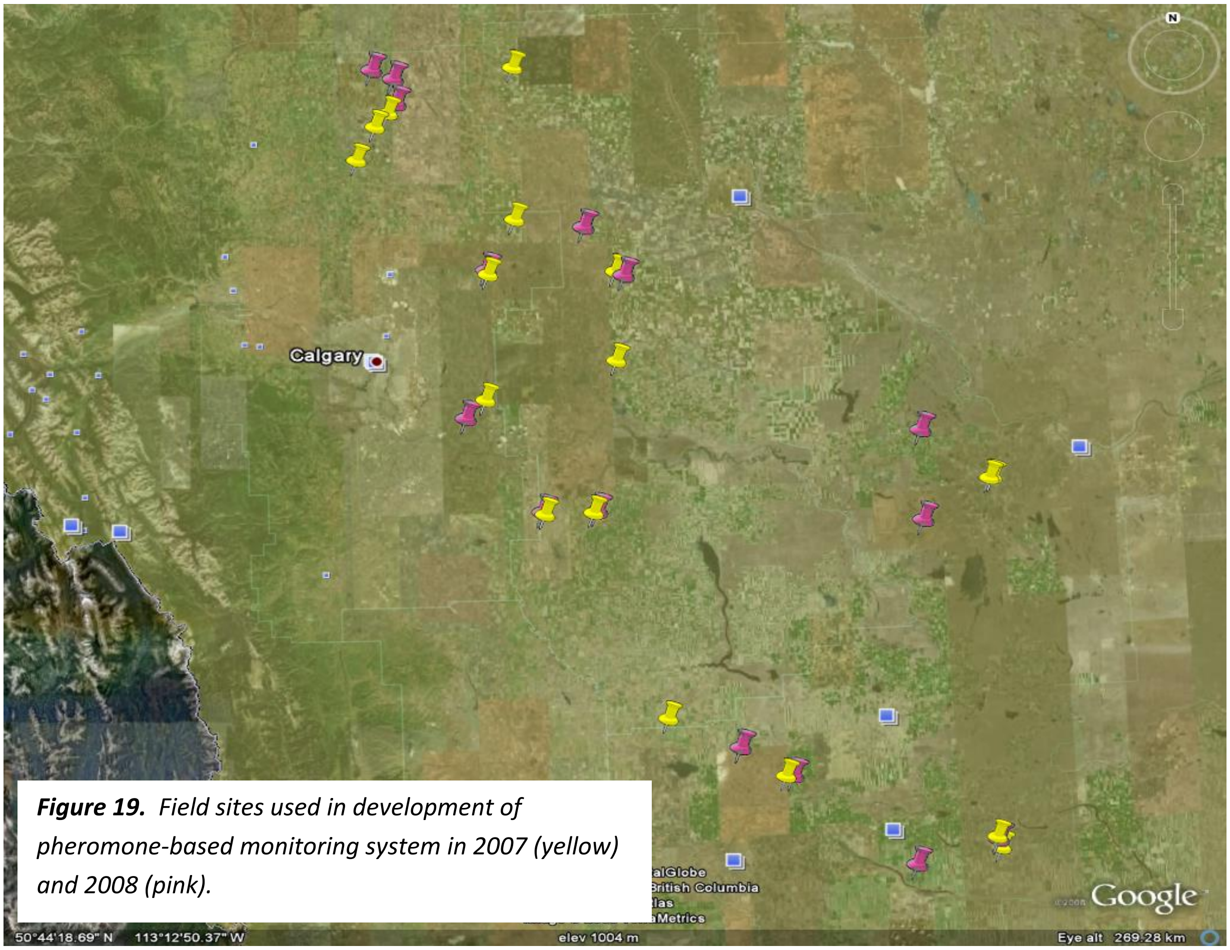


***Figure 19.** Field sites used in development of pheromone-based monitoring system in 2007 (yellow) and 2008 (pink).*

Results:

In 2007, when Alberta experienced moderate densities of diamondback moth, season-long capture of males in pheromone-baited traps did significantly predict the number of immatures sampled at these same sites (Figure 20). In 2008, when densities were low throughout the province, there was no significant relationship between the number of male moths captured in pheromone-baited traps and immature subsequently sampled at sites (Figure 21). Immatures sampled throughout the season were in varying stages of development indicating that multiple generations occur over the field season (Figure 22).

