CANOLA COUNCIL OF CANADA, CANOLA AGRONOMIC RESEARCH PROGRAM
FINAL REPORT FOR:
Development of a semiochemical monitoring and detection system for the diamondback moth on canola,
February 28, 2008

Principal investigator: M. Evenden
Collaborators: L. Dosdall, A. Keddie, S. Meers, K. Fry, M. Hartman and G. Gries
Graduate Students: C. Miluch, A. Abdukakharov

Objective of Research: The overall objective of this research is to develop a semiochemical-based monitoring system for diamondback moth (DBM) on canola in western Canada. This research will optimize the synthetic pheromone-based lure currently used to trap male moths and will determine if trap catch can be predictive of larval densities and crop damage. In addition, a combined formulation containing sex pheromone and host plant volatiles will be tested for its attractiveness to both male and female moths. Since the Interim Report submitted in November 2007, we have conducted further analyses and the results of those analyses are reported here. In this final report for year 2 of the study, our experiments for year 3 are outlined and plans for further funding to support the project are proposed.

Summary of Results (2007): In 2007, we related male moth trap capture throughout the flight season to immature samples of larvae and pupae in a season-long experiment. Although a predictive model is still being developed, it appears that there is a correlation between male moths captured in pheromone-baited traps and infestation levels of immatures in canola. In 2007, we again compared different commercially available diamondback moth pheromone lures in a trapping experiment with sites throughout the province. As in 2007, the most attractive lures were those from PheroTech (currently used in the Prairie Pest Monitoring System) and APT. In 2007, the APT lures were slightly more attractive than the PheroTech lures. However, lures from both of these companies were not as attractive as calling virgin females in a field cage experiment. This finding suggests that there is something missing from the commercial pheromone blends and we are continuing to analyze the female-produced pheromone to find these differences. Different ratios of pheromone blends were tested in a field trapping experiment and blends with a higher concentration of the acetate component were most attractive. Other trapping experiments demonstrated a dose response to pheromone by male diamondback moths and pheromone release from grey septa was more attractive than red septa that are currently used to release the commercial pheromone from PheroTech lures. To date, we have been unable to demonstrate increased attraction to pheromone combined with host cues (colour and green leaf volatile) but we will try testing these cues at different doses and at different times of the season in 2008.

Achievements Obtained (2007):
- APT lures were the most attractive of the commercial lures tested in 2007.
- The most attractive commercial lures are not as attractive as calling females.
- Establishment of a correlation between moth catch and immature densities.
- Determination of a dose response to pheromone released from red and grey septa.
- Pheromone released from grey septa was more attractive than the same pheromone released from red septa at the higher doses tested.
- Pheromone blends with higher acetate concentrations were more attractive than blends with higher aldehyde concentrations.

Personnel who conducted experiments (2007):
- Christine Miluch (MSc student) conducted field work on the development of the predictive model (Exp. 3) and conducted experiments 4 and 5.

- Ellis Chan (summer student) assisted with all experiments
- Artem Abdukakharov (PhD student) conducted female pheromone gland extracts
- Regine Gries (Pheromone chemist, Simon Fraser University) analyzed female pheromone gland extracts
- Scott Meers (Alberta Agriculture) helped to coordinate collaborators in experiment 1
- Maya Evenden (PI, University of Alberta) conducted the field cage study (Exp. 2), blend comparison study (Exp. 6) and coordinated data collection for experiment 1.

EXPERIMENTAL PLAN: In 2007, six field experiments were conducted:

Experiment 1 Five different commercial pheromone lures that target male DBM were compared for attractiveness to male moths. 20 field sites were established across Alberta and traps were erected in the beginning of May and monitored weekly until the middle of August. Lures used to bait traps were changed at 6-week intervals. A six-week lure replacement regime was tested instead of the commercially recommended three-week period because our data from 2006 showed that older lures were potentially more attractive than fresh lures.

Experiment 2 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 International; 2) one commercial pheromone lure from Applied Pheromone Technologies; 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.

Experiment 3 The capability of pheromone-baited traps to predict larval densities was tested at 20 field sites in southern-central Alberta. The commercially available lure from PheroTech International that is currently used in the Prairie Pest Monitoring program was used to bait traps. Three traps were erected at each site in the beginning of May and traps were monitored every 2 weeks until the middle of August. Throughout the monitoring period lures were changed at six-week intervals. After DBM presence was detected by male moth capture, 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.

