Effects of ground predators, abiotic factors and plant density on the flea beetles, *Phyllotreta cruciferae* (Goeze) and *Phyllotreta striolata* (F.) (Coleoptera: Chrysomelidae)

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ABSTRACT

The crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae), and the striped flea beetle, *Phyllotreta striolata* (Fabricius) (Coleoptera: Chrysomelidae), are prominent economic pests to canola crops, *Brassica napus,* (L.) (Brassicaceae) in the Canadian prairies, by defoliating canola seedlings, resulting in recurring yield losses. Current management strategies in the Canadian prairies rely heavily on chemical control via both seed-coated and foliar spray treatments. To limit chemical pesticide applications due to potential adverse environmental effects, there has been a focus on alternative flea beetle management strategies including altering plant density and determining potential natural enemies. In addition, few studies have demonstrated how temperature and plant density affect flea beetle feeding and there is little known about the factors affecting stem feeding on canola seedlings. Laboratory studies in Petri dishes and microcosms were completed to examine effects of temperature, plant density and predators on cotyledon and stem feeding by both species of flea beetles in terms of damage intensity and prevalence. Both species defoliated similar amounts, suggesting no feeding difference in species. Feeding increased with temperature and more defoliation occurred on the underside of the cotyledons. *Pterostichus melanarius* and *Pardosa* spp. reduced flea beetle abundances as well as total cotyledon defoliation. In addition, there was less cotyledon and stem damage at higher plant densities. Field studies were completed in four regions of the Canadian prairies (Lethbridge and Peace River, AB; Saskatoon, SK; and Carman, MB) over 4 years (2018 – 2021). Evidence of both the resource concentration hypotheses and a dilution effect was found as flea beetle abundance increased with plant density, yet defoliation levels generally decreased. Yield increased with plant density regardless of the management treatment or region. Using seed-coated pesticide produced similar levels of defoliation, flea beetle abundance, and yield relative to using foliar spray alone at the 25% injury level. The knowledge gained on how flea beetles' feeding on stems and cotyledons is affected by temperature, plant density, and predators will help for in-field scouting and assessment ratings and provide information for future research into biological control potential with the tested predators. In addition, using plant density as a flea beetle management strategy either combined with current chemical practices or as an alternative, is effective to protect canola yield while decreasing the number of chemical applications.

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To my loved ones

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INTRODUCTION

The crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae), and the striped flea beetle, *Phyllotreta striolata* (Fabricius) (Coleoptera: Chrysomelidae), are invasive pests to canola crops, *Brassica napus,* (L.) (Brassicaceae) in North America. The crucifer flea beetle was first recorded in Canada in the 1920's (Wylie 1979, Knodel 2017) while the presence of the striped flea beetle was first discovered in Canada in 1668 (Rousseau and LeSage 2016), although recent molecular evidence suggests this is a Holarctic species (Li et al. 2024). Crucifer flea beetles were already a prominent pest by 1946 and with striped flea beetles, they are currently considered to be important pests of canola across Canada and the northern USA (Wylie 1979, Soroka and Grenkow 2013, Gavloski 2017). With the continued pressure these pests put on the canola production across the Canadian prairies, it is critical to have effective methods of scouting as well as effective management strategies that will ultimately protect canola yield.

Canola scouting methods are reliant on visual assessments of the upper side of the cotyledons and leaves based on a proportion, or a percent, of damaged tissue (Palaniswamy and Lamb 1992). There is little emphasis on stem damage even though it may be an important contributor to early plant death (Canola Council of Canada 2024). Additionally, there are few studies that have examined feeding differences between the two species or how temperature affects their feeding (Soroka et al. 2018, Toshova et al. 2009). Assessing how various factors including temperature and plant density affects flea beetle feeding on both the cotyledon and the stem will help streamline our current scouting practices to improve efficiency and help mitigate flea beetle outbreaks by early detection.

Predators of flea beetles have been scarcely recorded in previous literature, but there is promising evidence of wolf spiders (Araneae: Lycosidae) consuming flea beetles in winter canola fields in Sweden (Ekbom et al. 2014). Arthropod predators have the potential to supress pest populations as well as indirect defoliation reduction based on how the predator changes the way that the pest interacts with its host plant. There is an obvious gap in literature regarding predators in the Canadian prairies that are capable of consuming flea beetles to examine if they are a potential for biological control in addition to our current management strategies to help reduce abundances, reduce defoliation, or a combination of the two.

Current flea beetle management strategies in Canada rely on the use of neonicotinoids as a seed-coating insecticide (Soroka et al. 2008). Besides unwanted detrimental environmental

effects caused by overuse of neonicotinoids (Desneux et al. 2007, Knodel 2017), there is also concern of pest resurgence due to tolerant populations of *P. striolata* in Manitoba (Tansey et al. 2008). It is important to examine other strategies including manipulating plant density to provide alternative or complementary management strategies by limiting flea beetle abundance and defoliation while protecting the seed yield.

In this thesis, I investigate the effects of generalist predators, temperature, and plant density on the interactions between flea beetles and canola. My thesis is composed of four chapters. The first chapter is a literature review in which I examine aspects of previous publications on insect-plant interactions between flea beetles (Coleoptera: Chrysomelidae) and canola, predators of flea beetles and our current knowledge on factors affecting flea beetle abundances and feeding. The second chapter is the first research manuscript, which focuses on biotic and abiotic factors affecting flea beetle feeding. In this chapter I test the potential of different predators to attack flea beetles and the ways they affect their feeding. In addition, I examine the effects of temperature and seeding rate on the feeding behaviour of the two economically important flea beetle species, *Phyllotreta cruciferae* (Goeze) and *Phyllotreta striolata* (Fabricius). Chapter three represents the second research manuscript, which focuses on assessing the effects of seeding rate and different methods of insecticide application on flea beetle abundance, damage to canola and canola yield. Finally, chapter four consists of a general conclusion to the thesis where I examine the implications and significance of my findings for flea beetle management in canola.

CHAPTER 1. LITERATURE REVIEW

1.1 Distribution and importance of flea beetles in North America

The crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae), and the striped flea beetle, *Phyllotreta striolata* (Fabricius) (Coleoptera: Chrysomelidae), are invasive pests to canola crops, *Brassica napus,* (L.) (Brassicaceae) in North America. The crucifer flea beetle is native to Asia, Africa, and Europe and was introduced later than the striped flea beetle being first discovered in the 1920's in British Columbia, Canada (Wylie 1979, Knodel 2017). The striped flea beetle is native to Eurasia and was first introduced to North America before 1668 through Quebec, Canada (Rousseau and LeSage 2016). Crucifer flea beetles were recorded to be a pest of high abundance in 1946 and along with striped flea beetles, they are responsible for over 10% of annual crop losses in North America (Wylie 1979, Knodel 2017). Both species are considered to be important and recurrent invasive pests of canola across Canada and the northern USA (Soroka and Grenkow 2013, Gavloski 2017).

1.2 Biology of flea beetles

Flea beetles undergo one generation per year and can increase to more generations per year in warmer climates (Andersen et al. 2005, Sarwar 2017). The beetles overwinter as adults in the soil beneath leaf litter, in grassy field edges, and sometimes in old canola fields (Wylie 1979, Knodel and Olson 2002). Peak emergence from overwintering occurs when the ground temperature reaches 15 ℃ (Ulmer and Dosdall 2006). When the overwintered flea beetles emerge, they will find their host plants by either brief flights, walking or jumping (Knodel and Olson 2002).

In climates including southern Canada and northern USA, crucifer flea beetles are thought to have a partial second generation in the fall where the eggs are laid in early August (Andersen et al. 2005). Oviposition can occur anywhere from May to early July where the eggs are laid in the soil at the base of their brassicaceous host plant (Wylie 1979). After 12 days larvae hatch from the eggs where they will develop in the soil for 25 to 34 days, feeding on roots and root hairs of the host plant until they pupate after the third instar (Knodel 2017). The pupae last for another 7 to 9 days in the soil before the adult stage (Knodel 2017) (Fig. 1.1). The new generation of adult flea beetles have been observed to have a sex ratio of approximately 1.2 females to one male (Ulmer and Dosdall 2006, Nagalingam and Costamagna 2019).

1.3 Host plant characteristics and plant-insect interactions

Brassicaceae, also known as crucifers, is a plant family comprising of approximately 375 genera, including the well-known cultivated species of canola, broccoli, turnip, and kale (Zhou and Zhang 2001, LeCoz and Ducombs 2006, Ahuja et al. 2011). These cultivated *Brassica* species are important sources of fodder, oils and food, and have become very prominent and important crops in North America (Ahuja et al. 2011). Brassicaceae as a host plant family has numerous crucifer-feeding specialists as well as generalist herbivores that interact with it in terms of the utilization for food, egg-laying sites, larval-development sites and shelter (Sarfraz et al. 2006, Zalucki et al. 2021). Insects constantly pose a threat to *Brassica* crops yield and health, which makes knowledge on important pest species vital. Important insect pests on *Brassica* crops include the diamondback moth, *Plutella xylostella* (L.), Bertha armyworm, *Mamestra configrata* (Walker), Lygus bugs, *Lygus spp.*, crucifer flea beetle, *P. cruciferae* and striped flea beetle, *P. striolata* (Sarfraz et al. 2006, Ahuja et al. 2011, Soroka et al. 2015). Like all pests of plants, insect pests of cruciferous host plants must overcome plant defense mechanisms which include both chemical and morphological defenses.