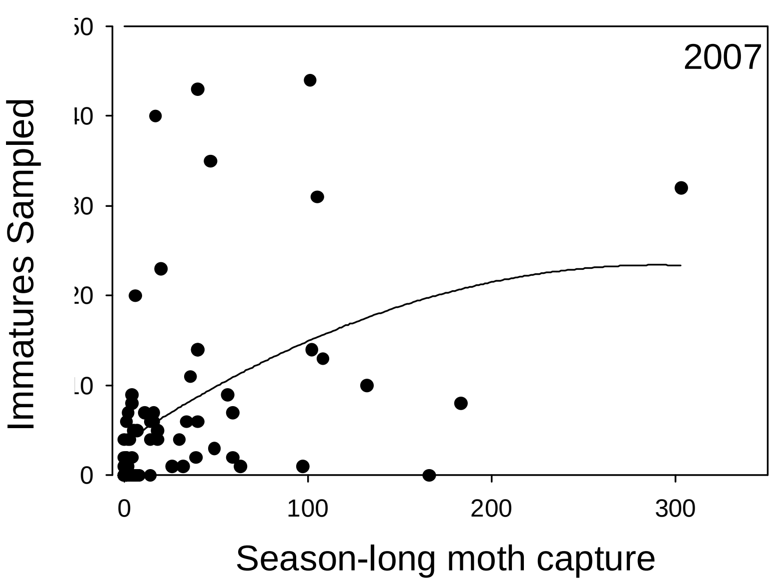


***Figure 20.** Relationship between male moths captured in pheromone-baited traps and the number of immatures sampled at each site in 2007.*

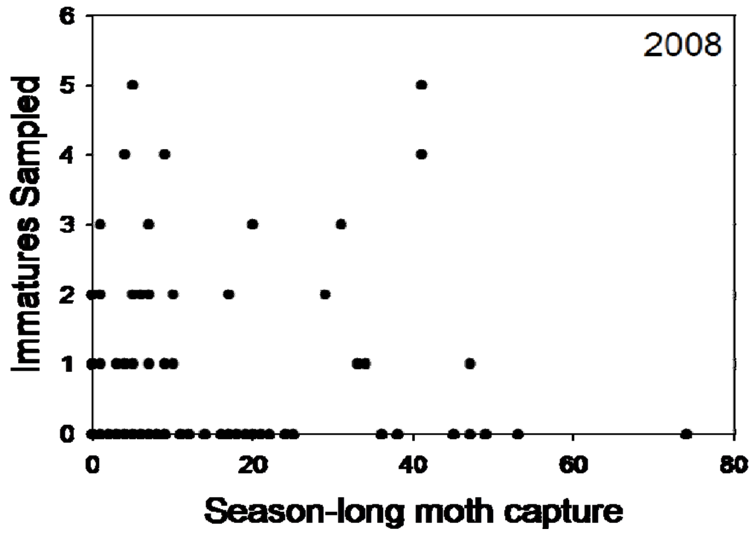


__Figure 21.__ Relationship between male moths captured in pheromone-baited traps and the number of immatures sampled at each site in 2008.

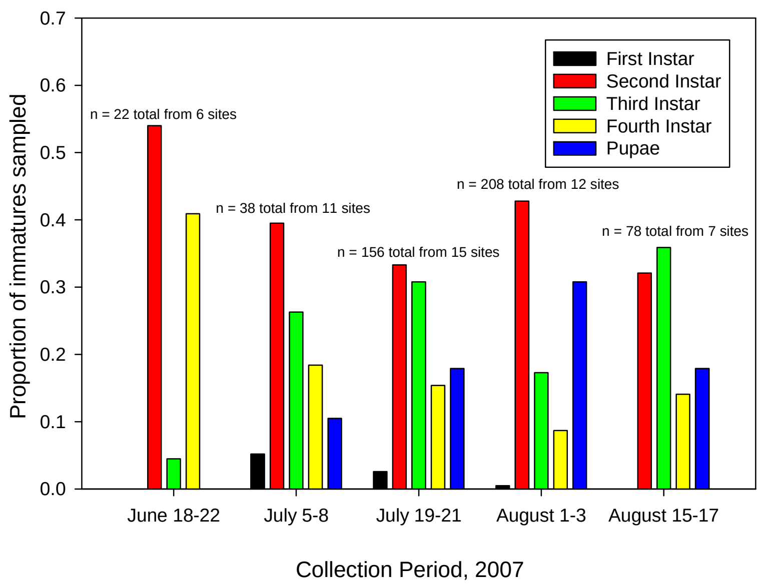
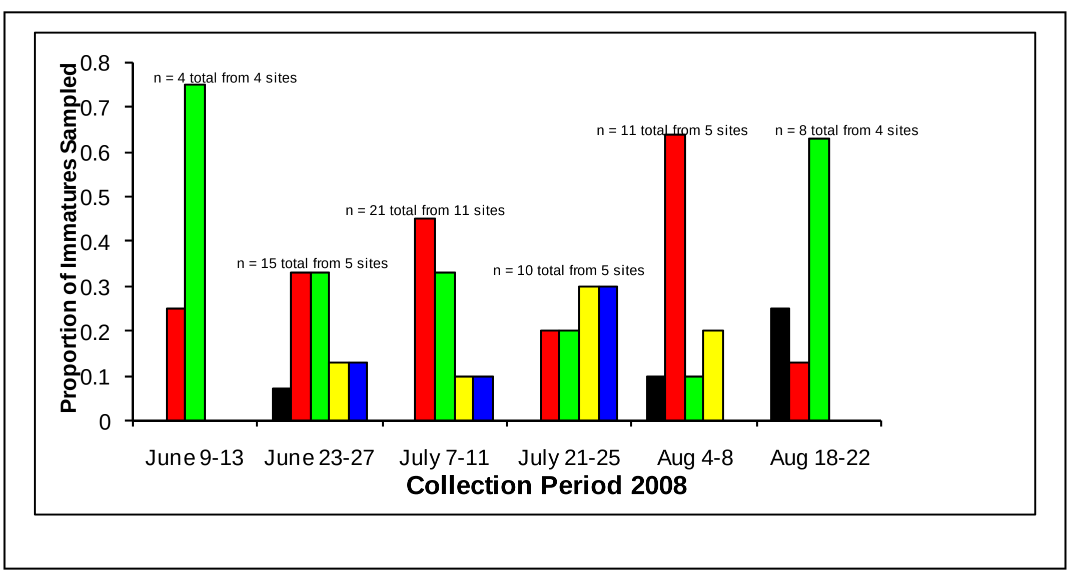