Experiment 4 The effect of pheromone dose and lure type on male DBM attraction were tested at 8 field sites in southern Alberta in July, 2007. The DBM pheromone blend produced by PheroTech International 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 a lures dosed with 100 µl of hexane alone. Lure type has been shown to affect the attraction of DBM 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.

Experiment 5 The effect of trap colour and the addition of a green leaf volatile to pheromone lures on male DBM attraction were tested at 8 field sites in southern Alberta in July. 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 a total trap catch over the 4-week period was used in analyses.

Experiment 6 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. Blends were chosen to encompass the pheromone blends that have shown to be attractive to DBM in cropping systems around the world.

RESULTS:

Experiment 1 The three most attractive monitoring lures were those from Applied Pheromone Technologies, PheroTech International and Trécé (Figure 1). Although these lures remained in the field twice as long as those tested in 2006, a similar pattern of male moth attraction to each type of lure was observed. However, this year the lures from Applied Pheromone Technologies were clearly the most attractive lure tested. Currently, the Praire Pest Monitoring system uses lures from PheroTech International.

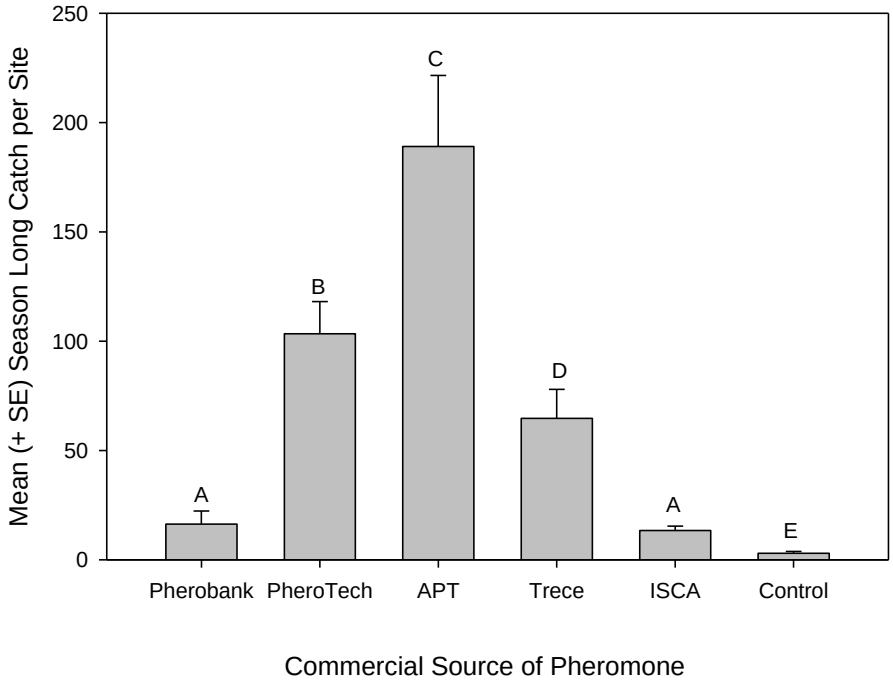

Figure 1. Mean season-long trap catch of DBM males in pheromone traps baited with different commercially available pheromone lures. Traps were located at sites throughout the province. Lures were changed at six week intervals throughout the monitoring period from May 1-August 15, 2007. Bars with the same letter are not significantly different (P>0.05).

Experiment 2 The female-baited trap captured more of the released males in the field cage study. Across all six replicates, 35 of the 125 released males (28%) were captured in female-baited traps. In comparison, 9 of 117 released males (7.6%) were captured in traps baited with the APT lures across all replicates. Three of 118 released males (2.5%) were captured in traps baited with the PheroTech Inc. lures across all replicates. These data indicate that the commercially available lures are not accurately mimicking the female-produced signal.

Experiment 3 Male moth captures correlated with immature densities over the flight season (Figure 2).