Brassica crops are one of many groups that contain glucosinolates, which are secondary metabolites derived from amino acids (Charron and Sams 2004). The glucosinolates produced can act as repellents for generalist insect pests but also attractants for crucifer-specialists such as flea beetles and diamondback moths since they rely on glucosinolates to aggregate, lay eggs and feed (Nielsen et al. 1979, Sarfraz et al. 2006, Beran et al. 2016). These compounds attract pests in the case of flea beetles first via airborne mustard oil, allyl-isothiocyanate (AITC), and then stimulates feeding through contact chemoreceptors found on their tarsi, which is a unique trait to Chrysomelidae (Vaughn and Hoy 1993, Yosano et al. 2020). The ability for these pests to use toxic glucosinolates for host location and feeding stimulation shows a trait that is unique to crucifer-specialists. Glucosinolates in the crucifer plants tissue forms toxic by-products with generalist insect feeding, but *P. striolata* have been observed to sequester glucosinolates from their crucifer host plants to produce hydrolysis by-products for their own protection (Beran et al. 2014). The sequestration observed by the striped flea beetle shows an example of coevolution between a specialist and their host plant to overcome the chemical plant defenses. While this adaptation helps these crucifer-specialists to overcome the crucifer's chemical defenses, the host plant's mechanical defenses are other barriers that the plant possesses to protect itself from pests.

Mechanical defenses that are possessed by plants in the family Brassicaceae include leaf colour, epicuticular waxes and elevated trichome numbers. It has been shown in previous studies that as the content of epicuticular wax increases, the damage caused by flea beetles, cabbage stink bugs, *Eurydema ventral* (Klt.), and onion thrips, *Thrips tabaci* (Lindeman), decreased significantly (Znidarcic et al. 2008). In addition, crucifer plants with pubescence have been shown to reduce damage due to insect feeding. An increased amount of trichomes on the leaves and stems of canola, *B. napus*, have been shown to decrease feeding by flea beetles (Soroka et al. 2011, Gruber et al. 2018) and larval diamondback moths as well as deterring oviposition by diamondback moth adults (Alahakoon et al. 2016). Mechanical traits of crucifer plants including waxes and trichome numbers could reduce insect feeding and oviposition, yet for the wide majority of *Brassica* crops grown, they are still vulnerable to attacks from various crucifer-specialists including *Phyllotreta* flea beetles.

The damage that flea beetles cause on canola could result in reduced yield if infestation levels are drastic enough (Lamb and Turnock 1982, Knodel 2017). When defoliating insects feed on their host plants, they likely produce more damaged tissue than what they consume. From this, they could end up causing irreparable damage in which the plant is unable to continue normal growth or result in plant death in cases of severe defoliation (Gavloski and Lamb 2000a, Dosdall and Stevenson 2005). Canola compensation primarily depends on the level of defoliation of the cotyledons and the state of the apical meristem and the root biomass (Gavloski and Lamb 2000a, 2000b). Canola, however, can compensate for the damage caused by flea beetles resulting in rapid regrowth and plant mass recovery when defoliation remains around 25% (Gavloski and Lamb 2000b). The pattern of defoliation that flea beetles feed in cause the most amount of biomass reduction in conjunction with the slowest and least amount of compensation in *B. napus* (Gavloski and Lamb 2000b) compared to other canola pests (Gavloski and Lamb 2000a).

1.4 Consumptive effects of generalist predators on Chrysomelidae

Predators have important effects on food webs by both consumptive (= lethal) and non-consumptive (= enemy-risk) effects on their prey (Culshaw-Maurer et al. 2020). Both consumptive and non-consumptive effects of predators have long been studied and utilized for controlling insect pests in agricultural systems (Thaler and Griffin 2008, Nivedita and Neelkamal 2021). Chrysomelidae is one of the largest families within the order Coleoptera, which includes

many important agricultural pests including the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), the cereal leaf beetle, *Oulema melanopus* (Linnaeus), and *Phyllotreta* flea beetles (Beenen and Roques 2010). Due to the abundance and level of threat chrysomelids pose on important crops, studies on their predators have been an important research topic for potential biological control. Insect predators of chrysomelids include the families Coccinellidae (Coleoptera), Nabidae (Hemiptera), Staphylinidae (Coleoptera) and Carabidae (Coleoptera) along with wolf spiders (Araneae: Lycosidae) (Hazzard et al. 1991, Kheirodin et al. 2019, Chaves-Fallas 2020).

Most previous studies of predators of Chrysomelidae have focused on their consumptive impacts. Generalist predators including carabids and coccinellids have been shown to successfully reduce egg abundances of the chrysomelid *L. decemlineata* in a previous study, which found that general predators consumed 37% of the eggs in the first generation and 58% for the second generation (Hazzard et al. 1991). In addition, Kheirodin *et al.* (2019) found that ground beetles, rove beetles and several species of lady beetles were successful predators of the eggs and larvae of the chrysomelid *Oulema melanopus* in laboratory experiments. They attributed larval predation to Nabidae, Carabidae, Coccinellidae and Lycosidae predators and egg predation to Coccinellidae and Carabidae. General predators of chrysomelids in the field can be detected with the use of DNA-based gut-content analysis (Ekbom et al. 2014). Some chrysomelids, however, such as the Western corn rootworm, *Diabrotica virgifera virgifera* (LeConte), have had no predators detected using molecular gut-content analysis. Oliveira-Hofman et al. (2020) found that zero out of 235 carabid specimens tested positive for the presence of *D. virgiferae virgiferae* DNA.

There are many species of ground beetles present in agricultural systems, but their importance to control Chrysomelidae pest under field conditions remains mostly untested. Certain species, however, such as *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae), might be a more successful candidate for flea beetle predation due to its ability to catch and consume flea beetles in a Petri dish in previous laboratory studies (A.C. Costamagna, personal communication) along with their abundance in field captures (Sebolt and Landis 2004, Kheirodin et al. 2019, Blubaugh et al. 2021). This species is native to Europe but is now widespread across North America and is a very important habitat generalist within crop fields (Busch et al. 2021). While *P. melanarius* has been found to be a successful predator of chrysomelid eggs in wheat

(Kheirodin et al. 2019), they have been found to have no significant effect on the number of flea beetles in broccoli fields (Blubaugh et al. 2021). Studies on the effect of *P. melanarius* on flea beetles are scarce, thus, there are significant gaps in the research showing the ability of these predators to consume flea beetles.

1.5 Non-consumptive effects of generalist predators on Chrysomelidae

When a predator directly consumes its prey, it has the ability to change the community structure by reducing prey abundance and community diversity (Blaustein 2002). In addition to direct consumptive effects, predators have the potential to indirectly affect their prey in a negative way via non-consumptive effects (Fill et al. 2012, Culshaw-Maurer et al. 2020). Indirect effects can be seen in predator-herbivore relationships where caterpillars feed less when exposed to higher perceived predation risk with non-lethal predators (Thaler and Griffin 2008). The reduction in feeding is likely caused by changes in the normal patterns of movement due to stress or to avoid detection (Barnes et al. 2002, Kahl et al. 2020). When cucumber beetles (Coleoptera: Chrysomelidae) are in the presence of wolf spider chemical cues, they reduced their feeding on the host plant (Snyder and Wise 2000) and showed increased emigration (Kahl et al. 2020). Other Coleopteran species exhibit other negative effects due to non-trophic interactions by reducing their activity, mating, and feeding despite the predators being non-lethal (Dupuy and Ramirez 2019).

The presence of generalist predators results in chemical cues that prey species are able to detect in order to avoid consumption (Buchanan et al. 2017). These cues include tactile, visual, or chemical cues, whether the predator was previously exposed or simultaneously present with the prey (Hermann and Thaler 2014). The striped cucumber beetle (Coleoptera: Chrysomelidae) relies mainly on tactile cues to avoid predation by wolf spiders (Williams and Wise 2003) while the spotted cucumber beetles (Coleoptera: Chrysomelidae) likely used visual cues to differentiate between low and high-risk predators (Snyder and Wise 2000). Chrysomelids have been shown to possess the ability to both recognize a predator and change their feeding behaviour according to the level of potential threat, where high risk predators will decrease their feeding rate more than low risk predators (Snyder and Wise 2000). Consumptive and non-consumptive effects by generalist predators on flea beetles regarding their potential for biocontrol and effects on crop

yield has not been investigated fully resulting in a lack of knowledge on their potential to aid in pest management.

1.6 Effects of abiotic factors on Chrysomelidae

Abiotic factors including temperature, wind direction and photoperiod all play a role in an insect's development, flight patterns, reproduction and feeding behaviour. Insects rely on various sensory cues including visual, tactile and olfactory to locate their host, making abiotic factors potentially important in modifying their success (Obonyo et al. 2008). Beetles in the family Chrysomelidae are affected in various ways depending on external factors, which will affect the way that chrysomelid pests find and feed on the host plant (Valente Pereira et al. 2002, Zhou et al. 2018).

