PROJECT DETAILS

- **Title**: Improved integrated crop management with beneficial insects (part 1)
- **Funders:** Agriculture and Agri-Food Canada
- **Research program:** Growing Forward
- **Principal investigator:** Lloyd Dosdall, University of Alberta
- **Collaborators/additional investigators:** Jim Broatch, Héctor Cárcamo, and John Spence
- **Year completed:** 2013

Final report

Many studies have been undertaken in canola agro-ecosystems to assess the impact and control of pest species of insects, but relatively few studies have investigated the importance of beneficial insects in these systems. The predators and parasitoids of insect pests hold major advantages for the integrated management of insect pests. Once established, natural enemies can become permanent fixtures of canola agro-ecosystems, and can bring about control that is very specific and cost-effective. This research project focuses on diamondback moth in canola and the parasitoids that help keep its populations regulated. Diamondback moth can cause considerable reductions in yield of canola depending on the year and location, and insecticide applications are currently the only control strategy available to producers. The importance of diamondback moth as a pest of the crop is predicted to increase as climate change effects become more manifest. Parasitoids of diamondback moth are poorly studied in canola, even though the parasitoid *Diadegma insulare* is known to sometimes completely terminate diamondback moth outbreaks in western Canada. Two other parasitoid species, *Microplitis plutellae* and *Diadromus subtilicornis*, also attack diamondback moth and sometimes inflict high levels of parasitism. However, in spite of the importance of *D. insulare*, *D. subtilicornis*, and *M. plutellae* for managing diamondback moth outbreaks in canola, very little is known of their life histories and habitat requirements. The aim of this project is to examine the biology, abundance levels and distributions of parasitoids of diamondback moth in prairie canola. Research on this Canola Cluster project began in April 2010 and continued throughout the 2010-2012 field seasons. Surveys were undertaken in Alberta and Saskatchewan to determine the parasitoid fauna of diamondback moth in canola, and to assess the levels of parasitism in different sites and ecoregions. Laboratory colonies of diamondback moth and its dominant parasitoid, *Diadegma insulare*, were established the University of Alberta (Edmonton) and Agriculture and Agri-Food Canada (Saskatoon). Laboratory analyses of the developmental biology of the parasitoid under constant and fluctuating temperature conditions were undertaken in Edmonton and Saskatoon to determine developmental temperature thresholds for the insects and the effects of fluctuating temperatures on their development. Research also was undertaken at the University of Alberta to determine the effect of plant stress on

diamondback moth and parasitoid development and fitness.

Diamondback moth has a widespread distribution throughout all ecoregions of Alberta and Saskatchewan where canola production occurs. Results from the project show that the moth is most abundant in the Moist Mixed Grassland ecoregion and least abundant in the Boreal Transition ecoregion of both Alberta and Saskatchewan. The principal parasitoid of diamondback moth on the prairies is the wasp species *Diadegma insulare*. *Microplitis plutellae*, *Diadromus subtilicornis*, and *Cotesia* sp. were also found to be important parasitoids. The study found several species of parasitoid not previously known to attack diamondback moth. The discovery of *Cotesia* parasitizing diamondback moth larvae in western Canada is novel; this species appears responsible for a substantial level of the total parasitism of diamondback moth in the region. The wasp *D. insulare* is most abundant in the Moist Mixed Grassland ecoregion of Alberta, but in Saskatchewan *D. insulare* is most abundant in the Boreal Transition ecoregion. Parasitism levels of diamondback moth larvae and pupae are highly variable, and depend on the site, year, and time of season.

Females of *D. insulare* are attracted to odors emitted by diamondback moth larvae and by larval frass (excreta), but not to odors produced by larval silk. Males are not attracted to odors of diamondback moth, larval frass, or larval silk.

Our results indicate that in canola fields, adults and larvae of diamondback moth tend to be clustered in their distributions early in the season, with distributions becoming more even as the season progresses. Sulphur content of canola leaf tissue appears to be an important factor in determining diamondback moth distributions: moth numbers tend to be correlated with areas of canola fields high in sulphur content. Distributions of larvae of diamondback moth and its principal parasitoid, *D. insulare*, are closely correlated.

