PROJECT DETAILS

- **Title**: Identification and assessment of the role of natural enemies in pest suppression in canola with specific reference to diamondback moth management
- **Funders:** Alberta Canola and SaskCanola
- **Research program:** Canola Agronomic Research Program
- **Principal investigator:** Maya Evenden and Sharavari Kulkarni
- **Collaborators/additional investigators:** Hector Cárcamo
- **Year completed:** 2022

Final report

Introduction

A variety of insect pests infest canola on the Canadian Prairies including native and invasive species (Dosdall et al. 2011). These include flea beetles, lygus bugs, bertha armyworm and diamondback moth, which may cause crop losses worth several million dollars. Pest management decisions can be particularly difficult for insect pests that show routine migrations in their life history (Chu 1986). For example, diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) migrates to western Canada on wind currents from the U.S.A annually (Dosdall et al. 2001). Losses incurred vary yearly and levels of infestation differ from region to region. While the pest assumes minor status in some years, heavy crop losses as high as $45 to 52 million CAD can occur during outbreaks (WCCP 1995). Use of chemical insecticides is the primary tactic during outbreak years and past records indicate insecticidal intervention over wide geographic areas (1.8 million ha area in 2001) (WCCP 2001). Factors complicating diamondback moth management include its reproductive potential, multiple generations in a growing season and capacity to develop resistance to several insecticides with diverse chemistries and modes of action (Dosdall et al. 2011). Reliance on a single management tactic such as chemical control for diamondback moth management is likely to fail (Talekar and Shelton 1993), and implementation of integrated pest management (IPM) is essential (Philips and Mengersen, 2014).

Integrated pest management tactics can help to mitigate insect pest losses in effective, economic and sustainable manner, and one of the main pillars of a successful IPM strategy is conservation biocontrol through the role of natural enemies in pest suppression. Pest management services provided by insect natural enemies amount to approximately $5 billion worldwide (Losey and Vaughan 2006). A sound IPM plan should therefore integrate crop and pest management practices that encourage the activity of natural enemies in field crops (AAFC 2015). A two-pronged approach that includes the following components is essential: 1) to identify the natural enemy fauna associated with major pests and investigate factors to promote conservation biological control and 2) to estimate natural enemy contributions to pest suppression by incorporating natural enemies into management decisions and action thresholds using approaches such as dynamic action threshold (DAT) (Hallett et al. 2013).

Quantification of such contributions can help producers make informed decisions about timing and selection of appropriate pest management tactics. Beneficial insects including predators, parasitoids and pollinators provide valuable services including pollination and pest suppression in canola (Canola Council 2015), and several species of predators and parasitoids have been documented. Natural enemy surveys for diamondback moth have identified the presence of species of ladybird beetles, lacewings, carabids, minute pirate bugs and several egg and larval parasitoids.

In western Canada *Diadegma insulare* (Cression) (Hymenoptera: Ichneumonidae) is the major larval parasitoid species of diamondback moth (DBM) and parasitization levels up to 45% have been recorded (Dosdall et al. 2011). Several other parasitoid species have been recorded to use DBM as a host including *Microplitis plutellae* (Muesbeck) (Hymenoptera: Braconidae), and *Diadromus subtilicornis* (Gravenhorst) (Hymenoptera: Ichneumonidae) (Dosdall et al. 2011). *Diadegma insulare* also migrates to western Canada, and DBM outbreaks are influenced by the timing of their arrival. Hence, the management tactics for DBM need to take into account the role of parasitoids like *D. insulare* and other natural enemies. This requires quantification of contributions of *D. insulare*, and also of other important parasitoid and predatory species to understand their role in natural suppression of DBM populations.

Natural enemies vary in their ability to suppress pest populations depending on their voracity (Hallett et al. 2013). Currently, the information on seasonal abundance and association of natural enemies with DBM populations is limited. No specific studies quantify contributions of major predatory and parasitoid species in DBM management. Further, critical information of natural enemy assemblages in canola with respect to crop growth stages, specific insect pest species and associated background vegetation is scant. Limited understanding of the role of natural enemies in pest suppression in canola agroecosystems has been identified as a major knowledge gap (Canola Research Summit, Canola Council 2011). It is important to understand which natural enemies contribute to diamondback moth population regulation and describe their functional response.

In view of the current knowledge gaps, this investigation aimed to survey natural enemy taxa (including predators and parasitoids) associated with diamondback moth in canola in Alberta and assess the changes in assemblages with respect to crop growth, background vegetation and abiotic factors (temperature, precipitation, relative humidity). The research team also aimed to investigate seasonal abundance of parasitoid species and their association with DBM populations. Factors enhancing levels of parasitization and parasitoid foraging for DBM in canola agroecosystems have not been fully investigated. For example, sugar resources are important for longevity and fecundity of parasitoids and provision of sugar-rich food/nectar resources through plant resource diversification can influence parasitization (Winkler et al. 2006). Information on the effects of resource provision on conservation biological control by the main natural enemy species, such as *D. insulare*, is scarce. Also, plant volatiles emitted by plants infested by DBM or other generalist pests attract parasitoids including *D. insulare* and improve their host searching ability in crops like collard (Hu and Mitchelle 2001).

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Mechanisms of semiochemical-based foraging by *D. insulare* in canola have not been specifically studied, and can help to strengthen conservation biological control. For example, in collards when populations of DBM are low in fields, plant volatiles released due to damage caused by other generalist pests can attract *D. insulare* adults to the field areas, and improve the encounter chances with DBM larvae (Hu and Mitchelle 2001). This investigation aimed to address these gaps by studying the host-parasitoid and predator-prey associations for DBM.

This investigation contributes important data to the development of dynamic action threshold model that can provide a holistic approach for DBM management. Currently, the Prairie Insect Pest Monitoring Network (PPMN) uses catch trap and field surveys for DBM monitoring. The pheromone trap catches indicate peak moth influx and provide information on when to monitor DBM larval populations. This research can improve the currently available action thresholds and strengthen IPM efforts for DBM management.

Objective 1: To monitor natural enemy populations associated with diamondback moth (DBM) in canola with particular focus on larval parasitoids.
Objective 2: Development of functional response models to understand relationships between DBM and its natural enemies to develop dynamic action thresholds.
Objective 3: To study non-consumptive effects of predator and parasitoid on DBM.
Objective 4: To understand the effects of varying larval densities of DBM on foliar damage in canola and yield.

Methods

Objective 1: To monitor natural enemy populations associated with DBM in canola with particular focus on larval parasitoids

Methodology: Natural enemy populations associated with DBM in canola in Alberta were monitored using biweekly surveys during July and August of 2018, 2019 and 2020 to coincide with peak moth influx and larval activity with respect to canola growth stages. The surveys were conducted in southern Alberta. The sites for the survey were determined using adult moth capture data from Alberta provincial surveys and the pheromone trap network of the Prairie Pest Monitoring Network. Samples were taken using sweep-net (15 cm diameter heavy duty sweep net, BioQuip Product Inc, Crompton, CA, USA) sampling and hand collection of larvae. Two hundred, 180° sweeps were used to collect larvae from each field (n=18 in 2018; n= 14 in 2019; n=10 in 2020) surveyed. At each field, larvae were hand collected through random selection of 5 locations where 0.30 m2 of plant foliage was searched. Larvae collected in the field were reared in the laboratory and observed for parasitization. Major natural enemy species were recorded, and their abundance was calculated.

Objective 2: Development of functional response models to understand relationships between DBM and its natural enemies to develop dynamic action thresholds

Methodology

***Plutella xylostella* colony and maintenance**

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The DBM colony was started in 2018 with individuals obtained from an established colony at the Agriculture and Agri-Food Canada (AAFC) Research Station in Saskatoon, Saskatchewan, Canada. The colony was maintained on greenhouse grown canola, *Brassica napus*, plants (Var. Q2) at 22o C, 60% R.H., and 16L:8D photoregime. Plants were grown in Sunshine Potting Mix no. 4 (Sun Gro horticulture Canada Ltd, Alberta, Canada) in individual 15.3 cm diameter pots. Plants were supplied with 150 ml water daily and fertilized with 1/g of 20:20:20 (nitrogen: phosphorus: potassium; Miracle Gro, Marysville, Ohio, U.S.) per plant every two weeks, starting at three weeks after germination. Larvae fed on canola plants in laboratory rearing cages (Bug dorm ,32 x 32 x 77 cm). Adult moths were housed in separate cages with canola plants as an oviposition host and were provided with a 30% sugar solution through a dental wick. Larvae fed on canola plants in laboratory rearing cages (Bug dorm ,32 x 32 x 77 cm). Adult moths were housed in separate cages with canola plants as an oviposition host and were provided with a 30% sugar solution through a dental wick.

Natural enemy colonies and maintenance

Colony of *Coccinella septumpuctata*:

Adult seven-spot ladybird beetle (LBB), *Coccinella septumpunctata* (Coleoptera: Coccinellidae) were field collected using sweep nets and hand collection from research plots at the University of Alberta farm (53.50°N, 113.52°W) from June 2018 through August 2021. Adults were maintained for breeding under lab conditions (22o C and 60% R.H. and 16L: 8D photoperiod) in plexiglass cages (15 cm3). Prior to use in bioassays, beetles were fed a mixed diet consisting of aphids and both DBM eggs and larvae. Cages were inspected daily for oviposition and egg hatch. Individual beetle larvae were separated and transferred to plastic 20 ml rearing cups lined with moist Whatman filter paper, where they were maintained on the same diet. Beetle developmental stages were recorded, and the fourth instar larvae and adults were used in the experiments. Eclosed adults were fed on a similar diet for 10 days prior to the initiation of experiments. Both adults and larvae were starved for 24 h prior to use in experiments.

Colony of *Chrysoperla carnea*:

Adult common green lacewings, *Chrysoperla carnea* (Neuroptera: Chrysopidae) were field collected using sweep nets from research plots at the University of Alberta farm from June 2018 through August 2021. Adults were maintained for breeding under lab conditions (22o C and 60% R.H. and 16L: 8D photoregime) in plexiglass cages (15 cm3). Prior to use in bioassays, lacewings were fed on DBM eggs. Cages were inspected daily for oviposition and egg hatch. Individual lacewing larvae were separated and transferred to 20 ml plastic rearing cups lined with moist Whatman filter paper, where they were maintained on the same diet. Larval developmental stages were recorded, and the fourth instar larvae were used in the experiments. Larvae were starved for 24 h prior to use in experiments.

Damsel bug, *Nabis spp.:*

Adult damsel bugs, *Nabis* spp. (Hemiptera: Nabidae), were field collected using sweep nets from research plots at the University of Alberta farm from June 2018 through August 2021 and from canola fields in Southern Alberta. Field collected bugs were fed DBM larvae for four days and then starved for 24h prior to the experiment.

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Carabid beetle *Pterostichus melanarius:*
Pterostichus melanarius (Carabidae: Coleoptera) were collected in weedy patches and canola field margins using dry pitfall traps (12 cm in diameter by 14 cm in depth) at the University of Alberta South Campus Farm (53.50°N, 113.52°W) during the summers of 2019 and 2020. Before initiating the experiments, adult beetles were kept in plastic containers (Gladware; 14 cm by 12 cm by 10 cm; 1.89 L capacity) and starved for 24hr.

Colony of *Diadegma insulare*
The initial colony of *Diadegma insulare* (Hymenoptera: Ichneumonidae) was established from field-collected DBM pupae obtained from AAFC, Ottawa (Ontario, Canada). Wasps that emerged were allowed to mate and then mated female wasps were released into a cage (32.5 x 32.5 x 77cm, Megaview Science Coo, Taiwan) containing a canola plant and third instar DBM larvae as an oviposition host.

Colony of *Diadromus subtilicornis*:
Initial colony of *Diadromus subtilicornis* (Hymenoptera: Ichneumonidae) was established from field-collected DBM pupae obtained from AAFC, Ottawa. Wasps that emerged were allowed to mate and then mated female wasps were released into a cage (32.5 x 32.5 x 77cm) containing DBM pupae for parasitization. The wasps were provided with 30% honey solution. The parasitized pupae were collected after 5 days and used to establish the colony.