Wind direction has been shown to affect the way that *Ophraella communa* (LeSage) (Coleoptera: Chrysomelidae) searches for its host plant and oviposition locations (Zhou et al. 2018). The olfactory cues that *O. communa* relies on for host location were affected by the wind, resulting in a higher concentration of this pest in the plants that were located downwind (Zhou et al. 2018). This suggests that windy days might be unfavourable for chrysomelid pests as it disrupts important olfactory sensory cues that are emitted by their host plants. Similarly, a previous study has also shown that there is an increase in capture heights of *P. cruciferae* with less wind, demonstrating that wind significantly affects flea beetle flight behaviour as they travel less (Tansey et al. 2015).

Previous studies show that increased temperature increased the feeding of two chrysomelid species, *L. decemlineata* and *Acanthoscelides obtectus* (Say), with the greatest amount of feeding occurring between 20 – 28 °C (Logan et al. 1985, Soares et al. 2015). A study done by Tansey et al. (2015) shows that an increase in temperature also significantly increases the capture heights of *P. cruciferae* indicating more flight travel with warmer temperatures. Since many chrysomelids rely on chemical cues from the host plant, the effect of temperature on the plants' glucosinolate concentration may be important in how the pest's feeding changes. It has been found that a higher temperature significantly increases the concentration of glucosinolate levels in *Brassica* crops, which may aid in increased feeding stimulation in chrysomelids such as flea beetles (Valente Pereira et al. 2002).

While abiotic factors have been shown to have varying effects on the family Chrysomelidae, few studies demonstrate the effect that factors such as temperature have on flea beetle feeding in canola. In a field study, it was found that warmer temperatures increase the likelihood of *P. cruciferae* captures in fields but decreased the abundance for *P. striolata,* suggesting key species differences (Soroka et al. 2018). In addition, Soroka et al. (2018) found that every 1°C increase in April increased the likelihood of *P. cruciferae* appearance. In contrast, increased temperatures in May and June will decrease the abundance of *P. cruciferae*, suggesting that temperature effects vary based on the time of season. The response of *P. striolata* to weather conditions such as temperature is thought to be more plastic than *P. cruciferae*, which could suggest that they are better at adapting to changes in various abiotic factors such as cooler weather early in the season (Soroka et al. 2018).

When using traps baited with the flea beetle attractant, allyl-isothiocyanate (AITC), it was found that more *P. cruciferae* are captured in warmer temperatures (Toshova et al. 2009). Flea beetles are more attracted to the host plant's volatiles in warmer temperatures, which could be explained due to a significantly increased rate of AITC release at increased temperatures (Dai and Lim 2014). An increased concentration of AITC present will aid the flea beetle in host-location and increase the likelihood of feeding as the temperature increases. While flea beetles use the glucosinolates in the canola cotyledons to find the plants and feed, it has been found that the absence of them in different canola cultivars do not decrease the flea beetle feeding rate (Bodnaryk and Palaniswamy 1990). Thus, it is important to determine various strategies to limit flea beetle damage to protect canola.

1.7 Feeding damage to canola

Most of the economic damage caused by flea beetles on canola plants is through feeding on the cotyledons and the stems of the seedling stage, which occurs during the two weeks post emergence (Lamb 1984, Brandt and Lamb 1993). The damage produced by flea beetles are shot-hole pits as well as plant tissue death (necrosis) resulting from extensive feeding (Knodel and Olson 2002). In the seedling stage, the plant relies fully on the cotyledons for photosynthesis making any feeding damage deleterious for the plant's fitness (Dosdall and Stevenson 2005). Canola has little resistance to herbivory at this stage, which can cause wilted plants, delayed maturity and reduced yield whereas it can withstand flea beetle damage more effectively after the

four-leaf stage (after 2-3 weeks) (Lamb and Turnock 1982, Lamb 1984, Soroka and Grenkow 2013). Unfortunately, the emergence of flea beetles coincides with the vulnerable seedling stage of canola resulting in a high abundance on the plants (Kinoshita et al. 1979a, Lamb 1988).

The distribution of the feeding plays an important role in the extent of damage to the plant (Brandt and Lamb 1993). The plant is more prone to death when the feeding is on the stem since it may cause breakage or loss of nutrients to the plant (Brandt and Lamb 1993). Therefore, it is important to understand the factors that regulate stem damage to prevent seedling death and to improve scouting techniques.

1.8 Aggregation behaviour and impact of olfactory cues

Aggregation behaviour is crucial for herbivores such as *Phyllotreta* to increase searching efficiency for their food source and for mating (Fletcher 2006, Fernandez and Hilker 2007). Male aggregation pheromones produced by *P. striolata* and *P. cruciferae* attract both sexes when they are feeding on or in contact with the host plant (Bartelt et al. 2001, Beran et al. 2011). There are six sesquiterpenes emitted by the male flea beetles, and there are three compounds that are attractive and trigger physiological responses when combined with volatiles from the host plant such as AITC (Beran et al. 2011). Flea beetles rely on glucosinolates found in *Brassica* host plants to locate and aggregate to their food source and egg-laying sites (Nielsen et al. 1979, Beran et al. 2016). As a result of the glucosinolate pheromones combined with the male-produced sequiterpenes, flea beetles are more likely to aggregate and immigrate into high density areas and move from small patches to large patches (Kareiva 1985, Fletcher 2006). The movement of *Phyllotreta* into large and dense patches are either a result of random movement facilitated by walking, jumping, or flying (Kareiva 1985) or by attraction to the plant volatiles (Bukovinszky et al. 2010).

The importance of olfactory cues to host location by *Phyllotreta* is further shown by the impact of adding non-host volatiles on their feeding behaviour. When placed in monocultures, adult flea beetles are more likely to remain on the plants and feed compared to host plants grown with natural vegetation or ones interplanted with non-host plants (Tahvanainen and Root 1972). *Phyllotreta cruciferae* are more likely to be found in large patches with no vegetational diversity while *P. striolata* are able to colonize smaller plots, but both are most successful at aggregating monocultured crops of their host plant (Cromartie 1975). Intercropping with non-host plants

including tomato, squash, sweet potato and beans reduce the abundance of flea beetles in *B. oleracea* crops but may not be a successful management strategy due to the significant decline in host plant yield (Tahvanainen and Root 1972, Latheef and Ortiz 1984, Latheef et al. 1984).

1.9 Damage distribution within the field

The specific feeding behaviours of *P. cruciferae* and *P. striolata* and their interactions with their brassicaceous host crops have seldom been studied besides some insight into feeding distribution on canola seedlings (Brandt and Lamb 1993). Since flea beetles tend to overwinter under the leaf litter in the field borders and grassy edges, damage is typically more extreme at the field margins (Knodel and Olson 2002). Temperature may affect the in-field damage distribution, however. Damage tends to be limited to the outer margin of the canola fields when the weather is cooler, but as the ambient temperature warms, there is typically an even level of damage throughout the entire field (Knodel and Olson 2002).

1.10 Feeding preferences

When the flea beetle begins feeding on canola plants, it has been found that there are preferences as to where they will feed on the cotyledon or leaf. *P. cruciferae* was determined to have a stronger preference for the upper surfaces of cotyledons with the same amount of damage on the bases and the tips and less in the centre of the cotyledons (Brandt and Lamb 1993). Brandt and Lamb (1993) also determined that there is a stronger preference for only the bases of true leaves. In addition, flea beetles prefer plants that are already wilted, which includes wilting from water stress that occurs during hot and dry summers (Palaniswamy et al. 1998). This preference might be correlated by the increase in the production of the stress hormone, abscisic acid (Qaderi et al. 2006, Xu et al. 2020) which in turn increases the production of glucosinolates (Bodnaryk 1994). Since flea beetles use glucosinolates to find their host plant, the increase of stress hormones is likely to result in more feeding damage (Nielsen et al. 1979).

When stem damage was examined of four species of Brassicaceae, it was found that *P. cruciferae* feeds equally across the stem (Brandt and Lamb 1993). The studies on stem damage preferences are scarce, resulting in little insight into what factors promote flea beetle feeding on stems. Future research on stem damage preferences is crucial for our understanding of flea beetle preferences and will aid their management.

1.11 Economic damage and chemical control

Because of the high intensity of feeding at the seedling stage, flea beetles rank as one of the costliest pests in North America (Lamb and Turnock 1982). The estimated losses are over 300 million dollars US dollars each year in North America alone (Lamb and Turnock 1982, Knodel 2017, Olfert et al. 2018). The amount of defoliation present in the field needs to be closely monitored to ensure that the damage threshold is not being exceeded to limit economic losses. Monitoring should begin when the canola is newly emerged when the temperature is around 14℃ (Knodel and Olson 2002). Since the canola seedlings are at their most vulnerable from emergence to the four-leaf stage (Sekulic and Rempel 2016), monitoring should continue until the majority of the crop reaches this stage. To prevent economic losses caused by flea beetle feeding, management practices involve controlling adult beetles on canola in the cotyledon stage. Canola growers in Canada first use seed-coating systemic insecticides, such as the neonicotinoids imidacloprid, clothianidin, and thiamethoxam, which are used on almost all canola fields (Sekulic and Rempel 2016). Foliar insecticides will then be applied to the plant when the average flea beetle damage exceeds 25% defoliation per seedling but they are likely applied at a more excessive rate than is needed (Lamb and Turnock 1982, Dosdall and Stevenson 2005). The 25% injury threshold is in place in Canada to maximize yield while minimizing pesticide applications (Knodel and Olson 2002) but more recent studies have shown that lower thresholds such as 15-20% may be more effective (Ekbom 2010, Tangtrakulwanich et al. 2014).