Diamondback moth females prefer to lay their eggs on older plants (six-week-old) than on younger plants (four-week-old). Females of diamondback moth also prefer to oviposit on plants not under moisture stress compared with water-stressed plants. Although diamondback moth larvae did not show differences in developmental rate from larva to adult on stressed or unstressed plants, development of the parasitoid *D. insulare* was faster when host larvae fed on unstressed plants than on stressed plants. Body size parameters of *D. insulare* were greater when host larvae were reared on unstressed than on stressed plants, suggesting that fitness of *D. insulare* when its larval hosts were reared on unstressed plants was better than on stressed plants.

Diamondback moth adults and larvae are more heat tolerant than adults of *D. insulare* and parasitized diamondback moth larvae. *Diadegma insulare* is a more effective parasitoid at lower than higher temperatures.

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Both insects are able to tolerate high temperatures if these are brought on gradually as opposed to suddenly. The molecular response through heat shock protein gene expression mirrors this ability to cope with high temperatures. These results suggest that diamondback moth could fare well in the event of temperature increases predicted to occur with the onset of climate change, while the effectiveness of *D. insulare* as an efficient parasitoid of the moth could decrease.

We found that sweep net sampling is more time-consuming than pan trap sampling or sampling with sticky cards for diamondback moth and its parasitoid fauna. However, sweep net sampling provides a more realistic estimation of the diamondback moth and parasitoid population densities, and their population structures.

The project resulted in development of innovative practices and recommendations in insect pest management for farmers and agrologists. These are summarized as follows:

- canola fields vary considerably in the densities of diamondback moth and the species composition and abundance of its parasitoid fauna, even in the same year and among fields in the same geographical region. Levels of parasitism in diamondback moth larvae and pupae can be relatively high early in the season. It is therefore important for growers to carefully monitor pest and natural enemy populations to ensure that unnecessary insecticide applications are not made.
- the most efficient approach for monitoring canola for diamondback moth and its parasitoid fauna is to take sweep net samples in production fields. Because populations of moths and parasitoids can be clustered or aggregated in their distributions, rather than distributed uniformly, it is important to sample at several locations within each field.
- while sulphur is an important soil nutrient required for canola production, its presence in plant leaf tissue appears to be attractive to diamondback moth. Canola producers should therefore apply levels of sulphur to soil based on soil sample recommendations, but should avoid applications that exceed recommended levels.
- when plants are under moisture stress, they are less attractive for egg-laying by diamondback moth females. Fields that are drought-stressed at the time of diamondback moth influxes on winds from southern North America will likely be subjected to reduced infestation levels of these pests. Parasitoids that develop in diamondback moth larvae reared on drought-stressed host plants require more time to progress from egg to adult, and are less fit than parasitoids that develop on unstressed plants. Careful crop monitoring is important when water stress occurs, because control of diamondback moth by natural enemies may not be as efficient compared to conditions with sufficient soil moisture levels.

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Objective 1: Determining how densities and levels of parasitism by *Diadegma insulare*, *Microplitis plutellae*, and *Diadromus subtilicornis* vary in canola agroecosystems across western Canada.

In 2010, 2011, and 2012, commercial fields of canola and mustard were sampled throughout Alberta and Saskatchewan to make collections of diamondback moth and its parasitoids across the various eroregions. For instance, in southern Alberta fields were sampled near Bow Island, Coaldale, Lethbridge, Etzikom, Foremost, Grassy Lake, Medicine Hat, Orion, Seven Persons, Skiff, and Wrentham. Many more fields were sampled in central Alberta. In Saskatchewan, canola fields were sampled near Saskatoon, Wakaw, Choiceland, and several additional sites. A global positioning system device (Garmin® 43 GPS 12) was used to record the location of each field. In total, approximately 50 fields were sampled each year in each province.

Using a sweep net, collections of approximately 50-100 diamondback moth larvae were obtained per field, returned to the laboratory, and reared in individual Petri dishes to obtain parasitoids and to determine the incidence of parasitism. Late in the season (late August), collections of diamondback moth pupae were made in several fields in southern Alberta. Pupae were collected by hand-picking specimens from canola plants, and in the laboratory they were placed in rearing containers, and reared to produce either diamondback moth adults or parasitoids. These collections targeted specimens of the parasitoid *D. subtilicornis*, because it attacks the pupal stage of diamondback moth. Specimens were maintained in a growth chamber where they were held at 25±1°C, photoperiod 8/16 h (D/L), with approximately 60% RH.