2.1 Functional response bioassays
Change in feeding rate with prey density is termed a functional response (Abrams, 1982; Holling, 1966) and it helps to quantify the contributions of insect predators to biological control, select the best type of biological control agents, and understand the overall stability of the association between predator and prey (Abrams, 1982; Schenk and Bacher, 2002; Fernández-Arhex and Corley, 2003). The research team hypothesize that functional responses of natural enemies will vary as a function of prey density. Predator and parasitoid species were exposed to DBM prey at their respective preferred life stages (egg, larvae, or pupae) at different densities (Table 1). For all bioassays an individual natural enemy was introduced into a transparent plastic container (15x8x8 cm) with a mesh lid containing moist Whatman filter paper and a 5 cm canola leaf disc at one of the tested prey densities. All prey densities were tested concurrently for each natural enemy species. The containers were held in chambers set at 22 °C and 60% R.H for 24 h. Data were collected on the number of prey items consumed over a period of 24 h. Each experiment was replicated 25 times. For each prey density treatment, controls were maintained at the same densities in the absence of the natural enemy to gauge natural mortality (n=10).

Data analyses: Functional response curves were determined by using logistic regression (Juliano 2001). In addition, because prey were not replaced during the experimental period, the research team used non-linear regression to fit the random model (Roger, 1972).

$$N_e = N_0\left(1 - exp\left(a(N_e h - T)\right)\right)$$

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where:
- Ne= the number of prey consumed or killed
- N0 = the initial density of prey
- a = attack rate
- h = handling time
- T= total time available for predation

Both attack rate (a) and handling time (h) were analysed using a bootstrap method in FRAIR package in R (version 3.3.) (Pritchard et al. 2017).

Table 1 Experimental design for functional response of predators and parasitoids to DBM eggs and larvae

Natural enemy	DBM life stage	DBM (prey density)	No. of replicate	Exposure time
Carabid beetle *Pterostichus melanarius*	egg	5, 10, 15, 20, 40, 60	25	24 h
	larvae	10, 15, 20, 30, 50,60	25	
LBB adults (*Cocinella septumpunctata*)	egg	5, 10, 15, 20, 40, 60	20	24 h
	larvae	10, 15, 20, 30, 50,60	25	
LBB larvae (*Cocinella septumpunctata*)	egg	5, 10, 15, 20, 40, 60	25	24 h
	larvae	10, 15, 20, 30, 50,60	25	
Chrysoperla carnea	egg	5, 10, 15, 20, 40, 60	20	24h
	larvae	10, 15, 20, 40, 60	20	
Damsel bug (*Nabis* sp.)	egg	5, 10,15, 20, 40, 60	15	24 h
	larvae	10, 15, 20, 40, 60	15	
Diadegma insulare	larvae	5,10,20,30,40	20	24 h (1 d)
		5,10,20,30,40	20	72 h (3 d)
Diadromus subtilicornis	pupae	2,4,6,8,10,14	15	24 h (1 d)
		2,4,6,8,10,14	10	72 h (3 d)

2.2 Effect of temperature on the functional response of LBB on DBM larvae

Methodology:

The effect of temperature on the functional response of both larval and adult LBB was tested in similar bioassays. Replicate LBB larvae and adults were provided with DBM larvae at one of six larval densities (10,15, 20, 30, 40, 60 larvae) and were kept in a growth chamber maintained under three different temperatures 10, 22 and 32°C. The number of larvae consumed in each container at each temperature (n=25) over a period of 24 h was recorded. For each prey density x temperature treatment, control larvae of DBM were maintained without a predator (n=10).

2.3 Functional response of parasitoids of DBM
Methodology:

Diadegma insulare
The experiment was carried out in a growth room from May 2020 to January 2021. Early flowering canola plants (8 weeks old) were kept individually in cages (32 x 32 x 77 cm, MegaView Science Co. Ltd, Taiwan) and provided with 30% honey solution. Five densities of third instar DBM larvae were randomly introduced to a canola plant in each cage. After introducing larvae, a 3-5 day old mated female wasp was released inside the cage. The research team tested 2 exposure periods, 1and 3 days, respectively. After the exposure period, wasps were removed from the cage and the exposed larvae were allowed to feed on the canola plant until pupation. The number of *D. insulare* pupae and wasp emergence was measured. The experiment was replicated 15 times for each exposure time.

Diadromus subtilicornis
Four-to-five-day-old mated *Diadromus subtilicornis* females were exposed to different densities of 3-day old DBM pupae in 250 ml containers with a 5ml vial of 30% honey solution. The wasps were exposed to pupae for 1 day. After exposure, wasps were removed, and pupae were checked daily for wasp emergence.
Data analysis: The research team used FRAIR package (Pritchard et al. 2018) for the data analysis similar to that of predators.

2.4 The biology of a generalist parasitoid *Microplitis mediator* on DBM larvae
The research team tested the hypothesis that DBM host stage would affect the percent parasitism, developmental rate and survival of the generalist parasitoid, *Microplitis mediator* (Hymenoptera: Braconidae). *Microplitis mediator* is a generalist parasitoid that was recently found parasitizing DBM larvae in Ontario. The parasitoid was obtained from AAFC and reared in the laboratory at the University of Alberta on DBM larvae at 22° C and 16L:8D photoperiod.

Methodology:
Four-day old mated female *M. mediator* were exposed to 10 DBM larvae (1st- 4th instar) in a clear plastic container (500 ml) augmented with a 30% honey solution and a 5 cm canola leaf disc. Larvae in each treatment were all in the same instar development. Wasps were allowed to parasitize for 48 h, after which DBM larvae were individually transferred to small Petri plates with canola leaves. Larvae were checked daily and observed for pupation, mortality and adult parasitoid or moth emergence.

Data analyses:
The data from was analysed using linear mixed models in R (package lme). The percentage parasitism and egg to larval development time did not follow the normal distribution and were ARCSIN and log transformed, respectively. Means were compared post ANOVA using Tukey's HSD test.

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Objective 3: To study non-consumptive effects of predator and parasitoid on DBM

The research team conducted several studies to assess the non-consumptive effects of larvae and adults of LBB and the larval parasitoid, *D. insulare*, on DBM fitness. Non-consumptive effects are the effects that natural enemies have on prey items that do not result from the direct parasitism or predation of the prey (Werner and Peacor, 2003).

3.1 Ovipositional choice studies

In order to observe the effects of fertilizer treatment (host plant nutrition) and predator presence on the oviposition behaviour of the DBM, the research team conducted a laboratory choice bioassay from April 2021 to December 2021. There were four treatments: Treatment 1: low fertilizer canola plant without predator, Treatment 2: high fertilizer canola plant without predator, Treatment 3: low fertilizer canola plant with LBB adult, and Treatment 4: high fertilizer canola plant with LBB adult. Plants assigned to these four treatment combinations were setup in individual cages (32.5 x 32.5 x 77cm, Megaview Science Coo, Taiwan) such that each cage contained all four treatments. For the treatments involving predator presence (treatments 3 and 4), five LBB adults were confined individually to a mesh cloth bag and the bags were clipped to one of the plant leaves that received the low fertilizer treatment (treatment consumption when exposed to predator presence vs. absence treatments under controlled conditions from April 2021 to December 2021. The adult stage of the LBB was used as the predator in this experiment.
The experiment consisted of four treatments:
1) control: no predator was added in the cage
2) predator threat with chemical cues only: 3 LBB females confined in an opaque mesh bag
3) predator threat with chemical and physical cue: 3 LBB females with mandibles removed but not confined in a mesh bag
4) predator presence: 3 free LBB females in the cage

A plexiglass cube (34 cm3) with mesh opening containing a 5-week-old canola plant with 20 previously weighed DBM second instar larvae were assigned to each of the four treatments for 48 h. After 48 h, predators were removed. Each replicate cage represented all four treatments (n=14). An additional no-choice bioassay was conducted to study DBM larval survival, growth, and leaf consumption using the larval stage of LBB as the predator. At the end of both experiments, DBM larvae were removed and weighed, and mortality of DBM larvae was assessed. The leaf consumption was calculated using Leaf Byte software.

Data analyses: The effects of predator treatments on larval weight and leaf area consumed were analyzed with a linear mixed-effects model with predator treatment (presence vs. absence) as a fixed factor and block as a random factor, using the 'lmer' function in the 'lme' package (Bateg 2015). The model fitness was assessed by QQ plots and the normality and heteroskedasticity of model residue was tested using Shapiro-Wilk test and Leuven test respectively. Treatment means were compared using Tukey's post-hoc test.

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3.3 Effect of host nutrition and parasitoid presence on non-vulnerable stages of DBM
An oviposition no-choice study was conducted under laboratory conditions to test the hypothesis that nutritional status of plants and presence or absence of the parasitoid will influence DBM oviposition behaviour.

There were four treatments applied to individually caged canola plants in this experiment:
1) high fertilizer without parasitoid
2) low fertilizer without parasitoid
3) low fertilizer with 3-day old female of *D. insulare*
4) high fertilizer with 3 day old mated female of *D. insulare*.

Five pairs of <1 day old DBM adults were released in a cage for 24 h. After 24 h of exposure, the seedlings were removed from the cage. The eggs laid per plant were counted. The experiment was set as randomized complete block design. Each block consisted of all treatments and was replicated 10 times.

Data analyses: The research team analysed oviposition data using liner mixed effects model (lme package in R 4.0). The proportion of eggs laid by DBM females was log transformed to meet parametric requirements. The model fitness was assessed by QQ plots and the normality and heteroskedasticity of model residue was tested using Shapiro-Wilk test and Leuven test respectively.

Objective 4: To understand the effects of varying larval densities of DBM on foliar damage in canola and yield.
4.1 Field-cage study: The effects of different larval densities of DBM on canola yield
The research team conducted a field-cage study to determine the effect of different DBM larval densities on canola yield. The study was conducted at the St. Albert research farm, University of Alberta in the summers of 2019 and 2020 using a randomized complete block design with 5 treatments arranged in 4 blocks (Table 2).

Field plots (1.2 m x 3 m) were seeded to canola (*Brassica napus* L.) (cultivar: PV 581GC; Roundup Ready hybrid, Proven Seeds, Regina, SK) in mid-May (11 May 2019) and (14 May 2020) using a Hege seed drill (6 rows/plot, 20.32cm row spacing; Hege Company, Waldenburg, Germany) at a seeding rate of 8 g/plot resulting in a crop density of 75-80 plants/m2. Crop emergence was recorded for each plot. Plant density was calculated at canola growth stage 2.2 to ensure that 75-80 plants/ m2 were maintained at the center of plots and all other seedlings were removed manually. Weeds were also removed manually. Once the plots were thinned, field cages (1 x 1 x 1.5 m) were set up on the cropped area in the plots at canola stage 3.3-4.3. Cages were positioned over the crop canopy and the base of the cage was secured with soil to seal the cage from all sides and prevent the entry or exit of DBM from the cage.
The control treatments consisted of an uncaged control (Treatment 1; no cage) and a caged control (Treatment 2; plot caged but no DBM larvae added). The remaining treatments consisted of various densities of laboratory reared DBM larvae added to caged plots: 2 larvae per plant added to late budding canola plants (Treatment 3), 4 larvae per plant (Treatment 4), and 8 larvae per plant (Treatment 5) added at the late flowering stage. Plant stages were recorded weekly in a marked area of 0.30 m2 in each plot. Twenty leaves per cage were removed at canola growth stage 5.2. The leaf damage analysis was done using the leaf damage

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application, Bioleaf analysis (Machando et al. 2017). Twenty pods per cage were removed at the canola growth stage 5.2 and the number of damaged pods was counted and recorded. Insect density was monitored and recorded in uncaged control plots by shaking plants in an area of 0.30 m^2 at weekly intervals after cage placement and counting the number of larvae. Canola plants within the cages were harvested at pod maturity.