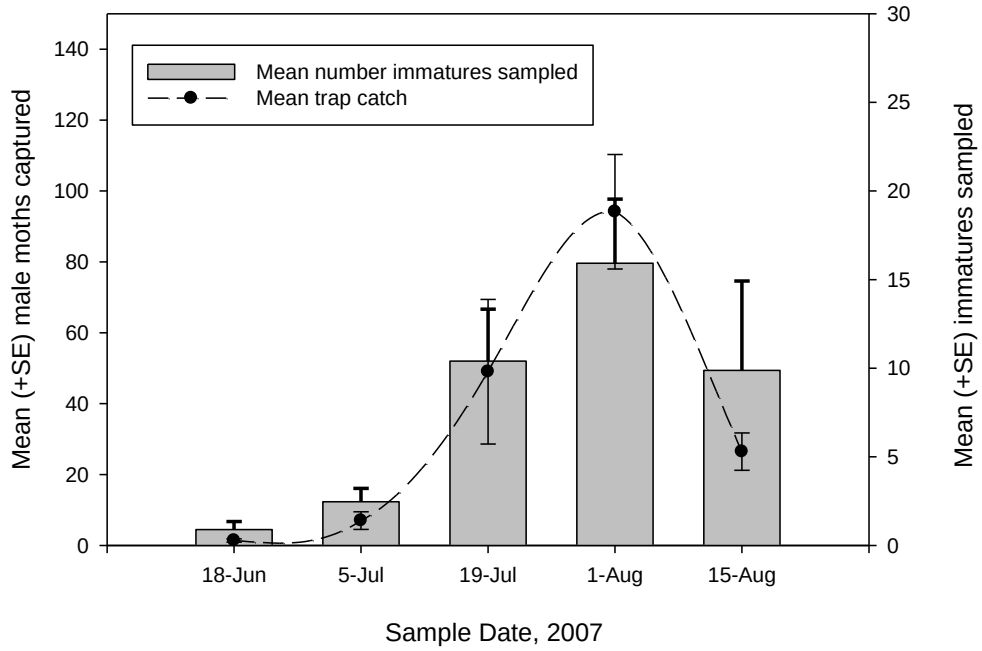

Figure 2. The mean number of male moths captured in pheromone-baited traps and immatures (larvae and pupae) per sampling interval at sites in south-central Alberta in 2007.

Our preliminary analyses indicate that male moth capture in pheromone-baited traps may be able to predict larval infestations (Figure 3). This information will form the basis of a pheromone-based predictive model to assist canola produces in forecasting DBM populations on canola. However, Chris Miluch is currently working with Dr. Yang from the Department of Agriculture, Food and Nutritional Sciences at the University of Alberta to develop a model using a negative binomial distribution, which appears to fit the data better. This model is still under construction and when complete we will be able to determine if three sites at each site are required predict larval densities.

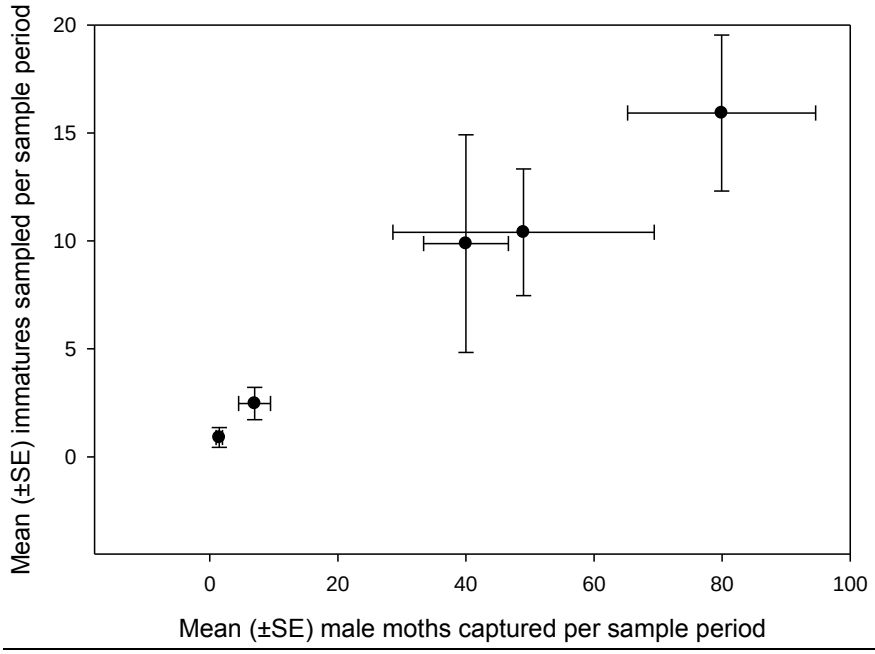

Figure 3. The relationship between the number of male moths captured and immature DBM sampled partitioned by sampling date in 2007.

All immature stages of DBM were found in the field throughout the sampling period. However, the proportion of individuals in each developmental stage varied with time and demonstrated that overlapping generations were present in the field (Figure 4).