In 2011 canola plants infested with newly-laid diamondback moth eggs were set out weekly for six weeks in research plots near Lethbridge to allow for possible parasitism by naturally occurring egg parasitoids. The plants were left in the field for about 48 hr, then returned to the laboratory to determine whether the eggs hatched into diamondback moth larvae or whether egg parasitoids emerged. Diamondback moth adults were swept from a canola field near Lethbridge, weekly for 6 weeks, preserved in alcohol, and later dissected to determine the possible occurrence of parasitoids within the moths.

Adult parasitoids were identified, labelled, mounted on insect pins, and sent to the Eastern Cereal and Oilseed Research Centre in Ottawa, ON for confirmation and further identification.

Objective 2: Determining olfactory cues used by females of *D. insulare* to locate larvae of diamondback moth.

A four-chamber olfactometer was used to evaluate the relative attractiveness of diamondback moth larvae, larval frass in the absence of larvae, larval silk, and a blank control to males and females of *D. insulare*. All tests

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were conducted in the laboratory at 21°C. The olfactometer was placed squarely under ceiling fluorescent lighting (GE T8 F32T8/SPX41, General Electric, Fairfield, CT). Compressed air was maintained at 0.24 L min^{-1} using an airflow regulator, filtered using activated charcoal and bubbled through distilled water to maintain consistent humidity before being pumped into the apparatus. Relative humidity in the device was measured at 1 s intervals over 2 h and determined to be 48.98 ± 0.009%. Airflow was run through a splitter and to odour sources and controls. Between runs, all parts of the apparatus were washed in dish soap and water, rinsed with water, then rinsed with 70% ethanol and dried.

Adults of *D. insulare* were introduced to the olfactometer, and wasp positions in the olfactometer were recorded after 20 min. Chambers containing test materials and controls were switched after each run. Data were analyzed with analysis of variance using a mixed model (SAS proc MIXED).

Objective 3: Determining the spatio-temporal distribution dynamics of diamondback moth and its parasitoids in canola.

The study was conducted in portions of three commercial fields of *B. napus* located near Lethbridge, AB in the Moist Mixed Grassland Ecoregion of Alberta. Seeding rate was 3-5 kg ha^{-1}, which resulted in canola plant stands of approximately 75 m^{-2} at each site. A grid layout was used within each field. Each grid comprised an area of 10,000 m^2 and was subdivided in a grid pattern to form 100 plots, each measuring 10 by 10 m. Sampling within each grid plot consisted of taking 10, 180° sweep net samples in a linear transect, placing the collected specimens in a labelled plastic bag, and storing samples temporarily in a freezer until larvae, adult moths, and adult wasps could later be identified, counted, and recorded. Samples of canola leaves were also removed from 10 plants within each grid cell. The leaves were stored temporarily in the freezer, and later dried and analyzed for contents of nutrients, including nitrogen, phosphorus, potassium, and sulfur. At each of three points within each grid plot, determinations of plant density were made by counts of plant numbers per 0.5 m^2.

Spatial Analysis by Distance IndicEs (SADIE) software was used to analyze spatial distributions of diamondback moth and its parasitoids. SADIE performs permutations of observed insect counts among sampling units and assesses observed arrangements in species count data by tests of randomization. Spatial patterns of single sets of counts of moths/parasitoids on a given date were determined using the main SADIE index, I_a, the subsidiary index, J_a, and the distance index, δ. Values of index I_a that approximate 1.0 indicate random distributions in a data set, but values that exceed 1.0 indicate aggregated arrangements. The index, J_a, distinguishes among patterns. When the value of J_a is greater than 1.0, it indicates a single major cluster, but values less than 1.0 indicate two or more clusters. Finally, δ refers to the distance between two centroids, *P* and *C*. The spatial

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analysis computes the location *P* from the *x* and *y* co-ordinates of sample units as the "middle" of the sample, and location *C*, as the centroid of the counts. The value of δ is then the distance between these two centroids. The patch index V_i and the gap index V_j are also calculated. The measure of association, X_k, was calculated for each sample location. Values less than 0.025 indicate significant association between parameters, and values greater than 0.975 indicate significant disassociation. We conducted tests of association/disassociation between diamondback moth and its parasitoids, between the insects and various host plant nutrients, and between insects and canola plant density.

Objective 4: Determining the most appropriate sampling methods for diamondback moth and its parasitoids.