Heavy rainfall and relatively colder conditions in 2019 and 2020 prolonged the crop growth and the crop was harvested in mid-September. At harvest, the cages were checked for the presence of DBM life stages and other insects, and if found, insects were removed from the cages and counted. Plants were manually harvested by clipping them at 5 cm above soil surface using field clippers. Clipped plants were shaken into plastic bags to collect any DBM life stages. The harvested plants were dried at 40°C in dryers at the University of Alberta, St. Albert research farm for 3 days. The dried plants were manually threshed to extract seed at South Campus research station, University of Alberta. The extracted seed from each of the treatment plots was weighed and expressed as seed yield in g/plot.

Table 2. Agronomic activities in the research plots at St. Albert Research Station, University of Alberta

Agronomic activity	2019	2020
Seeding (8gm/ plot)	11 May	17 May
Planting density	70-80/m^2	70-80/ m^2
Herbicide(s)	Glyphosate 180 g ai/ha	Glyphosate 180 g ai/ha Lontrel + Pro + Mustard
Fungicide (Proline 480 SC)	23 June	02 July
Cage set up	23 June	10 June

4.2 Growth room study: The effects of different larval densities of DBM on canola yield

The research team conducted an experiment on the effects of DBM larval densities on canola yield under controlled conditions using the growth room facility, Department of Biological Sciences, University of Alberta at 22°C and 16 h L:D**.** Canola (cultivar: PV 581GC; Roundup Ready hybrid, Proven Seeds, Regina, SK) was seeded in 3- L pots containing Sunshine Potting Mix no. 4 (Sun Gro horticulture Canada Ltd, Alberta, Canada). Plants were supplied with 250 ml water daily and fertilized at the rate of 3 g of 20:20:20 (N: P: K; Miracle Gro, Marysville, Ohio, U.S.) per pot every two weeks, starting at three weeks after germination. A total of 8 larval density treatments were included in the growth room study (Table 3). The range of larval densities and crop growth stages studied, allowed us to examine the effect of larval on crop yield in relation to crop staging. After the

addition of larvae to caged plants (cage: 32.5 x 32.5 x 77cm, Megaview Science Coo, Taiwan), the plants were watered daily with 200 ml water. A 30% honey solution was provided throughout the experiment. After plants started to dry out the DBM adults were collected using an InsectaVac aspirator (BioQuip, USA) and kept in the freezer at -18° C for 48 hrs, at which point they were counted. Canola was hand harvested near the roots. The immature stages of DBM were collected by carefully inspecting each plant as well as the walls of the cages. The canola pods from each plant were counted, dried and manually threshed using USA standard soil sieve (30 cm diameter, Cole Parmer Sieve, Analytica Scientific Company,Montreal, Qubec, Canada). The canola seeds were weighed and seed yield was averaged across the replications and reported in g/treatment.

Table 3. Treatments included in determining economic threshold studies under controlled conditions

Treatment	
T1	0 larvae (control)
T2	2 larvae per plant at early flowering stage, larvae allowed to feed and form pupae in the cage
T3	4 larvae per plant at early flowering stage, larvae allowed to feed and form pupae in the cage
T4	2 larvae per plant at early flowering stage, larval feeding discontinued, and larvae removed once they neared pupation
T5	4 larvae per plant at early flowering stage, larval feeding discontinued, and larvae removed once they neared pupation
T6	2 larvae per plant at late flowering/early pod stage
T7	4 larvae per plant at late flowering/early pod plant
T8	8 larvae per plant at late flowering/early pod plant

Data analyses: Data were analyzed using R version 4 (R Core Development Team 2020). General mixed effect model (R Studio 4.1) was used to analyze all models from field studies. The data of DBM adult count in 2019 and 2020 did not meet normality assumptions and hence log transformed. Larval density was a fixed effect whereas block was a random effect for all the models. Data was analysed using lme package. The normality of model residuals was checked by using Shapiro-Wilk test. Model fit was confirmed by using QQ-plots. The means were compared using Tukey's *post hoc* test (Tukey 1977). For the growth room study, canola yield and canola pod numbers were analysed using General mixed effect model (R package lme). Larval density was a fixed effect and block was a random effect. The normality of model residuals was checked by using Shapiro-Wilk test. Model fit was confirmed by using QQ-plots. The means were compared using Tukey's *post hoc* test (Tukey 1977).

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Results

Objective 1: To monitor natural enemy populations associated with diamondback moth (DBM) in canola with particular focus on larval parasitoids

Results: In all these three years of the study, there was very low DBM activity in Alberta. Extreme weather conditions from severe drought in 2018 to heavy rainfall in 2019 and 2020 affected the DBM influx and colonization. This was also evident from low trap captures reported by the Prairie Pest Monitoring Network throughout these 3 years. In the 2018 survey, the research team found higher parasitism of DBM by a new parasitoid species *Diaoclogaster claritibia* (Hymenoptera: Braconidae) that was recorded in commercial fields of canola by Dr. Cárcamo and his team. For 2018 and 2019, *D. insulare* was the major parasitoid contributing 85% and 90% parasitism, respectively.

Objective 2: Development of functional response models to understand relationships between DBM and its natural enemies to develop dynamic action thresholds

In this study, the research team found that prey consumption rates by predators was a function of prey density. Most of the predators (except *P. melanarius* and *C.carnea* that showed Type III) exhibited a Type II functional response, in which predation rate increased with increasing prey densities and the consumption rate plateaued at the highest densities tested (Fig. 1). Many other generalist predators exhibit Type II functional responses, while some present Type III response patterns (Ma et al., 2005; Smout & Lindstrøm, 2007; Santos et al., 2016; Nunes et al., 2019). Coccinellid predators have Type II functional responses in response to varying densities of aphid prey items (Asante, 1995; Işıkber, 2005.; Xue et al., 2009). Another generalist predator of DBM, *N. kingbergii,* also exhibits a Type II functional response to increasing densities of DBM larvae (Ma et al., 2005).

Larvae of the subtropical green lacewing, *Ceraeochrysa cincta* (Schneider) (Neuroptera: Chrysopidae) show a Type II functional response on DBM eggs and second instar larvae (de Oliveira Pimenta et al., 2020). Similarly, the earwig, *Euborellia annulipes* (Dermaptera: Anisolabididae) has a Type II functional response on DBM larvae (Nunes et al. 2020). Seasonality in prey density patterns can affect predator consumption patterns (Ma et al. 2005), and the functional response of generalist predators at low prey density will be largely determined by the searching capacity of the predator (Ma et al., 2005). In this study, most prey items (both larvae and eggs) were consumed by *P. melanarius*. Other predators, including larvae and adults of *C. septumpunctata* also preyed on many DBM.

Fig. 1. Functional response curves of generalist predator species consuming life stages of DBM a) *C. carnea* on DBM eggs, b) *C. carnea* on DBM larvae, c) *P. melanarius* on DBM eggs d) *P. melanarius* on DBM larvae e) *Nabis spp*. on DBM eggs f) *Nabis spp.* on DBM larvae

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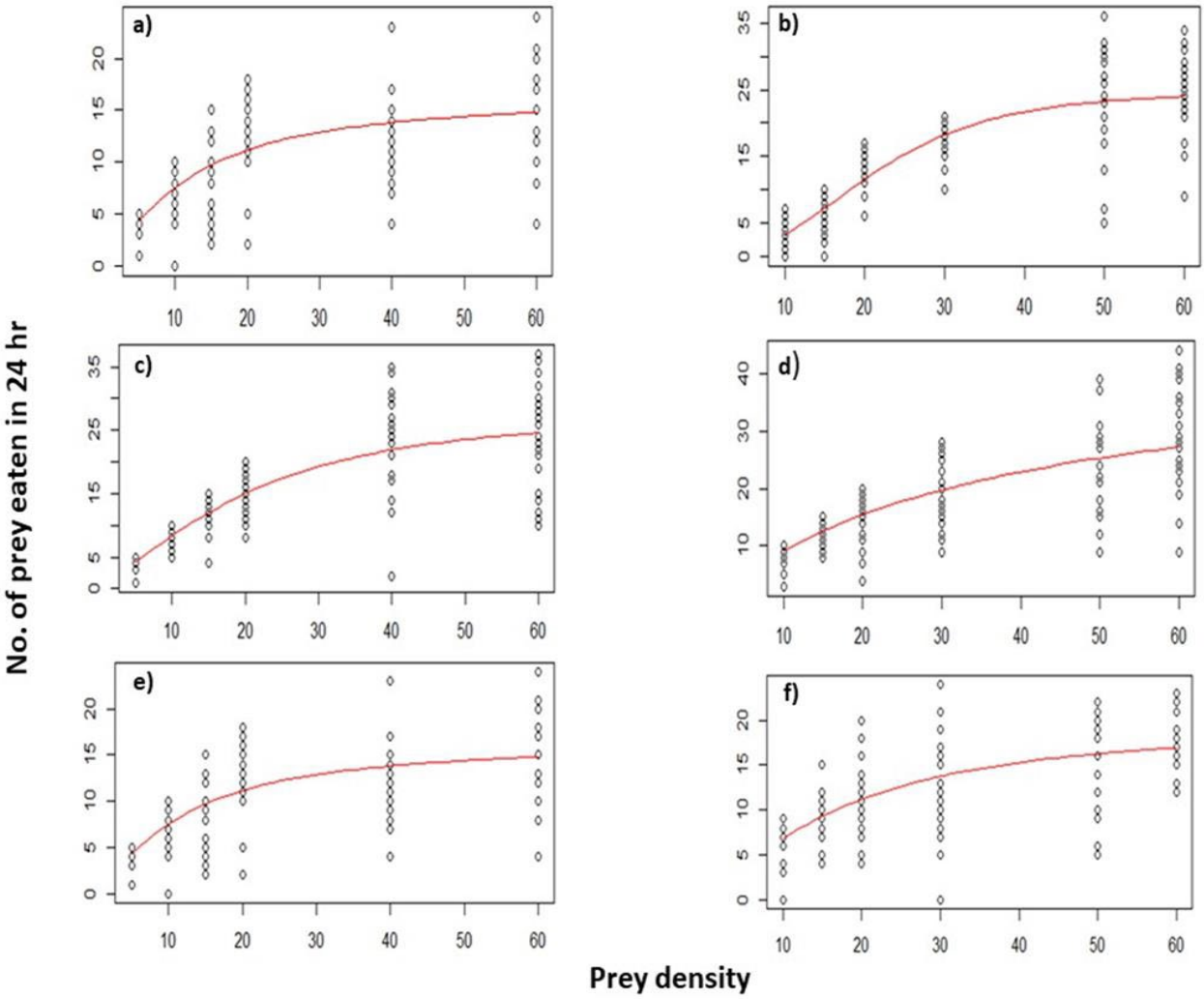

Parameters defining functional responses including attack rate and handling times were estimated. Both the attack rate and handing time differed with predator species. Maximum prey consumption which indicated the maximum number of prey that a predator is able to consume in 24 h was calculated by using the computed handling time (Th) from Roger's equation (Tables 4 and 5).