The main control strategy used in over 90% of the canola planted in the Canadian prairies is insecticide-coated seeds (Soroka et al. 2008). The reliance on neonicotinoids as an insecticide coating may lead to future pest resurgence as there is tolerance to some compounds shown in populations of striped flea beetles in Manitoba (Tansey et al. 2008). In addition, the overuse of pesticides can create detrimental effects on natural enemies and beneficial pollinators (Desneux et al. 2007, Knodel 2017) and lead to pest resurgence (Reddy et al. 2014). Thus, it is crucial to determine and understand different strategies that may provide flea beetle management.

1.12 Management practices for flea beetles

Alternatives to chemical control of flea beetles include increased seeding rates, no-till planting, row spacing, seed cultivars and early planting dates (Dosdall et al. 1999, Dosdall and Stevenson 2005, Lundin et al. 2018). Increasing the row spacing has been shown to decrease flea

beetle damage per plant when increased from 10 cm to 20 – 30 cm (Dosdall et al. 1999). Dosdall et al. (1999) also observed that wider row spacing resulted in more canola plants per row, meaning the effect of row spacing may be attributed to an increased canola density. Reduced or no-till planting is effective at reducing damage, especially when combined with increasing the seed rate of canola due to the increased levels of crop residue (Dosdall et al. 1999). Early planting has been shown to decrease flea beetle damage, but Lundin et al. (2018) found that this strategy usually results in a higher canola density, which may be a contributing factor to the lower defoliation levels. The negative impact of morphological traits such as increased trichome number on flea beetle feeding has been used to develop tolerant canola cultivars (Alahakoon et al. 2016). Two genotypes of *B. napus* were developed to increase trichome density, which resulted in a reduction of flea beetle feeding by 30 – 50% (Alahakoon et al. 2016). Even with these non-chemical management strategies, insecticidal control remains to be the most used practice in controlling flea beetle damage in Canada.

The manipulation of planting density has shown promising results in decreasing defoliation in canola. Dosdall and Stevenson (2005) have shown that increasing the seeding rate results in decreased flea beetle damage per plant due to a dilution effect that results in a greater amount of canola tissue biomass for each flea beetle and therefore less damage to each plant when the seeding rate is higher (Knodel 2017). The beetles have been thought to be attracted to the greater amount of visual contrast between plants and the soil (Dosdall et al. 1999) or the increased amount of warmth emitting from bare soil (Ulmer and Dosdall 2006) that both occur from a lower seeding rate. Thus, increasing the seeding rate will limit the amount of bare soil and may decrease the amount of feeding on the plants. While the effect of seeding rate and patch size on flea beetle abundance and defoliation has been studied previously, the interaction between seeding rate and the abundance of flea beetles and their predators has never been studied. Determining the effect of seeding rate not only on the interactions between flea beetles and canola, but also on predator communities and final yield is important to fully understand the implications of management by seeding rate.

1.13 Biological control of flea beetles

In addition to mitigating flea beetle damage by insecticidal practices and cultural control strategies, natural enemies also have the potential to decrease their damage (Ekbom et al. 2014).

Due to the success and availability of insecticides in canola across Canada, biological control agents for flea beetles are seldom looked at as a viable IPM strategy. Natural enemies that have potential for biocontrol includes entomopathogenic nematodes (EPNs), entomopathogenic fungi (EPFs), parasitoids, and generalist predators. Nematodes in the genus *Steinernema* (Rhabditida: Steinernematidae) have resulted in lower canola damage (Antwi and Reddy 2016) and higher mortality of *Phyllotreta* flea beetles (Trdan et al. 2008). The EPF *Beauveria bassiana* resulted in 50 – 90% mortality in *P. cruciferae* (Miranpuri and Khachatourians 1995) and reduced in-field defoliation in Montana when used as a spray combined with *Metarhizium brunneum* (Reddy et al. 2014).

Parasitoids are an important component of biological control programs, yet are not used in IPM for flea beetles due to low parasitism rates (Wylie and Loan 1984) or lack of parasitoid establishment in Canada (Wylie 1988). The parasitoid, *Townesilitus bicolour* (Wesmael) (Hymenoptera: Braconidae), is a successful parasitoid of *Phyllotreta spp.* in Europe, but did not establish when introduced into Canada (Wylie 1988). The parasitoid commonly found in Canada that attack *P. cruciferae* and *P. striolata* is *Microctonus vittatae* (Muesebeck) but the incidence of parasitism is usually below 5% (Wylie 1981, Wylie and Loan 1984).

Phyllotreta flea beetles have very few observations of predators in the field (Ekbom et al. 2014). The few predation events documented include observations of *Collops vitattus* (Coleoptera: Melyridae) (Gerber and Osgood 1975), a damsel bug (Hemiptera: Nabidae) (Burgess 1982) and a lacewing larva (Neuroptera: Chrysopidae) (Burgess 1980), a spined soldier bug (Hemiptera: Pentatomidae) (Culliney 1986) and fall field crickets (Orthoptera: Gryllidae) (Burgess and Hinks 1987) partially consuming flea beetle adults. In addition, there is evidence of flea beetle consumption by wolf spiders due to flea beetle DNA identified in their guts, where 19.4% of the captured *Pardosa spp*. (Araneae: Lycosidae) were positive for flea beetle DNA (Ekbom et al. 2014). *Pterostichus melanarius* has been observed to feed on flea beetles in a Petri dish experiment (A.C. Costamagna, personal communication) as well as in spring barley fields (Chiverton 1984). The effect predators have on flea beetles in canola fields is relatively unknown, and there is little insight into how the presence of predators will affect flea beetle abundances and defoliation. Thus, it is important to determine the effect of *P. melanarius,* Lycosidae spiders, and other potential general predators on flea beetle species feeding to further our understanding in natural control methods in canola fields.

1.14 Objectives and Hypotheses

The objectives of this thesis are:

i. Determine the biotic and abiotic factors affecting the interactions between flea beetles and canola. This includes determining effective predators of flea beetles, examining how temperature, seeding rate and predators affect flea beetle behaviour, and determining differences between the two economically important flea beetle species.
ii. Determine how different seeding rates of canola and application rates of foliar insecticide will affect the density of flea beetles, plant damage, and yield.
iii. Gather insights from my findings and provide informed suggestions for Integrated Pest Management of flea beetles in canola in conjunction with knowledge from previous literature.

Based on the published research reviewed in the first chapter, my hypotheses are as follows: I expected to find that increased temperatures will increase the total area damaged due to increased flea beetle activity. I expected that *P. striolata* will feed more at lower temperatures due to their earlier emergence dates when compared to *P. cruciferae* and may be better adapted for cooler temperatures. I also expected that the flea beetles will feed more on the abaxial side of the leaf due to an instinctive tendency to remain shaded. I expected this feeding preference to be stronger at lower temperatures hypothesizing that flea beetles will move down the plant in cooler weather. Similarly, I expected to find an increase in stem damage at a lower temperature. I also predicted less damage per plant at higher planting densities when compared to lower planting densities due to a dilution effect. In terms of biotic factors, I predicted that the presence of the predators will decrease the prevalence and intensity of both cotyledon and stem damage due to consumptive effects and non-consumptive effects of flea beetle avoidance behaviour.

In terms of the effects of insecticide and seeding rate on flea beetle-canola interactions, I predicted that an increased seeding rate will decrease the amount of damage per plant due to a dilution effect. I also expected that a moderate seeding rate will produce the highest yield due to less overcrowding of the plants resulting in larger plants plus moderate dilution effects. Lastly, I expected that foliar insecticide treatments at 25% damage will decrease defoliation and overall flea beetle abundance.

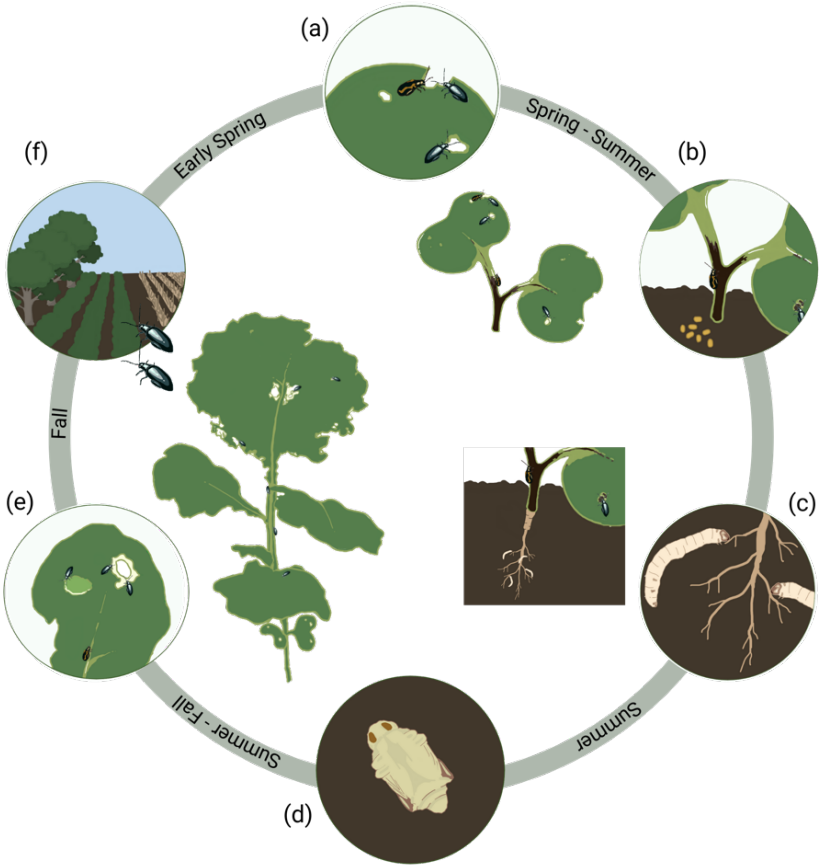


Figure 1.1. Life cycle of the striped and crucifer flea beetles (*Phyllotreta* spp.) on their host plant canola (*Brassica napus*). (a) Overwintered adults emerge and begin feeding on newly emerged canola seedlings; (b) eggs are laid in the soil at the bases of the plant; (c) beetles undergo three larval instars in the soil feeding on roots and root hairs; (d) pupation in the soil; (e) newly emerged adults begin feeding on late season canola including the leaves, stems, and pods. Mating occurs at this time; (f) adults migrate to aestivation sites including hedgerows, old canola stubble and grassy borders at the field borders. Adapted from Ortega-Ramos et al. (2021).