At Lacombe and Edmonton, AB populations of *P. xylostella* and its parasitoids were monitored with three methods: sweep net, pan trap, and sticky card sampling. The project was undertaken in fields seeded to *B. napus* canola, using a randomized complete block design, with plot dimensions of 10 by 10 m. Sampling began in early June when canola was in the rosette stage of development and continued throughout the season, with samples collected once per two weeks. Sweep net samples were taken with a 40-cm-diameter net, along a linear transect in each plot, so that each sample consisted of 10, 180° sweeps per plot. Sweep net samples were bagged, labelled, and kept in refrigerated containers while being transported to the laboratory. In the laboratory, approximately 200 mL of 70% ethanol were added to each sample bag; the samples were then inspected for insects, with specimens of diamondback moth and its parasitoids counted and recorded. One plastic yellow pan trap, measuring 30 by 24 by 7 cm, was placed on the soil surface of each plot. Pan traps were filled with a 50% solution of propylene glycol. Each sampling date, all specimens from each pan trap were removed by filtration through an aquarium net and insects were stored in 70% ethanol until specimens of diamondback moth and its parasitoids could be counted and recorded. Sticky cards were 7.6 cm provided by Contech Enterprises Inc., Delta, British Columbia. Three cards were placed in each plot, spaced about 50 cm apart, and attached to wooden stakes, held about 10 cm above the soil surface.

In each of the research plots, a sample of 10 entire plants was collected on each sampling date. Entire canola plants were cut off at the soil-stem interface, bagged individually, kept in refrigerated containers, and transported to the laboratory for processing. To remove insects from the plants, each plant was washed in 70% ethanol and thoroughly inspected. Specimens of diamondback moth and its parasitoids were counted and recorded.

Data were analyzed to determine the total numbers of specimens of *P. xylostella*, *D. insulare*, *M. plutellae* and *D. subtilicornis* collected with each sampling device each week, and to compare numbers of specimens of each

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P. xylostella life stage collected with the different samplers.

Objective 5: Determining how larvae of diamondback moth and *D. insulare* respond to abiotic stress, specifically lack of moisture (drought).

A greenhouse experiment was set up using a completely randomized design. Treatments comprised two species of Brassicaceae, *Brassica napus* and *Sinapis alba*, and two levels of stress, drought-stressed plants and irrigated (unstressed) plants. Insect cages (measuring 40.5 by 40.5 cm at the base and 80.5 cm in height, lined on the sides with 500-µm Nitex mesh screening) on a greenhouse bench were used to house single plants of either *B. napus* or *S. alba*, under drought stress or unstressed.

In Experiment 1, four- and six-week-old canola plants were used to investigate the effects of plant stress and plant age on oviposition by diamondback moth. Unstressed plants were watered daily until saturation, and stressed plants received no watering for the three days preceding the experiment. The pressure chamber method was used to determine the leaf water potential of stressed and unstressed plants. Twelve pairs of diamondback moth adults were placed in each insect cage on a greenhouse bench and allowed to oviposit for 48 h on stressed and unstressed plants that were either four or six weeks old. There were 10 replicates of each host plant treatment.

In Experiment 2, 10 larvae of diamondback moth were placed onto each plant, within an insect cage, when plants were in the 4-leaf stage of development. Larvae were newly eclosed second instars, and their developmental time was determined from second-instar larvae to emergence of new generation adults.

In Experiment 3, 10 second-instar larvae of diamondback moth, freshly parasitized by mated females of *Diadegma insulare*, were placed onto each host plant (in the 4-leaf stage of development). To obtain parasitized, second-instar larvae of diamondback moth, we first placed a potted plant of *Brassica rapa* ssp. *pekinensis* in a cage with 20-30 mated females of diamondback moth within a growth cabinet (at 25±0.5°C) in darkness for 6-8h for oviposition. Adult moths were then removed, and the plant was maintained at a constant temperature of 25°C for eight days until second-instar larvae emerged from their leaf mines. Second-instar diamondback moth larvae were then removed, suspended individually on their silken threads using a small brush, and exposed to mated, gravid females of *D. insulare*. Following parasitization, the larvae were then placed into the cages for use in Experiment 3.

Stressed and unstressed plants varied in the amount of water added to their soil during plant growth. The

plants were held in 10-cm-diameter plastic pots, and 88 mL of water were added daily to the irrigated plants, compared with 30 mL for the stressed plants. Levels of stress for each plant were quantified by measuring the leaf water potential of each host plant in a pressure chamber.