Table 4. Mean estimates ±SE, (95% confidence intervals) for attack rate (no. of prey per hour) and handling time (Th/day) of different predator species on DBM eggs. Means with different letters across the rows are significantly different calculated using "frairboot" from Frair package (Pritchard, 2015)

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Predator species	Type of FR	Attack rate(±S.E.) (C.I)	Handling time (C.I)	Maximum prey consumption per day (T/Th)
C. carnea	TypeII	2.58b(±0.06) (2.30-2.84)	0.04a(+0.001)	25
P. melanarius	TypeII	2. 95 b (±0.13) (2.46-3.53)	0.02a(0.001) (0.03-0.043)	50
Nabis spp.	TypeII	2.01a(±0.11) (1.8-3.4)	0.06c(±0.002) (0.05-0.07)	16
C.septumpunctata larvae	TypeII	1.94 a (±0.09) (1.64 -2.28)	0.03a (0.028 -0.04)	33
C.septumpunctata adult	TypeII	2.59 b (±0.13) (2.31 -3.84)	0.04a (0.04 -0.06)	25

Table 5. Mean estimates ±SE, (95% confidence intervals) for attack rate (no. of prey per hour) and handling time (Th/day) of different predator species on DBM larvae. Means with different letters across the rows are significantly different calculated using "frairboot" from Frair package (Pritchard, 2015)

Predator species	Type of FR	Attack rate (± S.E) (C.I)	Handling time (C.I)	Scaling coefficient (C.I)	Maximum prey consumption per day (T/Th)
C. Carnea	Type III	0.005 (±0.002) [a] (0.001-0.053)	0.04 (± 0.001)[a] (0.32-0.044)	1.9 (±0.19)[a] (0.62-2.04)	25
P. melanarius	Type III	11.33 (±0.16) [b] (6.08-13.81)	0.013(±0.001)[b] (0.007-0.017)	-0.59(±0.03) [b] (-0.002-0.017)	100
Nabis spp.	Type II	1.71(±0.14)[c] (1.33 -2.19)	0.03 (± 0.001) a (0.021-0.037)	NA	33
C. septumpunctata larvae	Type II	2.00 (±0.16) [c] (1.56-2.59)	0.04 [a](±0.002) (0.05 -0.07)	NA	25
C. septumpunctata adult	Type II	2.70 (±0.16) [d] (2.16-3.30)	0.02(±0.001) [c] (0.01-0.02)	NA	50

2.2 Effect of temperature on the functional response of LBB on DBM larvae

Results:

The functional response of LBB larvae on DBM larvae was influenced by temperature. The predator displayed a Type II functional response at 10 and 22°C, but it changed to a Type III response at 32°C (Fig.2). The attack rate (a´), and the handling time (Th) of LBB larvae on DBM larvae were both influenced by temperature. At the lowest temperature tested (10° C) the attack rate of LBB larvae was almost four times lower than at the highest tested temperature (32° C), while the handling time was also the longest at 10° C. Neither the attack rate nor the handling time differed statistically between 22 and 32° C when LBB larvae preyed upon DBM larvae. The handling time of LBB larvae at both 22 and 32° C was half the time of that at 10° C (Table 6). The lower handling time of DBM larvae at higher temperatures resulted in maximum consumption for adult of LBB at the higher temperatures (Table 6). LBB adults consumed ~33 larvae per day at 32°C whereas, the maximum consumption rate of LBB larvae was ~20 larvae per day at 32°C.

The functional response of LBB adults on DBM larval prey items showed similar trends at the different temperatures tested. Adults displayed a Type II functional response at the lowest (10°C) and moderate (22°C) temperatures, but had a Type III response at 32°C. The attack rate (a'), and the handling time (Th) for LBB adults on DBM larval prey were both influenced by temperature. At the lowest temperature (10°C), the attack rate of LBB adults was slowest and the handling time was the longest. Attack rates increased with temperature, but did not differ between 22 and 32°C. The highest attack rates were recorded at 32°C. The highest attack rates were recorded at 32°C. The handling time for adult LBB did not differ significantly between 22 and 32°C, but was approximately 3x faster than at 10°C.

Fig. 2 Functional response of larvae (a-c) and adult (d-f) of LBB on larvae of DBM under different temperatures

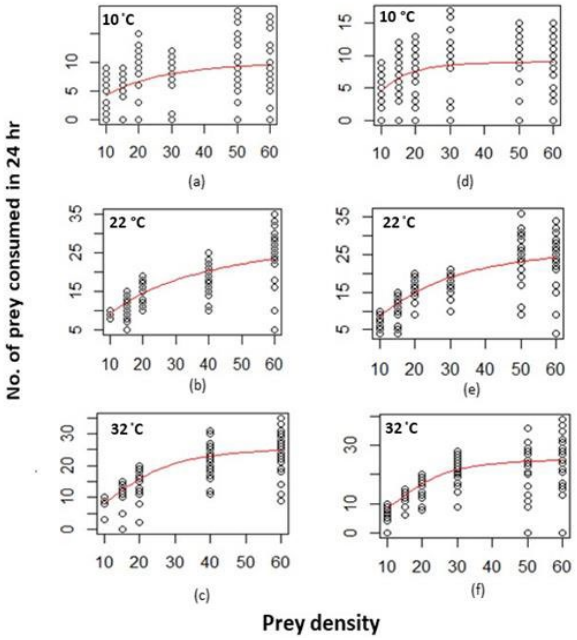

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Table 6. Mean estimates (±SE, with 95% confidence intervals in brackets) for attack rate (no. of prey per hour) and handling time (Th/day) by larva and adult of LBB at different temperatures. The means for the attack rates and handling times (Th-day) sharing different letters are significantly different across the rows for LBB larvae and adults

Life stage of LBB	Temperature (°C)	Attack rate (±C.I) (a´/h)	Handling time (±C.I) (Th-day)	Maximum prey consumption per day (T/Th-day)
Larva	10	0.50[a] (0.33 -0.79)	0.13 [a] (0.09 -0.18)	7.69
	22	2.00[b] (1.56 -2.59)	0.07 [a] (0.05 -0.07)	14.28
	32	1.96[b] (1.51 -2.55)	0.05 [a] (0.04 -0.06)	20.00
Adult	10	1.04 [d] (1.01 -1.35)	0.09 [a] (0.08-0.11)	6.25
	22	2.70 [e] (2.16-3.30)	0.05 a (0.04-0.06)	20.00
	32	2.16[e] (1.45-3.02)	0.02 [c] (0.01-0.02)	33.33

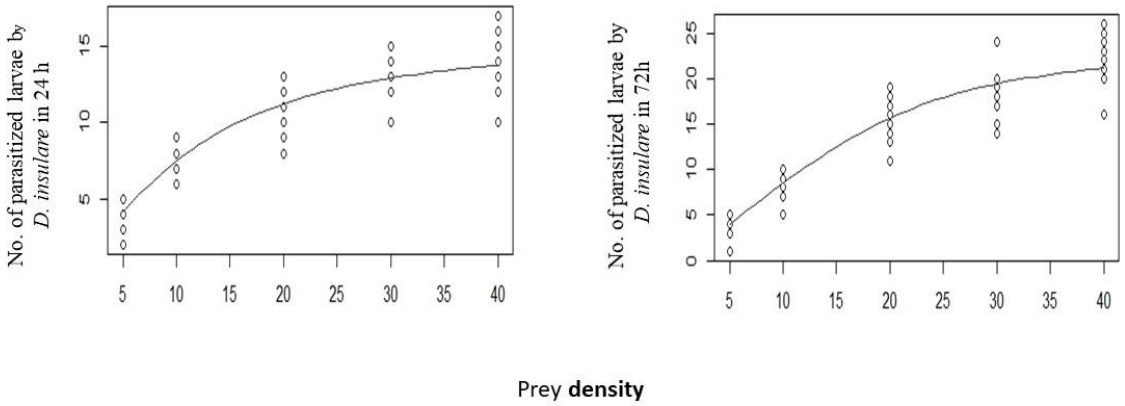
2.3 Functional response of parasitoids of DBM

In this study, the general shape of functional response did not change with the exposure period. Attack rate did not differ significantly, however handling time differed significantly to each other (Table 7). *Diadegma insulare* exposed for the longer exposure period (3 days) needed less time to handle the DBM larvae and showed Type II response for both 1 day and 3-day exposure period (Fig. 3).

Table 7. Mean estimates (with 95% confidence intervals in brackets) for attack rate (no. of prey per hour) and handling time (Th/day). The means for the attack rates and handling times (Th/day) sharing different letters across the rows are significantly different at 95% confidence interval (C.I) calculated using frairboot from Frair package (Pritchard, 2015)

Experimental period	Attack rate (±C.I.) (a´/h)	Handling time (±C.I.) (Th-day)	Maximum prey consumption per day (T/Th-day)
1 day	2.30a (0.33 -0.79)	0.09a (0.09 -0.18)	11
3day	2.00b (1.56 -2.59)	0.013a (0.05 -0.07)	23

Fig. 3 Functional response of *D. insulare* on larvae of DBM at 1 and 3 days exposure period



Diadromus subtilicornis

The research team found that general shape of functional response did not change with the exposure period. Attack rate did not differ significantly however handling time differed significantly to each other (Table 4). *Diadromus subtilicornis* exposed for longer exposure period (3days) needed less time to handle the DBM pupae and showed Type II response for both 1 day and 3 day exposure period (Fig. 4).

Table 8. Mean estimates (95% confidence intervals) for attack rate (no. of prey per hour) and handling time (Th/day). The means for the attack rates and handling times (Th/day) sharing different letters across the rows are significantly different at 95% confidence interval calculated using frairboot from Frair package (Pritchard, 2015)

Experimental period	Attack rate (±C.I) (a´/h)	Handling time (±C.I) (Th-day)	Maximum prey consumption per day (T/Th-day)
1 day	4.17a (3.87 -4.39)	0.17a (0.14 -0.20)	5
3day	3.75a (3.54 -3.95)	0.08a (0.06 -0.10)	13

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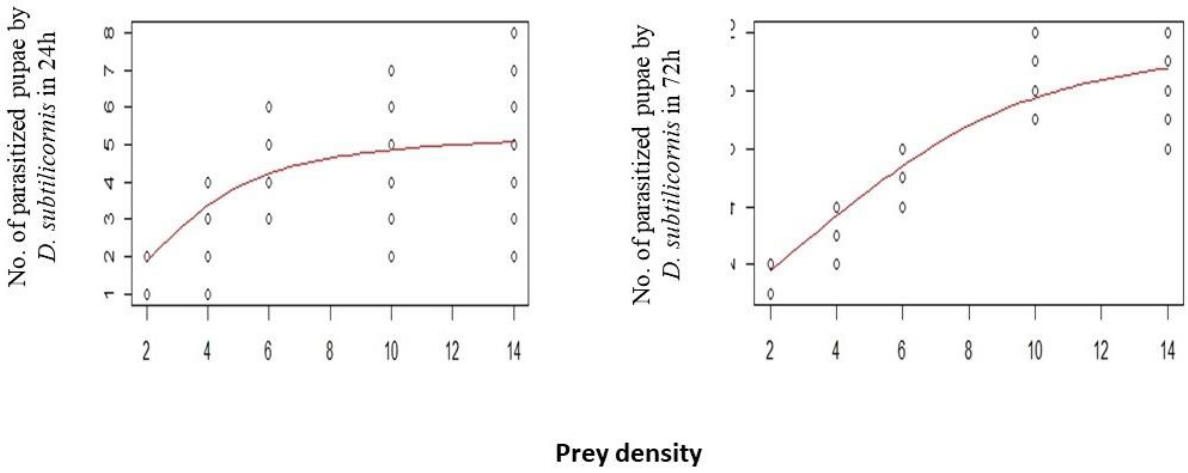
Fig. 4 Functional response of *D. subtilicornis* on pupae of DBM at 1 and 3 days exposure period



2.4 The biology of a generalist parasitoid *Microplitis mediator* on DBM larvae

Results:

Microplitis mediator parasitized all DBM larval instars. The mean percent parasitization of the second and third DBM larval instars, however, was twice that of the percentage parasitization of the first and fourth instars, respectively (Table 9). The data collected for adult wasp longevity and DBM larval mortality is currently being analysed. The developmental period of *M. mediator* from egg to larvae was not significantly different across treatments. The wasp pupae that resulted from eggs laid into fourth instar DBM took more time to develop.