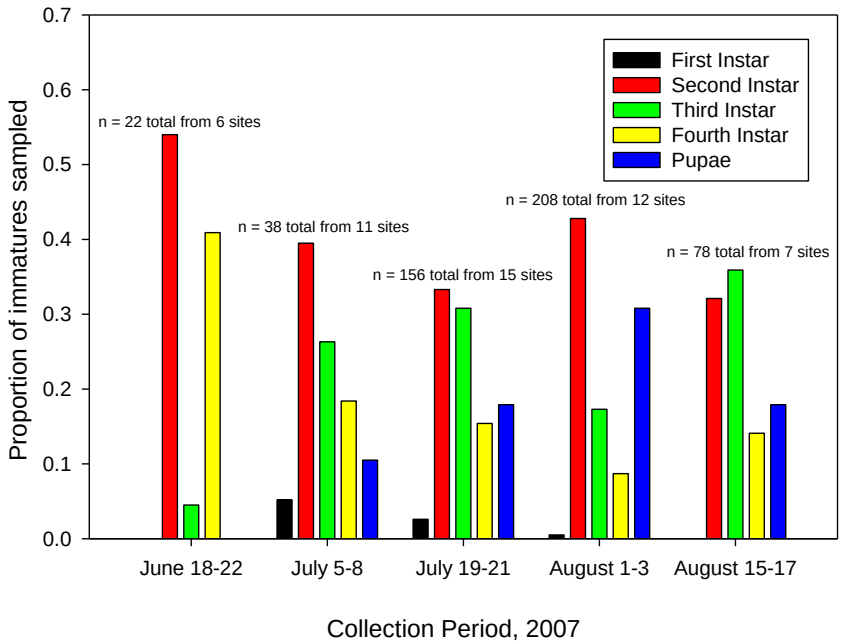

Figure 4. Distribution of immature stages in samples of 50 canola plants per site over the monitoring period from 18-Jun to 17-Aug, 2007 at sites in south-central Alberta in Experiment 3. Not all sites had immatures present at each sample date.

Experiment 4 There was a significant effect of pheromone dose on male moth capture and trap catch peaked at the pheromone dose currently used in the Prairie Pest Monitoring system. Trap capture declined at the highest dose tested. There was no main effect of lure type on the number of male moths captured but there was a significant pheromone dose*lure type interaction term ($P<0.001$). This significant interaction term was the result of increased trap capture in traps baited with grey lures at the highest doses tested (Figure 5).

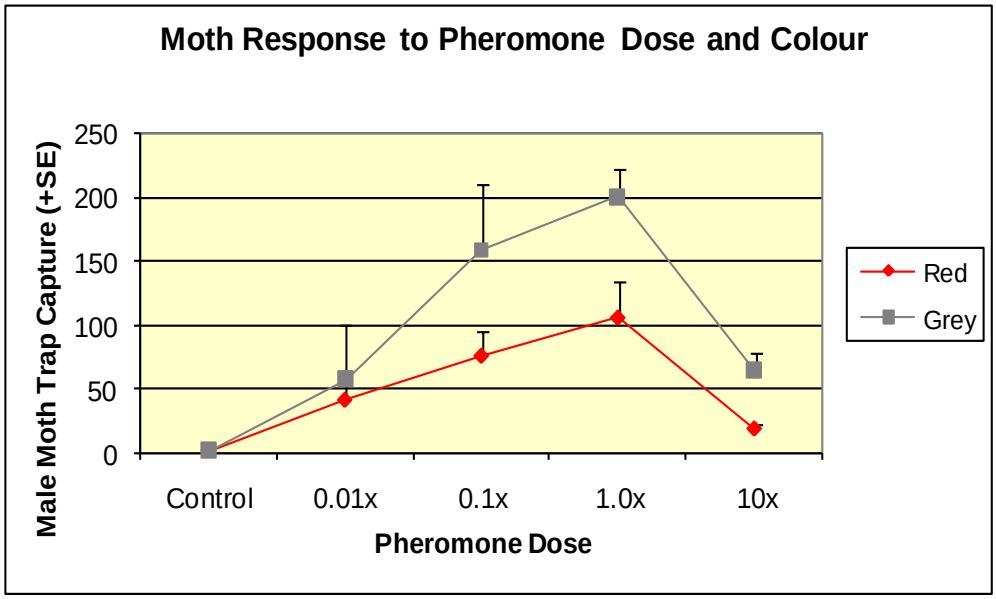

Figure 5. Mean number of male moths captured over a four-week period in traps baited with different doses of the commercially used pheromone (PheroTech International). Pheromone doses were applied to pre-extracted red and grey septa.