Diamondback moth oviposition preferences on stressed and unstressed plants of *B. napus* that were four and six weeks old were determined by comparing counts of eggs laid on each replicate host plant. Survival and developmental parameters of the non-parasitized (Experiment 2) and parasitized (Experiment 3) larvae of diamondback moth were determined. Data were analyzed using analysis of variance and a mixed model.

Objective 6: Determining how development of *D. insulare* is affected by temperature regime.

The published lower threshold temperature for diamondback moth development is 7.3°C, but there is no known upper threshold temperature for the insect, nor are there set minimum or maximum threshold temperatures for its larval parasitoid *D. insulare*. In Experiment 1, a laboratory study was undertaken to determine the effects of constant temperatures (7, 22, and 30˚C) and corresponding fluctuating temperatures (0-14, 15-29, and 23-37˚C) on the development of diamondback moth and *D. insulare*. Parasitized third-instar diamondback moth larvae were reared until adult mortality in individual thermal gradient cells at different temperature regimes. Larval mortality, parasitism success, pupal mortality, larval and pupal developmental time, adult longevity, and pupal and adult dry weight were recorded.

Experiment 2 considered survival and development of diamondback moth and *D. insulare* under various short-term (2 h) high extreme temperature regimes. Ten newly emerged diamondback moth or *D. insulare* adults were transferred into individual plastic cups (6 × 4 cm^2) along with a moistened dental roll. They were then acclimatized at 25°C for 24 hours. Following acclimation, the insects were exposed to different temperature regimes: an abrupt shift to 38 or 40°C, or increasing temperature over one hour to 38 or 40°C, and then staying at the peak temperatures for two hours in individually controlled thermal gradient cells. After the heat treatment, insects were immediately transferred to a 25°C incubator for two hours, at which time survival was evaluated. A similar protocol was followed for fourth instar parasitized and non-parasitized diamondback moth larvae, except that larvae were placed on a moistened filter paper in a plastic Petri dish (5.5 cm).

In Experiment 3 we examined the molecular responses of diamondback moth and *D. insulare* to elevated temperatures by monitoring the levels of the heat shock 70 protein gene (*hsp*70) in the organisms when they were exposed to differing thermal regimes. Primers for *hsp70* and *β*-actin genes of both insects were developed. After exposure to the thermal treatments of Experiment 2 and a 1 h recovery period at 25˚C,

insects were frozen in liquid nitrogen and stored at –80°C. Real time quantitative PCR analyses were run on the insects to determine their levels of *hsp70* gene expression.

In a fourth activity, we fit data that we generated for survival and development of diamondback moth and *D. insulare* at temperatures ranging from 2 to 38° to statistical models in order to determine upper and lower threshold temperatures for both insects. A DYMEX climate model also was developed for predicting diamondback moth and *D. insulare* occurrence in the field.

Main Findings: The main findings of our studies are described below.

Objective 1:

- diamondback moth is a widespread pest of canola, occurring across the prairie provinces in every agricultural ecoregion. In Saskatchewan, highest numbers were found in the Mixed Grassland Ecoregion, and lowest numbers occurred in the Boreal Transition Ecoregion. In Alberta, highest numbers of diamondback moth were found in the Mixed and Moist Mixed Grassland Ecoregions, and lowest numbers occurred in the Boreal Transition Ecoregion.

- parasitoid species are important in the natural regulation of diamondback moth populations. Parasitism levels ranged from 5 to 96%, depending on the year and site. Parasitism tends to be greater early in the season, rather than in mid-season or late in the growing season. However, parasitoids were reared from diamondback moth larvae collected as late in the season as November 4, 2011, indicating a wide tolerance to temperatures by parasitoids. The principal parasitoid species attacking diamondback moth on the prairies are *Diadegma insulare*, *Microplitis plutellae*, *Diadromus subtilicornis*, and *Cotesia* sp., believed to be *Cotesia vestalis*. The discovery of *Cotesia* parasitizing diamondback moth larvae in western Canada is novel, and this species appears responsible for a substantial level of the total parasitism of diamondback moth. One hyperparasitoid of ichneumonids and tachinids, *Mesochorus bilineatus*, was identified as a primary parasitoid of diamondback moth. *Conura* sp. was also responsible for a low level of parasitism (9.3%) from central Alberta in 2011. When parasitoids were sent to Ottawa for identity verification, taxonomists determined with the help of DNA analysis that *Microplitis plutellae* Muesebeck is actually numerous species in a species complex, the members of which have yet to be determined.