Table 9. Percent parasitism of DBM by *M. mediator* and parasitoid development and weight in no-choice study. Means within the same column followed by different letters are significantly different (P<0.05)

Treatment	% parasitism	Egg to larva (days)	Pupa (days)	Pupal weight (mg)
1st instar	19.5(3.18)a	8.26 (0.04) a	4.12 (0.09) a	3.38 (0.05) a
2nd instar	61.6 (3.18)b	8.58 (0.04) a	4.30(0.09) a	3.36 (0.05) a
3rd instar	55.3 (3.18)b	8.84 (0.04) a	4.91(0.09) ab	3.49 (0.05) a
4th instar	30.5 (3.18)c	9.47(0.04) a	5.01(0.09) b	3.50(0.05) a

Objective 3: To study non-consumptive effects of predator and parasitoid on DBM

3.1 Oviposition studies

In the first oviposition choice experiment conducted in the growth chamber, female moths chose plants treated with high fertilizer rate over the plants receiving low fertilizer rate ($F_{1,42} = 631.2$, $P < 0.005$). There was no effect of the presence of LBB ($F_{1,42} = 1.784$, $P=0.188$), or the interaction between fertilizer regime and the

presence of LBB adults (F1,42 = 2.056 P=0.206) on DBM oviposition choice (Appendix 1, Fig. 1). Females laid ~2 times more eggs on the highly fertilized plants (32.4%) compared to plants with low fertilization (17.2%).

3.2 Effect of the presence of different predator species on DBM larval survival, growth, and leaf consumption
The mean percentage larval weight gain was significantly affected by the presence of the LBB adults (F3,39 = 19.59, P<0.005). The presence of surgically manipulated LBB adults and direct exposure to the adults both reduced DBM larval weight by ~2% over 48 h (Fig.5). The amount of leaf tissue consumed by DBM larvae was affected by the predator cue treatment (F3,39= 43.41, P<0.001). The amount of leaf area consumed in the treatments with confined predators and the absence of predators did not differ significantly (Fig. 6). The amount of leaf area consumed, however, was reduced by ~24% in the treatment with an active predator that did not have mandibles. The lowest leaf area consumed was in the cages where the predator had direct access to prey.

The mean percentage of DBM larval weight gained was significantly affected by the presence of predator larvae (F3,39 = 17.24, P<0.05). However, the percent weight gain was not affected by either the presence of confined LBB larvae or the presence of surgically manipulated LBB larvae (Fig.7). Larvae exposed to LBB larvae had 19% weight loss compared to the control. Predator exposure affected the mean amount of leaf area consumed by DBM larvae (F3,39 = 17.24, P<0.05). Overall, across all the different predator exposure treatments, only direct predator exposure significantly reduced (34%) the leaf area consumed (Fig.8). The confined LBB larvae and surgically manipulated LBB larvae did not affect the leaf area consumed.

Fig. 5 Mean percentage weight gain by DBM larvae across different treatments. Vertical bars represent mean (± S.E). Four plants each assigned to one of the four treatments (T1: low fertilizer, T2: high fertilizer, T3: low fertilizer with LBB adults, T4: high fertilizer with LBB adults) were maintained in a cage. The different letters above the bars indicate significant differences between two treatments (Tukey's test: p<0.05)

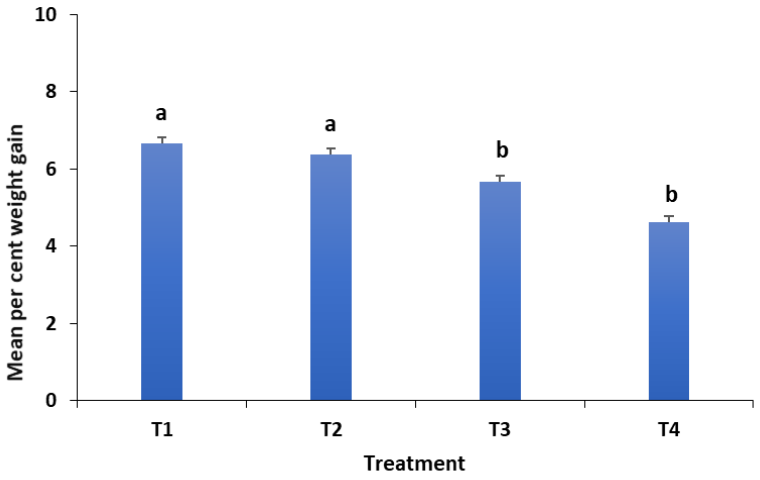


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Fig.6 Mean leaf area consumed by DBM larvae across different treatments (T1: low fertilizer, T2: high fertilizer, T3: low fertilizer with LBB adults, T4: high fertilizer with LBB adults). Vertical bars represent (± S.E). The different letters above the bars indicate significant differences between two treatments (Tukey's test: $p<0.05$)

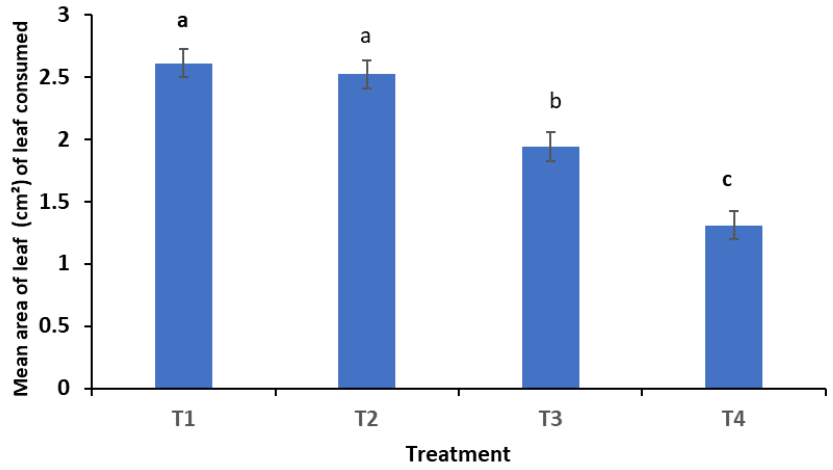

Fig. 7 Mean percentage weight gain by DBM larvae across different treatments (T1: low fertilizer, T2: high fertilizer, T3: low fertilizer with LBB larvae, T4: high fertilizer with LBB larvae). Vertical bars represent (± S.E). The different letters above the bars indicate significant differences between two treatments (Tukey's test: $p<0.05$)

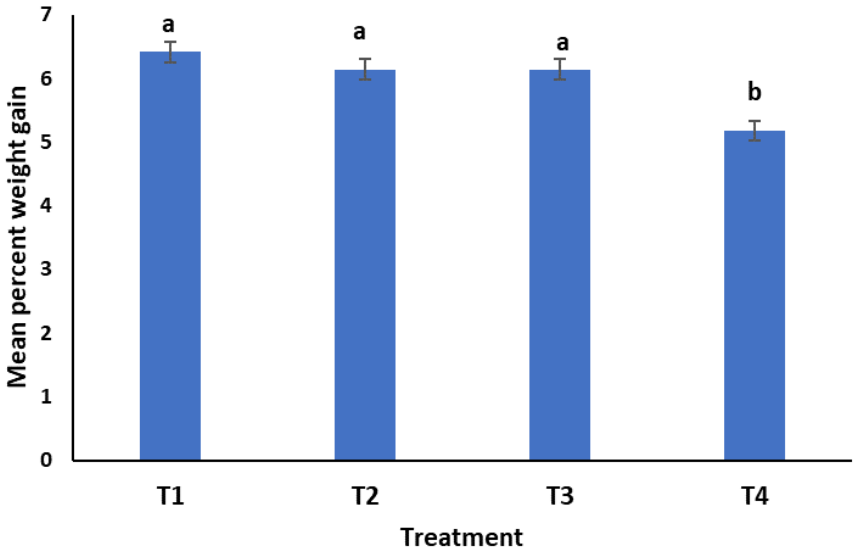

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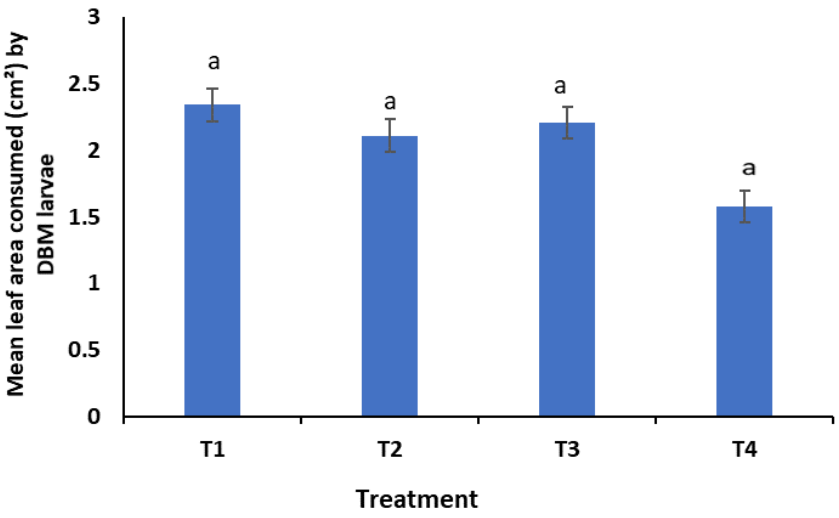
Fig. 8 Mean leaf area consumed by DBM larvae across different treatments (T1: low fertilizer, T2: high fertilizer, T3: low fertilizer with LBB larvae, T4: high fertilizer with LBB larvae). Vertical bars represent (± S.E). The different letters above the bars indicate significant differences between two treatments (Tukey's test: $p<0.05$)



3.3 Effect of host nutrition and parasitoid presence on non-vulnerable stages of DBM

The plant fertilization level had a significant effect on the oviposition behavior of *D. insulare*. Female DBM laid more eggs on plants that received the higher fertilization rate. Neither parasitoid presence nor the interaction of fertilization level and parasitoid presence significantly affected the proportion of DBM eggs laid.

These results demonstrate that bottom-up effects of host plant nutrition dictate oviposition choice mor than the predation threat. These results suggest "mother knows the best" hypothesis (Thompson 1988). Earlier study observing the effects of different fertilizer levels on DBM'S ovipositional choices indicated that host plant nutrition improved the oviposition choice of the DBM females (Sarfaraz et al., 2009). Similar results were also found in *P. rapae* where the effect of N fertilizer influenced the oviposition over threat (Lund et al. 2020).

In a no-choice bioassay, DBM larvae differed in their response to physical cues associated with the predators. When DBM larvae were exposed to LBB adults, the larvae consumed less foliage when they perceived the visual predation threat cue or were exposed to predation compared to when larvae perceived chemical cues or were in the no-predator control treatment. There were no differences in DBM foliage consumption in treatments where larvae perceived predator chemical cues or in the no-predator control treatment. Whereas, when DBM larvae were exposed to surgically manipulated LBB larvae there were no significant differences between the amount of foliage consumed except in direct exposure to the predator. This indicates that DBM larvae respond differently to different stages of the LBB.

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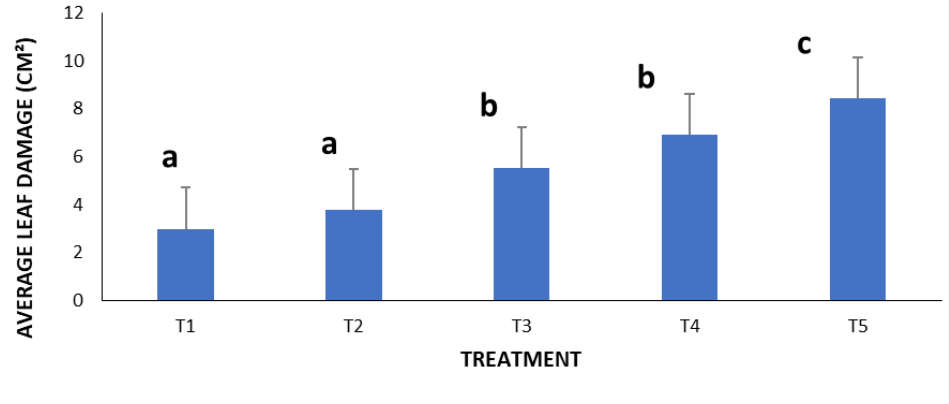
The larvae of Colarado potato beetle consumed less foliage when exposed to predation threat or risk (Hermann and Thaler, 2014). Previous studies revealed that the larvae of some other lepidopteran pests consume more foliage under predation risk in order to outgrow the consumable size (Xiang et al. 2015, Lund et al. 2020). The fact that DBM larvae responded differently to different predator threat treatments shows that the visual cues associated with LBB might play an important role in risk assessment and perception by DBM larvae compared to the chemical cues associated with LBB stages.