CHAPTER 2. EFFECTS OF BIOTIC AND ABIOTIC FACTORS ON THE INTERACTIONS BETWEEN FLEA BEETLES AND CANOLA

2.0 Abstract

The crucifer flea beetle, *Phyllotreta cruciferae* (Goeze), and the striped flea beetle, *P. striolata* (Fabricius) (Coleoptera: Chrysomelidae), are invasive pests to canola crops, *Brassica napus* (L.) (Brassicaceae), in North America. Determining how temperature and predators affect flea beetle damage to canola is essential to improve current management strategies but has been rarely studied. The prevalence (proportion of plants damaged), intensity (defoliation of damaged plants), and overall damage (defoliation of all plants) caused by flea beetles were examined under laboratory conditions on canola stems and cotyledons at different temperatures and plant densities as well as with or without generalist predators. Flea beetles preferred to feed on the undersides of cotyledons regardless of temperature and produced more stem and cotyledon damage at higher temperatures, with no difference between the species. *Pardosa spp.* (Araneae: Lycosidae) and *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) reduced flea beetle abundance and the intensity of cotyledon defoliation. *Pterostichus melanarius* also reduced overall stem damage, whereas *Pardosa spp.* reduced the intensity of stem damage. Under constant flea beetle densities, canola sustained less stem and cotyledon damage at a higher plant density. Our results suggest that agricultural practices that enhance ground predator populations and increase plant density may contribute to decreasing flea beetle damage to canola.

2.1 Introduction

Phyllotreta flea beetle species are some of the most damaging and chronic pests in canola crops, *Brassica napus* (L.) (Brassicaceae), across North America (Lamb and Turnock 1982, Lamb 1989). The most common species in the Canadian prairies are the crucifer flea beetle (CFB), *Phyllotreta cruciferae*, and the striped flea beetle (SFB), *Phyllotreta striolata* (Soroka et al. 2018). Flea beetles cause economic damage on canola seedlings before the four-leaf stage on the cotyledons, leaves, and stems of the plant (Lamb 1984, Brandt and Lamb 1993, Ortega-Ramos et al. 2021). Damage on canola seedlings by flea beetles can be found on the stems and on both sides of cotyledons and leaves but due to efficiency it will typically only be recorded from the upper sides. A previous study suggested that the upper sides of cotyledons and leaves are preferred by flea beetles and that stem feeding occurs evenly across the entire stem (Brandt

and Lamb 1993). Stem damage has the potential to be very important in determining plant health since heavy stem damage can cause breakage or loss of nutrients to the plant (Brandt and Lamb 1993). Factors regulating flea beetle-induced stem damage have seldom been studied, which leaves gaps in our understanding of how flea beetles feed on the entire canola seedling as well as a lack of insight into flea beetle monitoring.

The main chemical treatment currently used in Canada is coating seeds with systemic neonicotinoid insecticides and is used in over 90% of canola fields (Soroka et al. 2008, Cornelsen et al. 2024). Foliar insecticides are used to complement seed treatments when flea beetle damage exceeds 25% during the critical stage in canola growth, which is up to the four-leaf stage (Ekbom 2010, Tangtrakulwanich et al. 2014). In order to successfully integrate non-chemical control methods into current practices used by canola farmers, we need a better understanding of what affects the interactions that flea beetles have with canola as their host plant. There are numerous gaps in research, however, regarding what factors affect flea beetle feeding behaviour on canola, including the effects of temperature, plant density and generalist predators.

Fluctuating temperatures have the potential to change insect behaviours including foraging, locomotion, reproduction, and microhabitat selection due to either physiology-based changes or adaptive behaviours in changing environments (Abram et al. 2017). Higher temperatures increase the metabolic rate of insects, which in turn leads to an increase in development and feeding rates (Ma et al. 2021, Ahmed et al. 2022). Warmer temperatures in the growing season have been shown to increase the abundance of CFB but are associated with a decrease of SFB in canola (Soroka et al. 2018). In addition, it has been thought that SFB has a much more plastic response to temperatures than CFB resulting in earlier emergence in the spring, which suggests an important difference in species response to temperature (Soroka et al. 2018). There are no studies, however, demonstrating the direct effects of temperature on damage per plant, which is an important first step in understanding how to monitor canola seedlings more efficiently.

Several studies investigated cultural control regimes such as planting date, tillage regimes, row spacing, and plant density (Dosdall et al. 1999, Dosdall and Stevenson 2005) to integrate them into flea beetle management. Earlier planting dates have been shown to decrease defoliation, possibly due to the corresponding plant density increase (Lundin et al. 2018). Both

reduced or no-till planting as well as increased row spacing was found to reduce defoliation (Dosdall et al. 1999). Increasing plant density has been shown to decrease canola defoliation under field conditions (Dosdall et al. 1999, Dosdall and Stevenson 2005, Lundin 2020, Woodland et al. in prep). However, previous work has not monitored flea beetle abundance in response to plant density to test what mechanism explains the observed damage reductions. In this thesis (Chapter 3), I show that increased plant density results in a decrease of the number of flea beetles per plant, demonstrating a dilution effect (Knodel 2017). Previous studies were conducted under variable environmental conditions and naturally occurring flea beetle populations, resulting in a need to test these interactions under controlled conditions. Finally, none of these cultural management strategies are widely used to manage flea beetle populations in canola, which still rely strongly on chemical management strategies.

Generalist predators have been shown to be effective at reducing pest populations in 75% of cases using biocontrol in integrated pest management (Symondson et al. 2002) but their role in flea beetle suppression in canola has seldom been investigated. There have been observations of few predators including a damsel bug (Hemiptera: Nabidae) (Burgess 1982), lacewing larva (Neuroptera: Chrysopidae) (Burgess 1980), spined soldier bug (Hemiptera: Pentatomidae) (Culliney 1986) and fall field crickets (Orthoptera: Gryllidae) (Burgess and Hinks 1987) feeding on adult flea beetles under field conditions. More recently, a study reported flea beetle DNA in the gut contents of wolf spiders (Araneae: Lycosidae) collected in winter canola fields in Sweden (Warner et al. 2003, Ekbom et al. 2014). These previous studies indicate the potential of some predators to have direct, consumptive effects on flea beetles, but potentially there are also indirect, non-consumptive effects of predators on prey by altering prey behaviour (Schmitz et al. 1997) that may result in further reduction of canola damage. For example, cucumber beetles (Coleoptera: Chrysomelidae) reduce their feeding and are more likely to emigrate when exposed to predator chemical cues (Snyder and Wise 2000, Kahl et al. 2020). Other beetles have shown to reduce activity, including mating and feeding, when exposed to non-lethal predators (Dupuy and Ramirez 2019). There is a need for research on predator species that are capable of consuming flea beetles as well as their potential non-consumptive effects understand the importance of generalist predator conservation in canola agroecosystems.

Monitoring canola for flea beetle damage currently consists of rating the canola for percent defoliation where 0% means no damage and 100% means the cotyledon or leaf is

entirely defoliated, which is adapted from Palaniswamy et al. (1992). Monitoring will start at emergence and continue until the canola reaches the four-leaf stage, which is the vulnerable period of canola growth (Sekulic and Rempel 2016). The components of the overall defoliation observed can be variable. For example, flea beetles may produce a high amount of damage on a low number of plants, which may show a similar average to a low amount of damage on many plants. Therefore, overall damage should be examined in two different ways to relate to flea beetle feeding behavior: prevalence and intensity. Prevalence is the proportion of plants attacked and intensity is the amount of damage observed on each attacked plant (Lamb and MacKay 2010). These variables are both components of the first variable, "overall damage", where prevalence multiplied by intensity equals the overall damage. Separating prevalence and intensity to describe the damage is important especially considering that canola compensates for damage done at the plant level as it grows as well as the patch level with rapid regrowth and mass recovery when defoliation is below 25% (Gavloski and Lamb 2000a, 2000b). Prevalence provides insight into patch level compensation, while intensity indicates compensation at the plant level. In addition, non-consumptive predator effects may affect prevalence or intensity differently, which may explain if flea beetles change the number of plants they visit and decrease their feeding per visited plant in the presence of a predator.