- two of four diamondback moth life stages are attacked by parasitoids: the larvae are attacked by *D. insulare*, *M. plutellae*, and *Cotesia* sp., and pupae are attacked by *D. subtilicornis*. As well, two ichneumonid species *Itoplectes quadricingulata* (Provencher) and *I. conquisitor* (Say), typically pupal parasitoids of leafrollers and

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other Lepidoptera, emerged from diamondback moth pupae collected in Saskatchewan, a new host record for these parasitoids. We found no evidence of parasitoids that attacked diamondback moth eggs or adults.

Objective 2:

- females of *D. insulare* responded positively to odors emitted by diamondback moth larvae and by diamondback moth larval frass ($P < 0.05$); however, larval silk and the blank control did not elicit behavioral responses ($P > 0.05$).

- males of *D. insulare* showed no positive responses in the olfactometer to either diamondback moth larvae, diamondback moth larval frass, diamondback moth larval silk, or the blank controls ($P > 0.05$). Males tended to remain in a neutral position in the centre of the olfactometer arena rather than selecting one of the choice chambers.

Objective 3:

- diamondback moth populations tended to be aggregated in canola throughout the growing season, but populations of its principal parasitoid, *D. insulare*, were clustered in early flowering and its distributions later became more uniform as the season progressed. Populations of other diamondback moth parasitoids were not abundant enough to show patterns of aggregation and/or random distribution.

- the close spatial associations between densities of *D. insulare* and diamondback moth suggested that host abundance was the main determinant of parasitoid distribution patterns.

- distributions of diamondback moth in canola were positively correlated with sulphur content in canola leaf tissue.

- no link between host plant density and distributions of either diamondback moth or its parasitoids was found.

Objective 4:

- in terms of sample processing time, sweep net sampling required the most effort, and sticky card sampling the least (sweep net sampling > pan trap sampling > sticky card sampling). However, sweep net sampling provided the most realistic estimate of diamondback moth and parasitoid populations in canola crops because this sampling approach captured most life stages (larvae and adults of diamondback moth and adults of parasitoids), whereas pan trap and sticky card sampling only captured adults of the herbivore and

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its parasitoids. Parasitoids were rarely captured on sticky card samplers and infrequently in pan trap samplers, but they were routinely collected in sweep nets. None of the sampling methods tested were effective for estimating egg or pupal populations.

- when each of the sampling methods was compared to sampling of entire plants, sweep net sampling most closely represented the population structure of entire canola plants. Entire plant sampling did result in collections of eggs and pupae of diamondback moth which were absent in sweep net samples, but the entire plant samples did not capture adults of either diamondback moth or its parasitoids.

- sweep net sampling requires more processing time than that required for pan traps or sticky cards, but less processing time than is needed for entire plant sampling. In view of the benefits and drawbacks of each sampling method, it is recommended that sweep net sampling be recommended for sampling populations of diamondback moth in canola cropping systems.

Objective 5:

- females of diamondback moth laid significantly more eggs on 6-week-old plants than on 4-week-old plants ($P < 0.001$).
- females of diamondback moth laid significantly more eggs on unstressed plants than on plants subjected to water stress ($P < 0.001$).
- developmental times of nonparasitized diamondback moth larvae were similar on *B. napus* and on *S. alba* ($P > 0.05$), but development from parasitized second-instar larvae to pupae was more rapid on *B. napus* (approximately 9 days) than on *S. alba* (approximately 10.5 days) ($P = 0.0029$).
- developmental times of nonparasitized diamondback moth larvae were similar on stressed and unstressed plants ($P > 0.05$). However, development of larvae parasitized with *D. insulare* from second instar to pupa was significantly faster on unstressed plants than on plants subjected to moisture stress ($P = 0.0384$). No significant differences were observed in development of nonparasitized or parasitized larvae from pupa to adult ($P > 0.05$).
- adult body weights were similar for diamondback moth larvae reared on stressed and unstressed plants. However, mean body weights of males and females of *D. insulare* were significantly greater when their diamondback moth hosts were reared on non-stressed than on water-stressed plants ($P < 0.0001$). Adult body weights of *D. insulare* were significantly greater when reared as parasitized diamondback moth larvae on *S. alba* than on *B. napus* ($P = 0.0049$).