Objective 4: To understand the effects of varying larval densities of DBM on foliar damage in canola and yield.

4.1 Field-cage study: The effects of different larval densities of DBM on canola yield

In 2019, the mean leaf area consumption expressed in cm2 (leaf damage) differed significantly between DBM larval density treatments ($F_{4,12} = 77.59$, $p \leq 0.05$). The highest leaf damage was observed at 8 larvae/plant (T5) whereas the lowest defoliation was observed for uncaged control (T1) and caged control with no larvae added (T2) (Fig.9). As the larval densities increased there was gradual increase in average leaf area consumed. While the foliar damage did not differ between the treatments with 2 and 4 larvae/ plants (T3 and T4, respectively) both treatments differed significantly from treatments with 8 larvae/plants. The leaf area consumption ranged between 3-8 cm^2.

Fig. 9 Effects of different DBM larval densities on canola leaf area consumed (cm2) in field cage study conducted in 2019. Bars marked with different letters are significantly different at $p < 0.05$



T1= control uncaged, T2= caged control, T3= 2 larvae/plant, T4: 4 larvae/plant, T5=8 larvae/plant

The mean number of DBM adults collected prior to harvest differed significantly across treatments (Fig. 10). The uncaged control and caged control recorded the lowest number of DBM adults (average range 0-1 adults/m2). Most moths emerged from cages infested with 4 and 8 larvae per plant (T4 and T5) with mean

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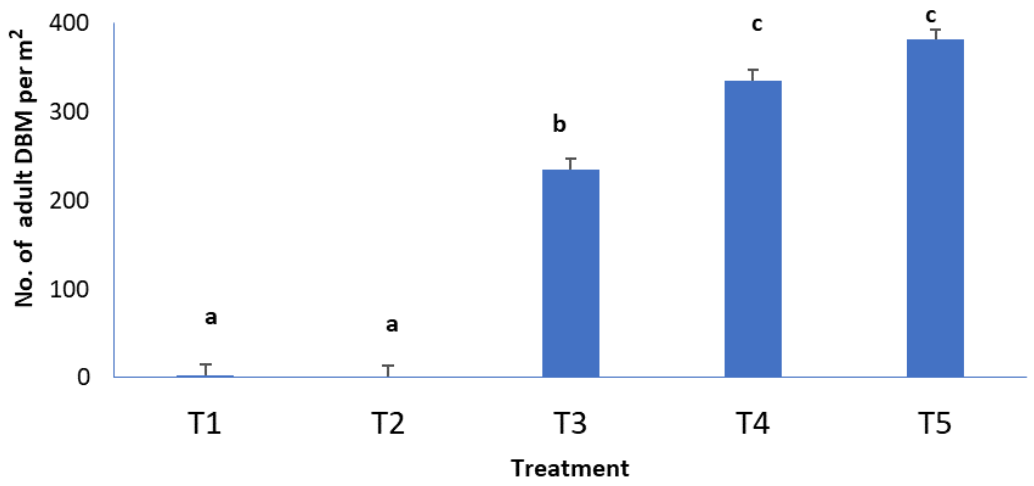
adult numbers ranging between 335-380 adults/m^2. The density of adults increased as the number of larvae per plant increased, indicating successful life cycle completion and subsequent adult formation. This also indicates that as the larval densities increase per plant, the risk of high densities of larvae colonizing canola plants in the next generation increases as more adults will emerge and oviposit in the cropped area. The research team could not recover immature stages (larvae and pupae of DBM) from cages and hence these stages are not included in the analysis.

Fig.10 Mean number of DBM adults per cage in the five larval density treatments (T1=control uncaged, T2=caged control, T3=2 larvae/plant, T4=4 larvae/plant, T5=8 larvae per plant) tested in the field cage study in 2019. Bars marked with different letters are significantly different at $p < 0.05$.



The mean canola yield differed significantly between treatments ($F_{4,12} = 5.61$, $p<0.05$). The highest yield was recorded in the uncaged control (115 g/m2) while the lowest yield was recorded in treatment 5 (67 g/m2) with 8 larvae/plant. The yield differed between caged and uncaged controls with uncaged controls recording significantly higher yields. Between the treatments receiving various larval densities, the highest yield was recorded when the larval density per plant was 2 larvae/plant (94 g/m2), which differed from both treatments 4 (4 larvae/plant) and 5 (8 larvae/plant). With 2 larvae/plant, the canola yield was approximately 1.5% higher than plants with larval densities of 8 larvae/plant. There was a linear decrease in yield as larval density per plant increased (Fig. 11).

Fig.11 Mean canola yield (g) per square meter in cages with different DBM larval densities (T1=control uncaged, T2=caged control, T3=2 larvae/plant, T4=4 larvae/plant, T5=8 larvae per plant) in the field cage study in 2019. Bars marked with different letters are significantly different at $p < 0.05$.

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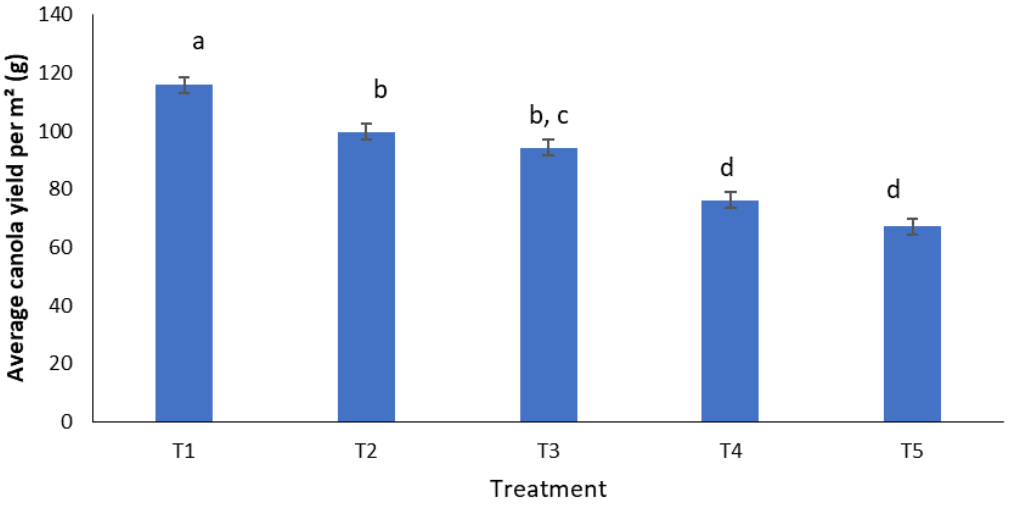

In 2020, the mean leaf area consumed in cm2 (leaf damage) differed significantly with DBM larval density treatments ($F_{4,12} = 81.59$, $p \leq 0.05$). As in 2019, the highest leaf damage was observed when the larval density was 8 larvae/plant (average 8 cm2), whereas the lowest defoliation was in uncaged and caged controls (range: 1-1.5 cm2) (Fig. 12). The mean leaf consumption did not differ when plants were infested with 2 larvae/plant vs. 4 larvae/plant. As soon as the larval density increased to 8 larvae/plant, there was approximately a 2-fold increase in leaf damage. Increased larval densities resulted in increased defoliation and larval densities beyond 4 larvae per plant can cause significant foliar damage. The density of adults increased as the number of larvae per plant increased (Fig. 13).

Fig. 12 Effects of different DBM larval densities (T1=control uncaged, T2=caged control, T3=2 larvae/plant, T4=4 larvae/plant, T5=8 larvae per plant) on canola leaf area consumed (cm2) in a field cage study conducted in 2020. Bars marked with different letters are significantly different at $p < 0.05$

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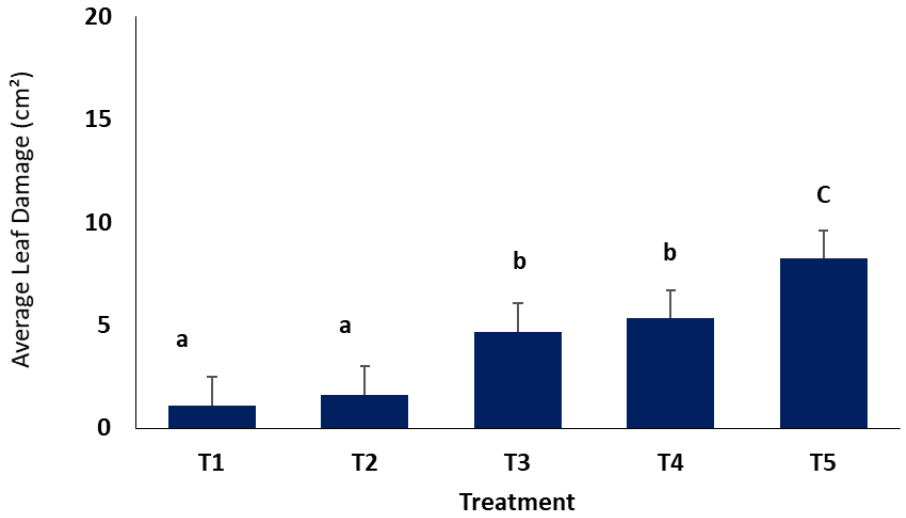
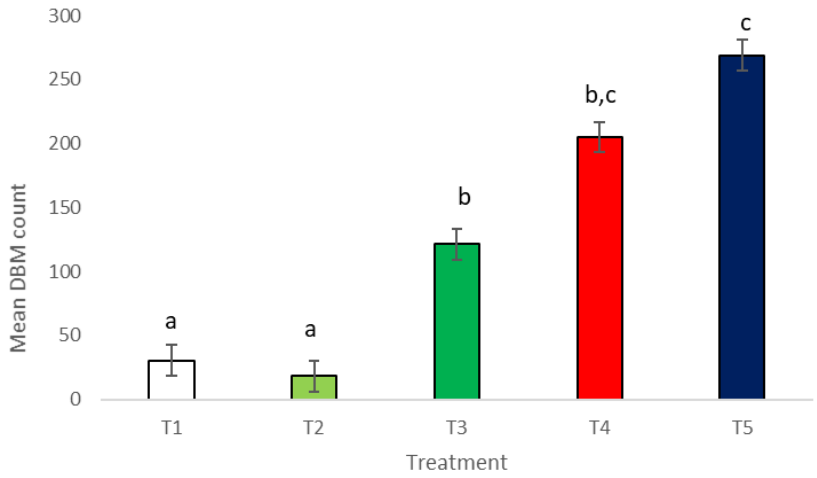


Fig. 13 The mean number of DBM adults per cage in the five larval density treatments (T1=control uncaged, T2=caged control, T3=2 larvae/plant, T4=4 larvae/plant, T5=8 larvae per plant) tested in the field cage study in 2020. Bars marked with different letters are significantly different at $p < 0.05$.



The mean canola yield per square meter differed significantly between treatments ($F_{4,15} = 10.61$, $p<0.005$). The mean canola yield per square meter in the control treatments (uncaged and caged control) were similar and did not differ from treatment 3 (2 larvae/plant) but differed significantly from treatment 4 (4 larvae/plant) (Fig 14). Both treatments 3 and 4 differed significantly from treatment 5 with 8 larvae/plant. The higher larval densities (4 and 8 larvae/plant) introduced at late flowering/ early pod stage (stage 4.2) of canola caused significant reduction in yield. At 8 larvae/plant, yield was 1.25 times lower than at larval densities of 2 larvae/plant.