This study aims to fill several gaps in our knowledge of the interactions of flea beetles with canola and generalist predators and how temperature affects their feeding on canola. Specifically, I tested (1) how temperature affects feeding on the cotyledons and stems, (2) how plant density affects flea beetle damage, and (3) how generalist predators affect flea beetle survivorship and damage. I hypothesized that: (1a) flea beetle damage will increase with temperature; (1b) striped flea beetles will cause more damage than crucifer flea beetle at low temperatures; (2) increased plant density will reduce plant damage by a dilution effect; (3a) predators will reduce flea beetle survivorship; and predators will reduce canola damage (3b) directly by consumptive, and (3c) indirectly by non-consumptive impacts on flea beetles. In addition, (4) I develop a visual method to quantify stem damage accurately and efficiently to be used in future research.

2.2 Methods

2.2.1 Insect and plant material

Both SFB and CFB used in experiments were collected from nearby canola fields around Winnipeg, Manitoba via sweep-netting. SFB were also collected from colony-reared cages maintained following the methods outlined in Nagalingam and Costamagna (2019). Field-collected flea beetles were maintained in BugDorm (BugDorm-4E4545, MegaView Science Education Services Co., Ltd., Taiwan) cages with canola at the cotyledon stage for food under the same conditions as the SFB colony. All colony-reared SFB used in experiments ranged in age from four to nine days after adult emergence. Predators were collected using live pitfall traps set in canola fields near Winnipeg, Manitoba, from July 7 to September 10, 2020 (experiment 3) and June 18 to Aug 28, 2021 (experiment 4). Pitfall traps consisted of a removable plastic container (7.5 cm height, 11.5 cm diameter) fitted inside a stationary larger plastic container (14 cm height, 11.5 cm diameter) to easily remove the trapped predators. The trap was covered by a wire mesh (2.5 cm^2 holes) at ground level to prevent larger animals from falling in as well as a plastic covering (16 cm^2) held approximately 5 cm above ground level by 4 metal nails to prevent rain from flooding the container. The pitfall trap contained dirt and leaf litter for refuge and either a water or beer-soaked sponge to provide moisture and attract predators. Predators were brought back to the laboratory and stored in individual vials that contained moistened filter paper and freeze-dried crickets for food. All predators and flea beetles were starved for 24 hours prior to trials. The moistened filter paper was replaced in the predator vials as necessary, and a damp sponge was added to the flea beetle cages for moisture.

Canola seeds were planted in mycorrhizae potting soil (PRO-MIX M, Premier Tech Horticulture; Quebec, CAN) in a plastic tray and grown for five days until they reached the cotyledon stage at 22℃, 16L:8D (70% RH). Only canola seedlings that ranged from 9 - 11 mm across the widest point of the cotyledon (length) and approximately 5 ± 1 mm (4 - 6 mm range) across the main vein (width) were used. They were then randomly transplanted into each microcosm according to the appropriate plant density or cut at the base of the stem where it meets the soil in the case of the predation assays in Petri Dishes. All experiments were conducted under controlled temperature and RH conditions (specified per experiment below) using reach-in Percival growth chambers (1-35LVL Percival; Perry, USA). Temperature regimes among growth

chamber were rotated among trials within and between experiments, so potential chamber effects were not confounded with temperature and RH regimes.

2.2.2 Stem damage estimation

A new method to estimate stem damage was developed using equation 1:

$$est.damage\ per\ stem\ [mm^2] = (\Sigma pits\ in\ size_i\ [mm]) \left(\pi\ \cdot\ 0.2\ mm\ \cdot \frac{l_i}{2}\right)$$

A rectangle (40 x 65 mm) of transparent plastic marked with three lengths was used to classify the pit length into one of three size categories (l_i): 1, 2 or ≥ 3 mm (Fig. 2.1). An average width of 0.2 ± 0.05 mm was measured from a random subsample of 60 pits from stems in experiments 2 and 4 (see below) and used as the standard width in the equation. The number of pits in each size category was recorded per stem. The length of each pit was multiplied by the width and by the number of pits per size category to achieve an estimated area of damage per stem (mm^2). On the same stems used above, the actual area of the stem pits was measured from digital pictures taken from two sides of the stem using the photo-editing software, GIMP (The GIMP Development Team 2019). The simple linear regression model used to compare the estimated damage (i.e. using the equation above) and the actual damage (i.e. measuring the digital pictures) showed a very accurate prediction of our simplified method of estimating stem damage ($F_{1,45}$= 424.7, p < 0.00001, R^2 = 0.90, n = 45; Y = 1.10X – 0.11; Appendix I). Therefore, equation 1 was used to estimate stem damage in experiments 2 and 4.

All data analyses for this experiment and subsequent ones were performed using R 4.2.1 (R Core Team 2023).

2.2.3 Microcosm trials

A microcosm setup was used for experiments 1, 2 and 4 using canola seedlings in a circular plastic pot (950.5 cm^3) and covered by a plastic covering made from the top portion of a 2 L plastic pop bottle (23 cm height x 11 cm diameter). Two squares were cut out and covered with a fine mesh to promote air circulation and avoid condensation (5 x 5 cm). A circle was cut on the side (2 cm diameter) and covered with a rubber stopper to remove flea beetles with an aspirator after the experiments. The top of the pop bottle was wrapped in a fine mesh and secured with a rubber band to promote airflow and prevent condensation (Fig. 2.2). After each experiment was completed, the microcosms were removed from their temperature-controlled

reach-in growth chambers to take measurements. The number of flea beetles alive were recorded and the canola plants were removed. The cotyledons were removed from the stem by cutting at the base of the cotyledon where it adjoins the petiole to take photos and quantify defoliation digitally (experiment 1).

2.2.4 Experiment 1: Effect of temperature and flea beetle species on canola damage

Microcosm studies were conducted to determine the effects of temperature on cotyledon defoliation caused by SFB and CFB. Four trials took place weekly from July 5 – 26, 2019 with each trial lasting 48 hours. Four constant temperature regimes (13℃, 18℃, 23℃, and 28℃) were combined in a factorial design with three flea beetle manipulation treatments (SFB, CFB, and control with no flea beetles) to assess flea beetle damage to canola. These temperature regimes encompass the range of temperatures experienced by flea beetles in the field above their activity threshold temperature of 10.2℃ (Kocourek et al. 2002). Each microcosm contained two canola plants at the cotyledon stage with five flea beetles of the appropriate species. Four reach-in growth chambers were used to accommodate the four different temperatures, where each chamber received three replicates of each treatment. All trials were conducted at 70% RH and a photoperiod of 16 L: 8 D.

At the end of each trial, photos were taken of each side of every cotyledon against white paper with an iPhone XR (12-megapixel; resolution = 828 x 1792 pixels). The photos were uploaded to the computer software, GIMP, to examine the area of defoliation. The defoliation was manually coloured pink on the photo editing software in order to make the damage drastically contrast with the healthy tissue. The pixels were separated into different colour shades using the R package Colordistance (Weller 2019) to group the multiple shades of green into one category. The proportion of all the pink pixels (damaged) to the green pixels (healthy) were computed to get an exact proportion damaged. Each cotyledon was measured across the widest point (length) and across the main vein (width) to obtain an estimation of the area of the cotyledon for efficiency. This measurement was taken due to the differences in size among temperature treatments from different rates of growth so I could obtain an unbiased estimation of the damage among the various sizes of cotyledons. The proportion damaged per cotyledon was multiplied by the estimated area of each cotyledon to determine the area damaged in mm^2. Defoliation was estimated separately for the upper and lower side of the cotyledons. The number of flea beetles alive were recorded.

A split-split plot ANOVA model was used to determine the effects of temperature (whole plot), flea beetle species (sub-plot), and the side of the cotyledon (sub-sub plot) on the area damaged per cotyledon per average flea beetle alive (mm^2), using trial as a random factor. Average flea beetle alive was calculated using the number alive at the start of the experiment (five) and the number alive after 48 hours, to correct for the slightly increased mortality in CFB at high temperatures when compared to SFB and the other temperature treatments (data not shown). Backward elimination of terms was completed with the use of AIC scores to determine the best model (Zuur et al. 2009). The main effect 'flea beetle species' was excluded from the model after no significant main or interacting effects were detected for this variable. Pair-wise comparisons between temperature treatments were done using estimated marginal means (EMM) adjusted by Bonferroni's method using the R package emmeans (Lenth 2023).

2.2.5 Experiment 2: Effect of temperature and plant density on canola damage

Five trials were conducted to test the effects of temperature and plant density on the intensity, prevalence, and overall damage to canola, between February 10 to March 30, 2021. Two constant temperature regimes (18℃ and 28℃ at 80 ± 5% RH) were combined in a factorial design with two flea beetle manipulation treatments (colony reared SFB and control with no flea beetles; CFB was not available at the time of this experiment), resulting in four treatments. The temperatures 18℃ and 28℃ were chosen because they were the most contrasting within the range of temperatures in southern Manitoba for the month of June (peak flea beetle emergence) (Government of Canada 2022) out of the four temperatures used in experiment 1. The two plant densities used were either five or ten canola plants per pot with either five flea beetles or none (control). Two reach-in growth chambers were used to accommodate the two temperatures, where each chamber received two replicates of each treatment for a total of ten replicates per treatment overall. All trials were conducted at 70% RH and a photoperiod of 16 L: 8 D.