Objective 6:

- diamondback moth can successfully complete development from second larval instar to adult at temperatures ranging from 4°C to 37°C. The temperature range for completing the life cycle (egg to adult) of *D. insulare* is 5 to 32°C. Larvae of either parasitized or nonparasitized diamondback moths exhibited more thermal tolerance than pupae and adults. Pupal mortality of *D. insulare* increased with increasing temperature; however, diamondback moth did not show such a response. The greatest parasitism success (67%) was found at constant and fluctuating 22˚C and fluctuating 7˚C, and the lowest (33%) at fluctuating 30˚C. Longer development times and greater pupal body masses occurred at lower temperatures for both insects. Significant differences occurred between constant and fluctuating temperature regimes for most parameters of both insects. Fluctuating compared with constant temperatures caused shorter development times and higher adult longevities for both insects at optimal and lower temperature ranges. Both insects experienced 0˚C at fluctuating 7˚C (0-14˚C) and survived. Comparing the successful parasitism capacity of the wasp and pupal survival and body mass of both host and parasitoid, it appears that *D. insulare* is a more effective parasitoid at lower temperatures.

- tolerance to high temperature stress by diamondback moth adults was greater than that of *D. insulare*. Survival of *D. insulare* adults was significantly reduced after a slowly increasing two-hour exposure to a peak of 38°C, a sudden increase to 38°C, a slow increase to a peak of 40°C, or a sudden increase to 40°C (in all cases followed by one hour recovery time) compared with exposure to a 25°C constant temperature. On the other hand, survival of diamondback moth adults was not affected by the tested extreme temperature profiles with the exception of a sudden increase to 40°C, where a significantly lower portion of moth adults survived compared with survival at the control temperature. There was no effect of two-hour heat stress of up to 40°C on either parasitized or non-parasitized diamondback moth larvae. Pupal mortality of *D. insulare* was highest when exposed to a sudden-onset 40°C temperature. Larval and pupal developmental times of *D. insulare* were affected by temperature stresses. Shorter larval developmental times were required when the stress temperature increased, with the fastest development occurring at sudden 40°C three times per week compared to exposure to the control temperature, but pupae took a longer time to adult eclosion. In contrast, developmental times of diamondback moth were not affected by such extreme temperatures. We conclude that the parasitoid *D. insulare* is more sensitive to high stress temperatures compared to its host, and the population expansion of *D. insulare* may be limited by warmer temperatures associated with climate change.

- in adults of both species, significantly more *hsp70* gene expression was observed when temperatures increased abruptly to 38°C compared to a gradual increase. In contrast, at 40°C significantly more

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expression was found in insects exposed to the gradual rather than the abrupt regime. *Hsp70* expression level was in agreement with the adult survival data and appears to be good indicator of stress levels. In parasitized and non-parasitized larvae, *hsp70* gene expression was significantly higher after abrupt shifts in temperature compared to gradual shifts to both temperatures. These results suggest that *hsp70* gene expression in DBM and *D. insulare* is responsive to extreme temperatures and may function as a potential biomarker for changing climatic conditions.

- the DYMEX models for both diamondback moth and *D. insulare* were developed and await validation with field data.

Development of Innovative Agri-products, Agri-processes and Practices:

The project resulted in development of some innovative practices and recommendations in insect pest management for farmers and agrologists. Some of these are summarized below.

- Canola fields vary considerably in the densities of diamondback moth and its parasitoid fauna, even in the same year and when the fields occur in the same geographical region. It is therefore important for growers to carefully monitor pest and natural enemy populations to ensure that unnecessary insecticide applications are not made.
- The most efficient approach for monitoring canola for diamondback moth and its parasitoid fauna is to take sweep net samples in production fields. Because populations of moths and parasitoids can be clustered or aggregated in their distributions, rather than distributed uniformly, it is important to sample at several locations within each field.
- Sulphur is an important soil nutrient required for canola production, but its presence in plant leaf tissue appears to be attractive for diamondback moth. Canola producers should therefore apply recommended levels of sulphur to soil, but avoid applications that exceed recommended levels.
- When plants are under moisture stress, they are less attractive for egg-laying by diamondback moth females. Fields that are drought-stressed at the time of diamondback moth influxes on winds from southern North America will likely be subjected to reduced infestation levels of these pests. Parasitoids that develop in diamondback moth larvae reared on drought-stressed host plants require more time to progress from egg to adult, and are less fit than parasitoids that develop on unstressed plants. Careful crop monitoring is important when water stress occurs, because control of diamondback moth by natural enemies may not be as efficient compared to when sufficient soil moisture levels occur.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
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