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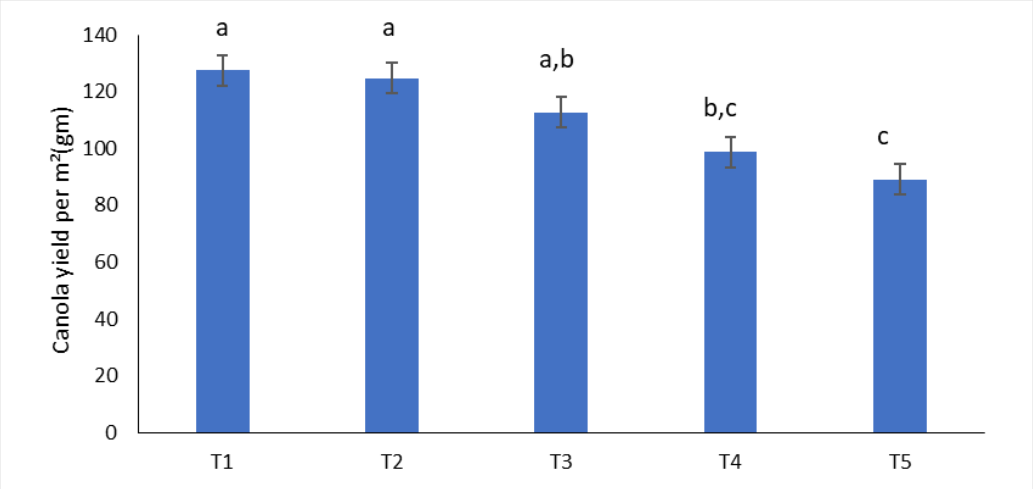
At canola podding stage, larval densities of 4 larvae per plant and above can be detrimental to the yield. With the current nominal threshold of ~2 larvae/plant at the podding stage (200-300 larvae/m2) the yield loss was not statistically different from the control treatments. However, with 4-8 larvae/plant translating to about 320-640 larvae/m2 at the podding stage, the yield loss was ~1.5 times more than the control and 1.25 times more than the current nominal threshold.

Fig.14 Mean canola yield (g) per m2 in cages with different DBM larval densities (T1=control uncaged, T2=caged control, T3=2 larvae/plant, T4=4 larvae/plant, T5=8 larvae per plant) in the field cage study in 2019. Bars marked with different letters are significantly different at $p < 0.05$.



4.2 Growth room study: The effects of different larval densities of DBM on canola yield

The yield produced per cage was significantly different across treatments (Fig. 15) ($F_{7,81}=17.98$, $P<0.05$). The caged control produced the highest yield, compared to all other larval densities except the treatment with 2 and 4 larval densities in which insect infestation was terminated after 8-10 days after larvae pupated. The yield was reduced by 19 percent and 28 percent in treatments when 2 and 4 larvae were introduced at early budding (3.2) growth stage, respectively. The yield in treatment 4 and treatment 5 did not differ from the control. Treatment 4 (2 larvae per plant) and treatment 5 (4 larvae per plant) were added at early budding stage and were removed from the cage after the larvae were close to pupation. This indicates that canola plant can compensate for early injury and the current threshold levels at early flowering stage could be relaxed up to 3-4 larvae per plant.

The number of pods produced per cage was significantly different across treatments (Fig. 16) ($\chi 2 = 680.98$, $df=7$, $P<0.05$). The caged control produced the highest number of pods (912 pods per cage), compared to all other larval densities except the treatment with 2 larvae/plant (average pods= 876 pods per cage) in which insect infestation was terminated after 8-10 days after larvae pupated. The lowest number of pods was

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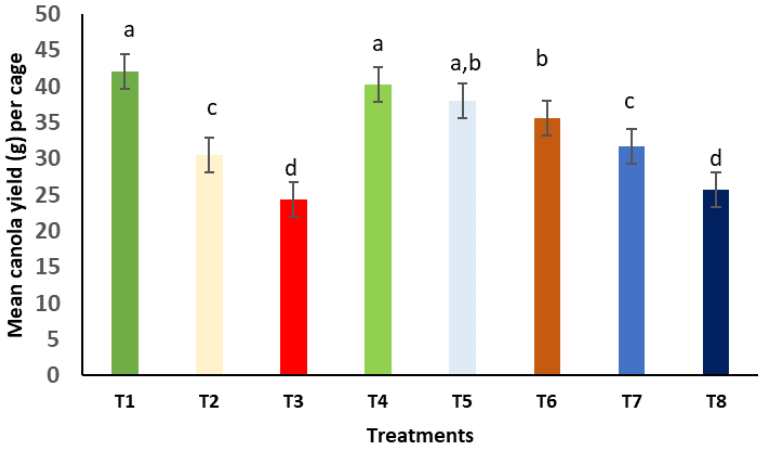
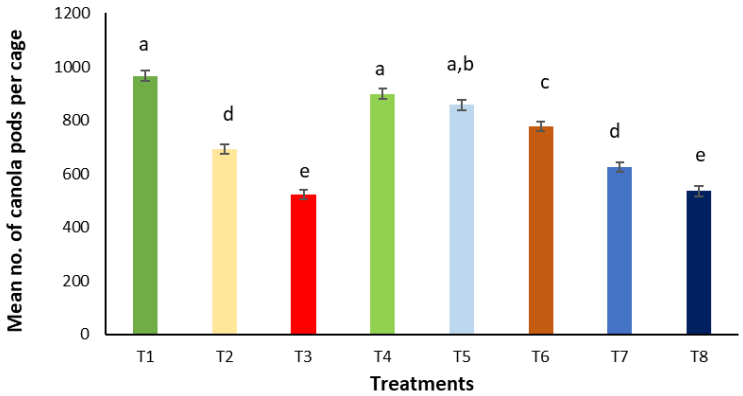
produced by plants with 4 larvae at the budding stage or 8 larvae in the early pod stage. The number of pods in these treatments were reduced by 46 and 43 percent, respectively.

Fig. 15 Effects of different DBM larval densities on canola yield (g) in cage study conducted in a growth room. Bars marked with different letters are significantly different at $p < 0.05$.



T1= caged control, T2= 2 larvae per plant at early flowering stage, T3= 4 larvae per plant at early flowering stage, T4= 2 larvae per plant at early flowering stage, larval feeding discontinued, and larvae removed once they neared pupation, T5= 4 larvae per plant at early flowering stage, larval feeding discontinued, and larvae removed once they neared pupation, T5= 2 larvae per plant at late flowering/early pod stage, T6= 2 larvae per plant at late flowering/early pod plant,T7=4 larvae per plant at late flowering/early pod plant, T8= 8 larvae per plant at late flowering/early pod plant

Fig. 16 Effects of different DBM larval densities on mean number of canola pods per cage (g) in cage study conducted under growth room. Bars marked with different letters are significantly different at $p < 0.05$**.**



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Conclusions and Recommendations

Objective 1: To monitor natural enemy populations associated with diamondback moth (DBM) in canola with particular focus on larval parasitoids

The DBM populations and moth influx were consistently low in all study years (2018-2020). Surveys conducted across southern Alberta in commercial canola fields resulted in minimal captures of all life stages. Low larval densities captured in sweep net samples correlate with low moth capture in pheromone traps deployed by the Prairie Pest Monitoring Network (PPMN) and the Alberta Pest Monitoring Network (APMN). The highest average capture of DBM larvae was in 2018 while the lowest capture was in 2020. Across all years surveyed, *D. insulare* was the most common parasitoid species found.

Objective 2: Development of functional response models to understand relationships between DBM and its natural enemies to develop dynamic action thresholds

This is the first study to provide quantitative estimates of individual consumption patterns of several predatory and parasitic species of DBM in canola, using mechanistic consumption models. The major outcome of this investigation is that the functional responses of predatory and parasitic species depend on the DBM life stage. The natural enemy guilds also differ in their responses to DBM egg and larval densities in terms of rates of predation and parasitism. All predators including *C. septumpunctata* (both larvae and adults), *C. carnea*, *P. melanarius* and *Nabis* spp. showed Type II functional response for DBM eggs indicating high initial consumption of eggs with increasing prey density followed by a plateau in consumption rates. The larval endoparasitoid, *D. insulare* also showed a Type II response to DBM larvae. Predator responses differed to DBM larval stages, wherein *C. carnea* and *P. melanarius* showed a Type III response while *Nabis* spp. showed a Type II response while feeding on larvae. A Type III response shows steady consumption at low larval densities with a linear increase in consumption at medium larval densities followed by a plateau at the highest densities.

Temperature further influenced prey consumption patterns in *C. septumpunctata* where even the predator stages differed in their consumption rates in response to temperature. For example, at low temperature (10oC) adults showed a Type II response to DBM larvae while it changed to Type III at high temperature (32oC). Knowledge of functional response patterns of key predatory and parasitic species of the DBM is vital for understanding their potential as components of biological control-based management strategies (Jiang et al. 2021). Enhancement of local densities of pre-existing natural enemy communities, also referred to as conservation biological control, can contribute to substantial control of pest populations (Kean et al. 2003). For pest species such as DBM that are associated with a broad guild of natural enemies, suppression of the pest population by both predation and parasitism can yield effective biological control (Hallett et al. 2014).

A combined action of the natural enemy guild of DBM in consuming eggs and larvae may reduce pest populations substantially, particularly in high infestation years. Quantification of the predatory and parasitic contributions can help to develop dynamic action thresholds (DAT) that incorporate natural enemies as mortality factors and reduce insecticidal applications and the associated costs (Hallett et al. 2014), and help to integrate agronomic or habitat management practices that conserve local natural enemy

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populations (Keller and Baker 2002). The findings have direct implications for conservation and augmentative biological control programs. Natural enemy populations can be conserved through habitat modification, provisioning of refuges, and supplemental food sources (Keller and Baker 2002). Further research on strategies to support local natural enemy populations will help to conserve populations of key predators and parasitoids of the DBM in canola (Kean et al. 2008; Keller and Baker 2002), and strengthen integrated management of this pest. Although augmentative predator release may not be feasible over large areas of crops like canola, these results can be extended to small acreage crucifer vegetables where augmentation of predators like *C. septumpunctata* or *C. carnea* can help to achieve DBM management. Relationships between temperature and predator consumption behavior are particularly important for implementing biological control strategies where temperature may influence establishment and successful colonization of the natural enemy (Hughes et al., 2009). More importantly, this investigation quantifies the contributions of predators and parasitoids in managing a key pest like DBM in canola and reiterates the importance of natural enemy biodiversity in crop ecosystems.

The biology of a generalist parasitoid, *Microplitis mediator* on DBM larvae

This investigation indicates that *M. mediator* can parasitize all DBM larval stages, however, second and third larval instars are most preferred. This species takes12 to 14 days to develop from eggs to pupae, with larval development taking~9-10 days. Although second and third instar larvae are the preferred DBM lifestage for M. mediator development, wasp pupal weight is similar when reared from any DBM larval instar. This parasitoid species can contribute to collective biological control services provided by the parasitoid guild of DBM in canola and further investigation needs to focus on its seasonal phenology, peak parasitism, and distribution dynamics in relation to DBM larval populations.

Objective 3: To study non-consumptive effects of predator and parasitoid on DBM

Recent studies suggest that non-consumptive effects associated with natural enemies can indirectly reduce prey fitness through sublethal effects (Hoki et al. 2014). Non-consumptive effects result from predator presence and the associated change in the prey behaviour and defenses (Fill et al. 2012). Such effects can cause significant fitness reduction in the prey population, and when combined with direct predation can negatively affect pest fitness and performance (Hoki et al. 2014). While the consumptive effects associated with generalist predators of DBM are documented, not much is known about the non-consumptive effects of most predatory species. This investigation provides the first report of non-consumptive effects of the presence of *C. septumpunctata* on DBM and its interaction with plant host quality. Different life stages of the predator (larvae or adults) were used to test DBM larval survival, growth and leaf consumption. There was no effect of the presence of *C. septumpunctata* adults or its interaction with fertilization regime on oviposition choice. Host plant nutrition had a significant impact on oviposition choice. The DBM females laid ~2 times more eggs on the plants with high fertilization compared to plants with low fertilization This underlines the effects of host fitness in terms of nutrient availability on DBM preference for colonization.