__Figure 22.__ Distribution of immature stages in samples of 50m canola plants per site during the monitoring periods in 2007 (a) and 2008 (b). Not all sites had immatures present at each sample date.



Conclusions:

1. *Trap capture in pheromone-baited traps at moderate population densities can be used to predict larval densities in the field.*

2. *Trap capture in pheromone-baited traps at low population densities did not predict larval densities in the field.*

3. *There are overlapping immature stages in the field throughout the field season, suggesting multiple generations of diamondback moth throughout the season.*

Chapter 4. Concluding Discussion and Recommendations

The overall objective of this research was to develop a semiochemical-based monitoring system for diamondback moth on canola in western Canada. Our research evaluated various commercial lures that are currently available to producers and determined the best lure type, age, and position in which to use these tools in the field. In addition, we tested other pheromone blends with various ratios of the known pheromone components with and without the addition a greenleaf volatile. Finally, this research evaluated the potential of diamondback moth trap capture in traps baited with commercially-available pheromone lures to predict larval infestation in canola in Alberta.

Major Findings:

1. *Lures from APT (now APTIV) and PheroTech (now Contech) were consistently the most attractive of the five commercial lures tested in field experiments in canola and these lures had a lower pheromone release rate than the less attractive lures.*

2. *Older lures (both PheroTech and APT) are more attractive to male diamondback moths.*

3. *Although traps baited with lures from PheroTech and APT captured the most male diamondback moths of the commercially available lures tested, they were not as attractive as calling virgin female diamondback moths.*

4. *Pheromone blends with the Z11-16:Acetate component as the major pheromone component are more attractive than pheromone blends with the Z11-16:Aldehyde component as the major pheromone component. All the commercial lures we have tested release Z11-16:Aldehyde as the major component.*

5. *The pheromone blend used commercially in PheroTech lures is more attractive to male moths when it is released from grey rubber septa, as compared to red rubber septa.*

6. *The pheromone dose currently used in PheroTech lures (100 µg) is the most attractive dose to male diamondback moths.*

7. *The addition of host cues (trap colour and greenleaf volatile) did not enhance the attractiveness of pheromone-baited traps when tested early in the season or during full bloom.*

8. *Traps positioned at 50 cm above the ground or moved with the crop canopy height captured more male diamondback moths than traps positioned at 2 m above the ground. The 2 m height is currently the industry standard in the Prairie Pheromone Monitoring Network.*

9. *Pheromone-baited traps can be used to predict larval densities in the field, but this was only successful at moderate population densities.*

Recommendations:

1. *PheroTech (now ConTech) lures were consistently among the most attractive of the commercial lures and their continued use in the Prairie Pheromone Monitoring Network is recommended.*

2. *Lures should be rotated on a 6-week rotation schedule and not on the 3-week rotation schedule as is currently used in the Prairie Pheromone Monitoring Network.*

3. *ConTech should dispense pheromone into grey rather than red rubber septa to improve the attractiveness of their product.*

4. *Producers should position traps at a height of 50 cm along the crop edge instead of the 2m height that is currently the standard used in the Prairie Pheromone Monitoring Network.*

5. *Development of a new pheromone blend that has the acetate as the major component should be the topic of future research and commercialization in collaboration with ConTech. All of the tested commercial lures release pheromone blends with the aldehyde as the major component and even the most attractive commercial lures are not as attractive as calling virgin females.*

6. *Producers are encouraged to use pheromone-baited traps to monitor diamondback moth flight and this information can predict expected larval densities in their crop. However, further research is needed to develop an economic threshold based on moth trap counts and continued larval sampling after moths are detected in traps is recommended.*

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