After the 24-hour period, the flea beetles were removed and counted to measure survivorship. The highest mortality observed was 1 flea beetle and mortality did not show differences across treatments (mean ± SE flea beetles alive): Low PD/18℃ = 4.8 ± 0.1, low PD/28℃ = 4.7 ± 0.2, high PD/18℃ = 4.9 ± 0.1, high PD/28℃ = 4.8 ± 0.1. Thus, defoliation was not adjusted by the average number of flea beetles alive. Percent cotyledon defoliation was visually assessed from 0-100% at 5% intervals, a version of the scale used by Palaniswamy et al.

(1992) where 0% = no defoliation and 100% = no living tissue due to complete defoliation. This assessment was used instead of the digital method described in experiment 1 due to its efficiency and the fact that it is following the method used for typical canola scouting. Stem damage was assessed as described above. For overall cotyledon defoliation (%), cotyledon defoliation intensity (%), overall stem damage (mm^2) and stem damage intensity (mm^2), a two-factor ANOVA model was used with temperature and plant density as fix main effects and their interaction. To test for the effects on prevalence for both cotyledon defoliation and stem damage, a type 3 Wald chi-square test was used with a binomial distribution (attacked versus non-attacked).

2.2.6 Experiment 3: Predation assays in Petri dishes

To determine potential predators of flea beetles in canola, nine Petri dish trials took place from July 7 – September 13, 2020. Generalist predators were collected from nearby canola fields as described above, including *Agonum spp.* (Coleoptera: Carabidae), *Amara spp.* (Coleoptera: Carabidae), *Bembidion quadrimaculatum* (Coleoptera: Carabidae), *Calosoma spp.* (Coleoptera: Carabidae), *Harpalus amputatus* (Coleoptera: Carabidae), *P. melanarius, Alopecosa spp.* (Araneae: Lycosidae), *Pardosa spp.* (Araneae: Lycosidae), and *Pirata spp.* (Araneae: Lycosidae). One predator and three adults of each flea beetle species, SFB and CFB, were placed in plastic Petri dishes (14 cm diameter). Each dish was one replicate. The number of replicates for each predator varied per trial based on the number of individuals that were captured in the field. Each trial also contained predator-free control replicates to estimate defoliation levels in the absence of predation. Petri dishes were kept in a reach-in growth chamber at 23 ± 1°C during the day and 19 ± 1°C at night, 80 ± 5% RH, and a photoperiod of 16L: 8D. A lower temperature for the night period in this experiment and the following experiment was added to mimic more typical field conditions (Government of Canada 2022).

Each Petri dish contained two canola plants at the cotyledon stage that were cut from the soil so there was no root material in the assays. Each cut end of the stem was wrapped in half of a moistened cotton ball to provide water for the plant. Each dish was sealed with one layer of parafilm after the plants and insects were placed in the dish. Observations were taken after 24 and 48 hours to count the number of flea beetles alive and confirmed that the beetles were consumed by looking for remaining pieces of the exoskeleton such as elytra or legs. After 48

hours, the Petri dishes were put in the freezer for 5 to 10 minutes to remove the canola without the risk of flea beetles escaping. Cotyledon defoliation was visually assessed as described in experiment 2.

A one-way ANOVA (type III partial sum of squares for unbalanced number of observations) model was used to determine the effect of the predator taxa (main fixed effect) on the dependent variables described above using the trial number x growth chamber as a random factor. Pair-wise comparisons were done using estimated marginal means (EMM) to compare the treatments to the control adjusted by Bonferroni's method using the R package emmeans (Lenth 2023).

2.2.7 Experiment 4: Effect of predation and plant density on canola damage

Twelve trials took place from June 23 to September 15, 2021. Trials 1-7 consisted of a single plant density (three plants per pot). Following the observations of effective predation in the first seven trials, a higher plant density treatment (six plants per pot) was added to the experiment to examine its effects on flea beetle feeding for trials 8-12. There was no significant difference in defoliation (t = 1.08, df = 31, p = 0.30), stem damage (t = 0.62, df = 26, p = 0.55) or flea beetle abundance (t = -0.26, df = 31, p = 0.80) at the low plant density treatments between trials 1-7 and 8-12. Therefore, to increase replication all trials (1-12) were used for the low plant density treatment when compared to the high plant density treatment (trials 8-12; Fig. 8 and 10).

Single predators were added to the microcosms and the number of replicates for each species varied based on the number of predators collected each week. Each microcosm either contained three flea beetles of each species or no flea beetles for the flea beetle-free controls. The microcosms were divided equally between two reach-in growth chambers held at the same temperature regime. The day temperature was 22 ± 1°C and the night temperature was 18 ± 1°C with a photoperiod of 16L: 8D (75% RH). The temperatures were each about one degree lower than experiment 3 due to minor growth chamber fluctuations. Each trial lasted for 48 hours.

Cotyledons and stems were assessed for damage as described in experiment 2. Defoliation per average flea beetle alive was initially assessed the same way as described in Experiment 1 but showed no difference in treatment effects compared to assessing overall defoliation not adjusted by flea beetle alive (data not shown); therefore, overall defoliation (%) is presented in the results. When stems were too damaged to accurately measure the pit sizes, the

individual stems were removed from the final dataset. Alternatively, when an estimated maximum area for damage (6.27 mm^2, obtained by a linear regression between stem and cotyledon defoliation at 100% damage) was used for the overly damaged stems, similar results were obtained; therefore, the analysis proceeded with those values removed. For single plant density trials, a generalized linear mixed model was used with predator species as a main fixed effect and week as a random effect. To test for differences in plant density in the later trials, plant density was also added as a second main fixed effect as well as a predator x plant density interaction term. The model was adjusted using White's method to adjust for the heteroscedasticity of the residuals (White 1980). The correction is applied to the coefficient of covariance matrix. To determine differences between the treatments and predator-free control group, estimated marginal means were performed adjusted with the Bonferroni adjustment. *Pardosa spp.* was not included in the analyses that contained both plant densities due to the lack of individuals during trials 8-12.

2.3 Results

2.3.1 Experiment 1: Effect of temperature and flea beetle species on canola damage

CFB and SFB similarly increased their damage in response to temperature increase (Fig. 2.3a and b), and there was no significant difference in cotyledon defoliation between flea beetle species ($F_{1, 26.6} = 0.38$, $p = 0.542$; Fig. 2.3e), and no significant interaction with the other treatments (flea beetle species x temperature x side: $F_{3,112.2} = 0.16$, $p = 0.924$; flea beetle species x temperature: $F_{3,23.7} = 0.52$, $p = 0.671$; flea beetle species x side: $F_{1,115.2} = 0.30$, $p = 0.586$), and thus the flea beetle species treatment was removed from the final model. Higher temperatures significantly increased cotyledon defoliation ($F_{3,26.6} = 31.64$, $p < 0.0001$), with the most defoliation observed at 28°C, the least defoliation at 13°C, and intermediate levels at 18°C and 23°C (Fig. 3c). More defoliation was observed on the abaxial than on the adaxial side of the cotyledons ($F_{1,118.3} = 24.55$, $p < 0.0001$; Fig. 2.3d). Temperature did not affect which cotyledon side was damaged more by flea beetles (temperature x side: $F_{3,116.2} = 1.07$, $p = 0.36$).

2.3.2 Experiment 2: Effect of temperature and plant density on canola damage

Temperature and plant density have interactive effects on overall cotyledon defoliation ($F_{1,32} = 5.92$, $p = 0.02$), with more defoliation found at a lower plant density at 28°C but not at

18°C (Fig 2.4A). There was a higher prevalence of cotyledon damage with high temperatures (Wald $X^2 = 33.1$, $p < 0.0001$), but no difference due to plant density (Wald $X^2 = 2.86$, $p = 0.091$) or plant density x temperature interaction (Wald $X^2 = 1.52$, $p = 0.217$; Fig. 2.4B). When examining cotyledon defoliation intensity, there was an interaction between temperature and plant density ($F_{1,31} = 6.72$, $p = 0.014$), resulting from higher damage at a low plant density at 28°C but not at 18°C (Fig. 2.4C), and suggesting that the overall cotyledon damage (Fig. 2.4A) was driven by damage intensity.

There was a significant interaction between the temperature and plant density on the overall stem damage, resulting in more stem damage at a low plant density at 28°C but not at 18°C ($F_{1,32} = 4.47$, $p = 0.042$; Fig. 2.4D). Higher temperatures significantly increased both the prevalence (Wald $X^2 = 13.9$, $p = 0.0002$; Fig. 2.4F) and intensity ($F_{1,3} = 20.68$, $p = 0.020$; Fig. 2.4E) of stem damage resulting in more attacked plants and more damage per attacked plant at a higher temperature. Neither plant density (Wald $X^2 = 1.27$, $p = 0.260$) or temperature x plant density (Wald $X^2 = 3.05$, $p = 0.081$) affected the prevalence of stem damage. Similarly, plant density ($F_{1,28} = 1.01$, $p = 0.323$) and temperature x plant density ($F_{1,28} = 1.17$, $p = 0.288$) had no effect on stem damage intensity. The overall trend of overall stem damage (Fig. 2.4D) appears to be more heavily influenced by the prevalence of stem damage (Fig. 2.4E).