Such bottom-up effects of host plant nutrition on DBM behaviour and colonization preference have been previously reported in canola (Sarfaraz et al. 2009). Plant nutritional status alone may be a

significant contributor to adult females' choice for oviposition compared to perception of fear due to predator presence. Physical presence of LBB adults or larvae reduced DBM larval weight gain by up to 2%. This was probably manifested through reduced feeding. Perception of fear due to adult LBB presence reduced overall leaf consumption by 24%. The mere presence of LBB larvae, reduced larval DBM weight by 19% compared to control which resulted in reduced leaf area consumed by ~35%. These results confirm that non-consumptive effects add to consumptive effects and together can negatively affect fitness gains in DBM larvae beside direct predation. Future studies on DBM natural enemy guilds should focus on collective influence of consumptive and non-consumptive effects on DBM fitness gains and interaction dynamics.

Objective 4: To understand the effects of varying larval densities of DBM on foliar damage in canola and yield.

The results of the field cage study and the controlled growth chamber study confirm that both foliar damage and yield reduction linearly increase as DBM larval density per unit increase. Significant yield reductions occur at larval densities exceeding 4 larvae/plant, with the highest yield reductions at 8 larvae/plant. Further, high larval density at the podding stage is detrimental to canola yield compared to that at flowering stage. Under field conditions, foliar damage in plants with 8 larvae/plants was ~4x higher than the plants at the action threshold with 2 larvae/plant at podding stage in both 2019 and 2020. These differences were reflected in yield reduction in both years. At the nominal thresholds of 2 larvae/plant, yield was 1.5% higher compared to that at 8 larvae/plant. The yields resulting from plants with 2 larvae/plant at podding stage were not different from the no-larvae control. This indicates that yield reductions at current nominal thresholds may not be as significant in years with low to moderate DBM infestation.

Growth room studies support these findings, as larval density of 2-4 larvae/plant at flowering-podding stage had more pods than plants infested with 8 larvae/plant resulting in significant yield reduction. Outbreak years of DBM may indicate high larval densities per plant and yield reductions can be substantial. Nominal thresholds tend to be conservative in most cases and are not necessarily based on pest density variations. Current nominal action threshold of 1-2 larvae/plant in canola for the DBM may be conservative at low pest densities and chemical intervention may be unwarranted, particularly if the yield losses are not significant. Depending on the level of infestation, the nominal thresholds for DBM in canola may need to be adjusted. Also, chemical control-based management of DBM should focus on pest monitoring and forecasts, estimates of pest density, commodity value, and the consideration to the role of natural enemies in the cropping systems.

These studies took a multipronged approach of estimating effects of larval densities on canola yield while also quantifying the contributions of natural enemies in the biological control. These studies create a foundation to further investigate how natural enemies can be fitted into action thresholds to convert nominal thresholds into dynamic thresholds. Dynamic action thresholds will provide realistic estimates of pest population densities for chemical intervention thus helping to time the pesticide applications such that they are least harmful to the activity of natural enemies. This will help to strengthen integrated management of DBM in canola agroecosystems while conserving natural enemy guilds.

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Literature Cited

- Asante SK (1995) Functional Responses of the European Earwig and Two Species of Coccinellids to Densities of *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae). Journal of Australian Entomology Soc 34:105–109
- Dosdall LM, Soroka JJ, Olfert O (2011) The diamondback moth in canola and mustard: current pest status and future prospects. Prairie Soils Crops 4: 66–76.
- de Oliveira Pimenta IC, da Silva Nunes G, de Magalhães GO, dos Santos NA, Pinto MMD & de Bortoli SA (2020) Effects of a Bt-based insecticide on the functional response of *Ceraeochrysa cincta* preying on *Plutella xylostella*. Ecotoxicology 29:856–865.
- Fill A, Long EY & Finke DL (2012) Non-consumptive effects of a natural enemy on a non-prey herbivore population. Ecol Entomol 37:43– 50. https://doi.org/10.1111/j.1365-2311.2011.01333.x
- Getman-Pickering ZL, Campbell AT, Aflitto, N, Ugine TA, & Davis, J. (2019) LeafByte: A mobile application that measures leaf area and herbivory quickly and accurately. bioRxiv. doi:10.1101/777516
- Hallett RH, Bahlai CA, Xue Y & Schaafsma AW. (2014). Incorporating natural enemy units into a dynamic action threshold for the soybean aphid, *Aphis glycines* (Homoptera: Aphididae). First published online, 11 Nov 2013. DOI 10.1002/ps.3674. Pest Management Science, 70: 879–888.
- Hermann SL, Landis DA (2017) Scaling Up Our Understanding of Non-consumptive Effects in Insect Systems. Current Opinions in Insect Science 54–60. doi: 10.1016/j.cois.2017.03.010
- Hermann S, Thaler J. (2014) Prey perception of predation risk: volatile chemical cues mediate non-consumptive effects of a predator on a herbivorous insect. Oecologia, 176 (2014), pp. 669-676.
- Hoki E, Losey J & Ugine TA (2014) Comparing the consumptive and non-consumptive effects of a native and introduced lady beetle on pea aphids (*Acyrthosiphon pisum*).Biological Control 70: 78-84.
- Hughes GE, Bale JS & Sterk G (2009) Thermal biology and establishment potential in temperate climates o the predatory mirid *Nesidiocoris tenuis*. BioControl 54: 785–795. https://doi.org/10.1007/s10526-009-9230-0
- Keller MA & Baker G (2002) Impact of conservation biological control practices on natural enemies: a case study of diamondback moth and its parasitoids. In First International Symposium on Biological Control of Arthropods, pp. 215– 220. Ed. FS USDA. Washington, DC: USDA Forest Service
- Ma J, Li YZ, Keller M & Ren SX (2005) Functional response and predation of *Nabis kinbergii* (Hemiptera: Nabidae) to *Plutella xylostella* (Lepidoptera: Plutellidae). Insect Science 12:281–286.
- Philip H, & Mengersen E (1989) Insect Pests of the Prairies. University of Alberta Press, Edmonton, Alta. 122

- Pritchard DW, Paterson RA, Bovy HC & Barrios-O'Neill D (2017) Frair: an R package for fitting and comparing consumer functional responses. Methods in Ecology and Evolution 8: 1528–1534.
- Nunes G, Truzi CC, Cardoso CP, Vieira NF, Ramalho DG, de Souza JM & de Bortoli SA (2020) Temperature-dependent functional response of *Euborellia annulipes* (Dermaptera: Anisolabididae) preying on *Plutella xylostella* (Lepidoptera: Plutellidae) larvae. Journal of Thermal Biology 93.
- Rogers D (1972) Random search and insect population models. Journal of Animal Ecology 41: 369–383.
- Vieira LC, Salom SM & Kok LT (2012) Functional and numerical response of *Laricobius* spp. predators (Coleoptera: Derodontidae) on hemlock woolly adelgid, *Adelges tsugae* (Hemiptera:Adelgidae). Biological Control 61: 47–54.
- Rahman MM, Zalucki MP & Furlong MJ (2019) Diamondback moth egg susceptibility to rainfall: effects of host plant and oviposition behavior. Entomologia Experimentalis et Applicata 167(8): 701-712.
- Santos BDB, Ramalho FS, Malaquias JB, Lira ACS, Pachú JKS, Fernandes FS & Zanuncio JC (2016) How predation by *Podisus nigrispinus* is influenced by developmental stage and density of its prey *Alabama argillacea*. Entomologia Experimentalis et Applicata 158:142–151.
- Sarfraz M, Dosdall LM, Keddie BA (2009) Bottom-up effects of host plant nutritional quality on *Plutella xylostella (*Lepidoptera: Plutellidae) and top-down effects of herbivore attack on plant compensatory ability. European Journal of Entomology 106: 583-594.
- Smout S & Lindstrøm U (2007) Multispecies functional response of the minke whale Balaenoptera acutorostrata based on small-scale foraging studies. Marine Ecology Progress Series 341:277–291.
- Talekar NS & Shelton AM (1993) Biology, ecology, and management of the diamondback moth. Annual Review of Entomology 38: 275-301
- Werner, E.E. & Peacor, S.D. (2003) A review of trait-mediated indirect interactions in ecological communities. Ecology 84: 1083–1100.
- Xiong X, Zhen JPM, Pengxiang L et al (2015) Chronic, predator-induced stress alters development and reproductive performance of the cotton bollworm, *Helicoverpa armigera*. Biocontrol 60:827–837. https://doi.org/10.1007/s10526-015-9689-

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Appendices

Table 1: Total number of DBM larvae collected during survey and percentage parasitism at each site in 2018.

Site no.	Location	Total no. larvae		Total larvae	No. of parsitized larvae	%Parasitization
		Sweeping	Handpicking			
1	Carmangay	18	4	22	3	13.6363636
2	Carmangay	16	4	20	2	10
3	Claresholm	4	0	0	0	0
4	Carmangay	37	6	43	5	11.627907
5	Baron	16	0	16	2	12.5
6	Granum	12	0	12	1	8.33333333
7	Stirling	24	2	26	1	3.84615385
8	Stirling	27	0	27	1	3.7037037
9	Lomond	13	0	13	3	23.0769231
10	Enchant	15	2	17	1	5.88235294
11	Vulcan	2	0	2	1	50
12	special area no.3	0	0	0	0	0
13	special area no.4	3	0	3	0	0
14	Vermillion county no.24	1	0	1	0	0
15	Vermilion River County No. 24	0	0	0	0	0
16	Vermilion River County No. 24	0	1	1	0	0
17	Vermilion River County No. 24	0	0	0	0	0
18	Camrose county no.22	11	0	11	0	0
19	Minburn county no.27	13	4	17	3	17.6470588

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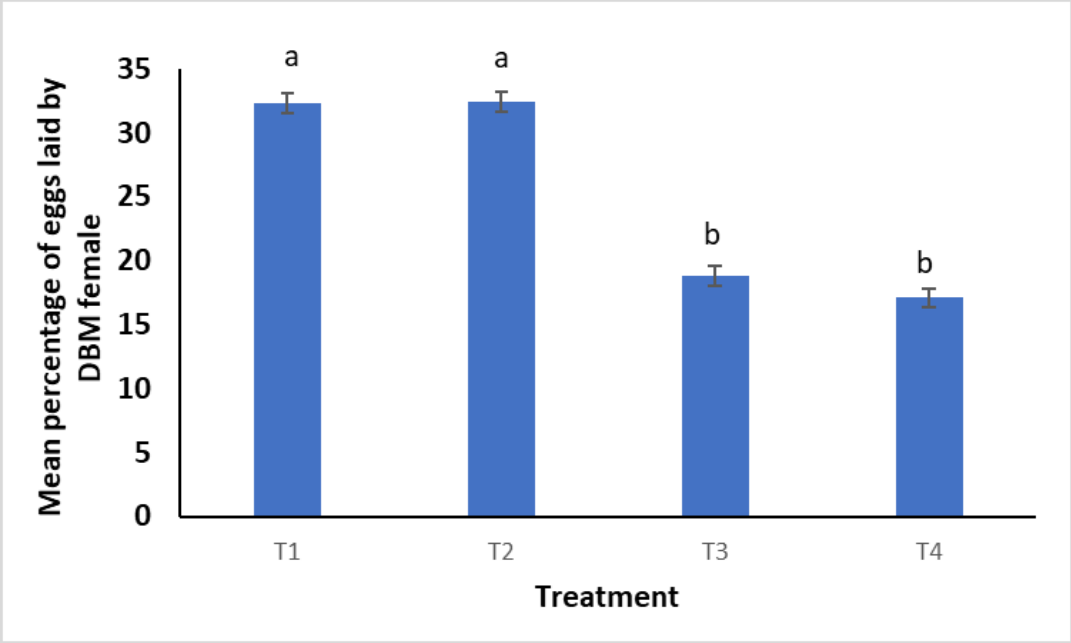
Fig. 1 Mean percentage of eggs gain by DBM female across different treatments (T1: low fertilizer, T2: high fertilizer, T3: low fertilizer with LBB adults, T4: high fertilizer with LBB adults). Vertical bars represent mean (± S.E). Four plants each assigned to one of the four treatments (were maintained in a cage. The different letters above the bars indicate significant differences between two treatments (Tukey's test: $p<0.05$).



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