Experiment 5 Significant moth trap capture occurred only in traps baited with pheromone (Figure 6). There was no increase in trap capture as a result of the incorporation of the green leaf volatile to the pheromone lure. Traps are still being processed to determine if any female moths were captured in traps baited with the green leaf volatile. Trap colour did not affect the number of male DBM captured (Figure 6) but there was a trend towards the painted traps being less attractive, regardless of colour.

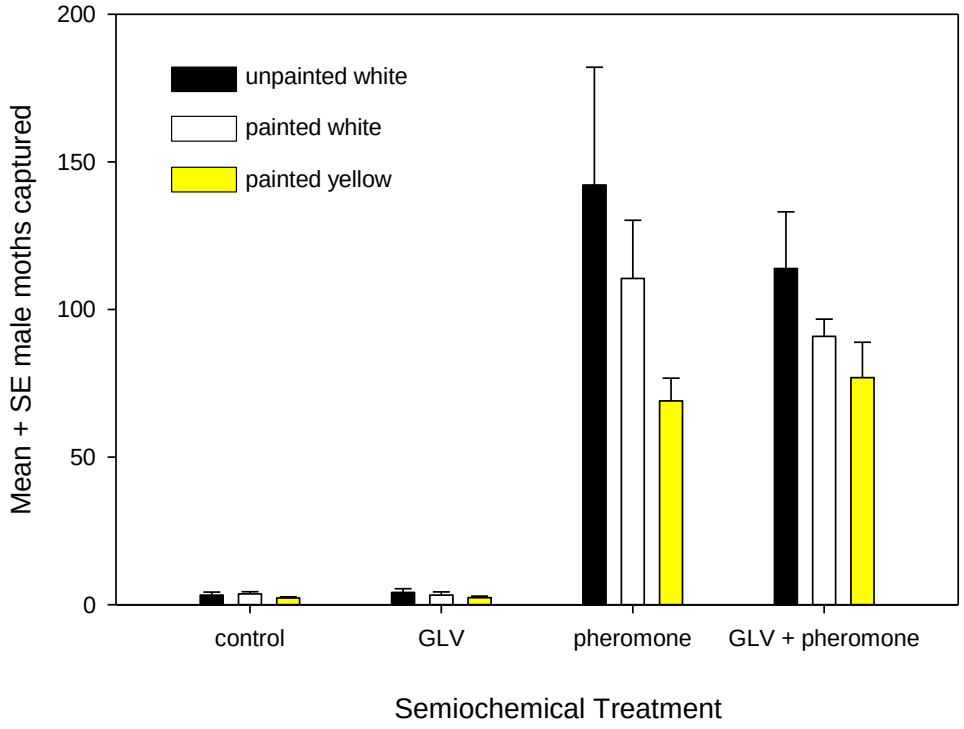

Figure 6. Mean number of male DBM captured in variously coloured traps baited with semiochemical treatments. The green leaf volatile (GLV) was *Z*3-hexenyl acetate and it was incorporated into lures in a 1:1 ratio with pheromone.

Experiment 6 The blend comparison experiment has now been analyzed (Figure 7). The most attractive blends tested contained 70:30:1 and 50:50:1 ratios of Z11-16:Acetate: Aldehyde: Alcohol (Figure 7). 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.

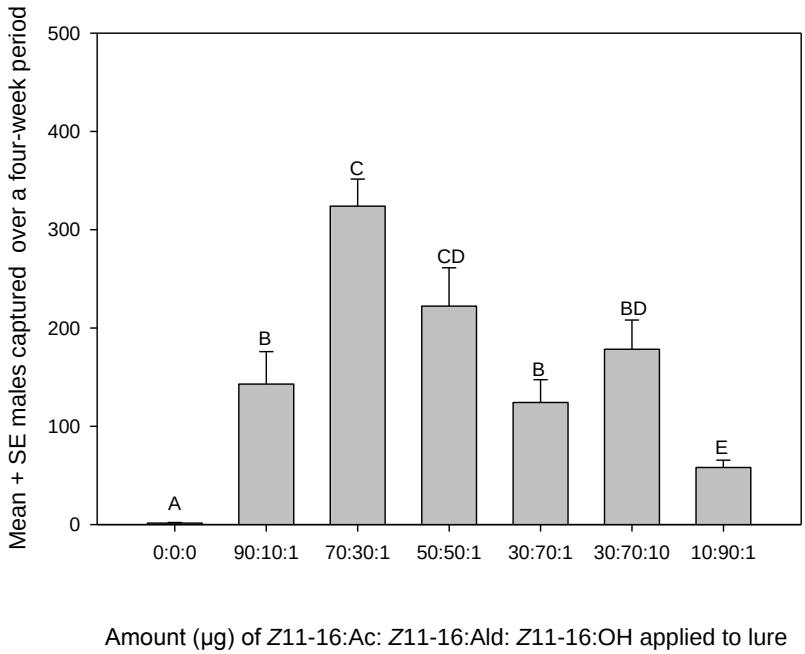

Figure 7. Mean number of male diamondback moths captured in traps baited with various ratios of the three known pheromone components over a four-week trapping period in 2007. Bars with the same letters are not significantly different (P>0.05)

Deviations from original plan:

- We have conducted all of the objectives for field experiments that were originally outlined in the proposal for Phase 2 of the project.
- However, we have so far not been able to develop a consistent laboratory assay to measure moth response to stimuli in the lab and so we are conducting all future tests in the field setting.
- We are still trying to quantify the proportion of each pheromone component that is released by females as female moths are much more attractive than commercially-available lures. However, we are recovering very low pheromone titres from calling females and have not yet been able to determine the proportion of compounds released by females.

EXPRIMENTS PLANNED FOR 2008:

Experiment 1: In 2008 we will continue with the development of a predictive model to determine DBM larval densities based on male moth capture in pheromone-baited traps. In 2008, at each of 15 sites in south-central Alberta there will be three traps baited with PheroTech lures changed at 6-week intervals (as in 2007) and three traps with PheroTech lures changed at 3-week intervals (as is commercially recommended) throughout the flight

season from May to mid-August. After the first moth catch, immature stages of the insect will be sampled at two-week intervals using a 50 plant sample from each site (as in 2007). Moth trap capture will be correlated with larval counts at each site to develop a predictive model in collaboration with Dr. Yang (statistician).

Experiment 2: A second season-long experiment will test the effect of trap height on male moth capture throughout the season. Three height treatments will be tested: 1) low (30 cm above the ground); 2) canopy height (trap moved with canopy height at each check); 3) high (1.5 m above the ground). The high trap position is what is currently in use for the Prairie Pest Monitoring system for diamondback moth. At each of 8 sites in south-central Alberta, there will be one trap baited with a PheroTech lure positioned at each of the three heights. Lures will be replaced at three-week intervals throughout the flight season.

Experiment 3: In 2007, we tested the attractiveness of host cues (green leaf volatile and trap colour) in combination with pheromone in a trapping experiment in July when the crop at all of our sites was in full bloom. In 2008, we will re-test the hypothesis that host cues may enhance trap capture in an experiment conducted earlier in the season (June) when there is limited competition of cues from the crop itself.

Experiment 4: In 2007, we tested one dose of a green leaf volatile (GLV) incorporated into the pheromone source to see if male moth response was enhanced and if we could attract female moths. In 2008, we will conduct a similar experiment except that we will test various doses of the GLV in combination with the pheromone. This experiment will be conducted over a four-week trapping period at peak flight in southern Alberta.

Experiment 5: The fifth field experiment in 2008 will compare red and grey rubber septa lures baited with the commercial dose of pheromone and aged for various periods prior to placement in traps. In 2007, we demonstrated that more moths were captured in traps baited with grey lures at the commercial pheromone dose. This experiment will tell us how attractive each of the lure types will remain throughout the season. Red and grey lures loaded with the commercial pheromone dose will be aged outside for 0, 2, 4, 6, and 8 weeks prior to placement in traps in the field in southern Alberta. Traps will remain in the field for four weeks and male moths captured in each treatment will be enumerated. Pheromone components released from each type of lure at each age treatment will be assessed in the laboratory.

Experiment 6: As PheroTech and APT lures have consistently been the most attractive pheromone lures in field trapping experiments comparing commercial lure types, we will conduct a field experiment to compare the longevity of attraction to these two lures. Lures will be field-aged as in Experiment 5 and differently aged lures from the two companies will be compared.

Experiment 7: In collaboration with the Gries lab at Simon Fraser University, we are continuing to examine female diamondback moth gland extracts in order to determine the female-produced blend. As we have demonstrated that calling female moths are more attractive than commercially available pheromone lures, we would like to determine what females are actually producing. If we are able to determine this, we will test this blend in the field and also compare it to response to calling virgin females in field cage studies.