There was a significant correlation found between cotyledon defoliation and stem damage, showing a predictive relationship between the two types of damage ($p < 0.0001$, $R^2 = 0.64$, n = 40; Y = 0.063X + 0.034; Fig. 2.5).

2.3.3 Experiment 3: Predation assays in Petri dishes

Ground predators reduced the abundance of flea beetles in Petri dish trials ($F_{9,82.6} = 11.27$, $p < 0.00001$), with four taxa differing from controls: *H. amputatus.*, *P. melanarius*, *Pardosa spp.* and *Pirata spp.* (Fig. 2.6). Due to the limited capture rate of *Pirata spp.* in the following field season, we chose to focus our efforts into examining *H. amputatus, P. melanarius* and *Pardosa spp.* as predators on flea beetles in the following microcosm experiment (Experiment 4).

2.3.4 Experiment 4: Effect of predation and plant density on flea beetles

Similar to the results from the Petri dish trials (Experiment 3), *P. melanarius* and *Pardosa spp.* also reduced flea beetle abundances in microcosms with low plant density (trials 1-7) after 48 hours compared to the predator-free control group ($F_{3,106.6} = 15.10$, $p < 0.00001$; Fig.

2.7). *Pterostichus melanarius* also reduced the mean number of flea beetles at both high and low plant densities ($F_{2,197.9}$= 17.3, $p < 0.0001$; Fig. 2.8). By contrast, *H. amputatus* did not reduce flea beetle numbers in microcosms either at low (Fig. 2.7) or both high and low plant densities (Fig. 2.8). There was no interaction found between predator species and plant density ($F_{2,198.5} = 1.17$, $p = 0.31$).

When placed in microcosms at low plant densities with *Pardosa spp.* and *P. melanarius*, flea beetles produced less overall cotyledon defoliation ($F_{3,125.6} = 3.43$, $p = 0.019$; Fig. 2.9A) and damage intensity ($F_{3,116.6} = 4.96$, $p = 0.003$; Fig. 2.9C) when compared to the control group. *Harpalus amputatus* did not affect overall damage or damage intensity by flea beetles at low plant densities (Fig. 2.9A and C). No predators resulted in a decreased prevalence of cotyledon damage at a low density (Wald $X^2 = 6.02$, df = 3, $p = 0.111$; Fig. 2.9B). With *P. melanarius*, flea beetles produced less overall stem damage ($F_{3,91.2} = 5.97$, $p = 0.001$; Fig 2.9D) and stem damage intensity ($F_{3,116.0} = 4.47$, $p = 0.005$; Fig. 2.9F) than in controls, but other predators did not affect these variables. *Pardosa spp.* and *P. melanarius* resulted in lower prevalence of flea beetle damage on stems than in the control, but there were no effects of *H. amputatus* (Wald $X^2 = 19.74$, df = 3, $p < 0.001$; Fig. 2.9E).

A higher plant density reduced the overall mean cotyledon defoliation ($F_{1,101.6} = 19.39$, $p < 0.001$; Fig. 2.10A), prevalence (Wald $X^2 = 5.34$, df = 1, $p = 0.021$; Fig. 2.10B), and intensity of cotyledon defoliation ($F_{1,90.4} = 14.86$, $p = < 0.001$; Fig. 2.10C) when compared to the lower plant density. The presence of *P. melanarius* reduced the overall mean cotyledon defoliation ($F_{2,171.6}$= 2.99, $p = 0.05$), while *H. amputatus* did not (Fig. 2.10A). Predators did not reduce the prevalence (Wald $X^2 = 0.49$, df = 2, $p = 0.781$; Fig. 2.10B) or intensity of cotyledon defoliation ($F_{2,172.73} = 2.28$, $p = 0.106$; Fig. 2.10C). There were no interactions between plant density and predator treatments for overall mean cotyledon defoliation ($F_{2,170.8}$= 1.81, $p = 0.167$), prevalence (Wald $X^2 = 1.97$, df = 2, $p = 0.373$), and intensity of defoliation ($F_{2,172.3} = 1.66$, $p = 0.193$).

A higher plant density also reduced overall mean stem damage ($F_{1,52.1} = 17.5$, $p = 0.0001$; Fig. 10D) and intensity of stem damage ($F_{1,43.9} = 8.95$, $p = 0.005$; Fig. 2.10F). The presence of *P. melanarius* predators reduced the overall stem damage ($F_{2,171.6} = 11.3$, $p < 0.0001$; Fig. 2.10D) whereas *H. amputatus* did not. There were no interactions between plant density and predator treatments for overall mean stem damage ($F_{2,163.0} = 2.98$, $p = 0.054$) and intensity ($F_{2,162.9} = 0.25$, $p = 0.783$). There was a significant interaction between plant density and predator for the

prevalence of stem damage (Wald $X^2 = 9.96$, $p = 0.007$; Fig. 2.10E), due to reduced prevalence of stem damage only in high plant density treatments when *P. melanarius* was present (Fig. 2.10E). *Pterostichus melanarius* and *Harpalus amputatus* reduced the stem damage intensity ($F_{2,161.6} = 6.04$, $p = 0.003$; Fig. 2.10F).

2.4 Discussion

Species Differences

Our results demonstrate that both flea beetle species, SFB and CFB, produced similar levels of defoliation on canola and increased their feeding at higher temperatures. There have been efforts to determine feeding differences between these two species as they are prominent pests in North America. Based on the presence of flea beetles on plants and their damage, Palaniswamy and Lamb (1992) suggested that CFB caused about twice as much damage as SFB. In contrast, Gavloski and Lamb (2000c) reported no differences between CFB and SFB feeding on canola seedlings under controlled conditions. Tansey et al. (2009) reported that SFB fed more than CFB on canola seedlings from seeds treated with Thiamethoxam and Clothianidin but found no differences between untreated controls exposed to overwintered individuals of both species. Both species show also a similar pattern of feeding on cotyledons, which was slightly higher (< 1%) on the abaxial than the adaxial cotyledon surface, suggesting that field estimates typically conducted on the adaxial cotyledon surface may be sufficiently accurate to estimate damage. Although I do not have a reason to think it affects the results, it should be noted that SFB was lab-reared and CFB was field-collected, since I was unable to collect enough SFB from the field at the time of these experiments. Although there is one method described to rear CFB under laboratory conditions (Kinoshita et al. 1979b), I have not been able to maintain a colony of this species. Despite the potential for variability introduced by using field collected individuals, previous studies with flea beetles use them extensively to compare species and quantify their damage (e.g. Lamb and Palaniswamy 1990, Gavloski and Lamb 2000c, Soroka et al. 2011), and therefore our results should be confirmed by tests using field collected populations for both species, until a viable rearing method for CFB is developed. Despite this potential caveat, our results, suggest that both species of flea beetles produce similar levels of defoliation to canola seedlings, supporting previous results found under different experimental conditions.

Temperature consistently increased damage by both flea beetle species, with more than four times more feeding on cotyledons at 28˚C than at 13˚C. Our research shows that feeding usually increased with temperature (except for cotyledon damage intensity at high plant density), which is supported by previous studies showing that more flea beetles are found in canola and are more active with warmer temperatures (Toshova et al. 2009, Canola Council of Canada 2023) implying that more feeding on the cotyledon may take place. Since there are more plant volatiles released at increased temperatures (Dai and Lim 2014), flea beetles might be responding to this cue with an increased feeding rate on the cotyledon when compared to lower temperatures with less plant volatiles released. In addition, our findings show that there is no difference in per capita feeding between the two species regardless of the temperature, and that both species increased their damage to canola at high temperatures. Future studies should focus on examining the effects of temperature on damage ratings of flea beetles under field conditions, considering the abundance and species composition of the flea beetle populations present as abundances may be affected by different temperatures.

Higher temperature also increased the damage caused by SFB on canola seedling stems. Previous observations suggest that stem damage is more likely under cooler and windy conditions, based on the premise that flea beetles will be less active to climb on top the plant to feed on cotyledons under those conditions (Canola Council of Canada 2023). In contrast, we found that under higher temperatures flea beetles produce both a greater number of pits per stem and more overall damage on the stems. As we did not investigate wind effects on stem damage, or temperature effects on stem feeding by CFB, these remain to be tested in future experiments.

Increased damage to cotyledon and stems at higher temperatures occurred based on different mechanisms. Stem damage was primarily driven by the prevalence of flea beetle attack while cotyledon damage was primarily driven by the intensity of damage per attacked plant. This might be explained by the presence of short trichomes on the stem (Gulden et al. 2007) compared to the absence of trichomes on the cotyledons (Gruber et al. 2006). The differences in trichome abundance on different plant parts might affect the feeding preference when comparing stems and cotyledons. Soroka et al. (2011) found decreased flea beetle feeding with increased trichomes suggesting that flea beetles may feed briefly on stems but prefer feeding on cotyledons due to the absence of trichomes. In addition, SFB consumed an order of magnitude more cotyledon than stem tissue, suggesting a preference for cotyledon tissue, which may result in

longer periods of feeding on cotyledons than on stems and increasing damage intensity rather than prevalence. This knowledge of the main driving factors of both stem and cotyledon damage in canola has implications for flea beetle monitoring practices. Since stem damage is driven by the number of attacked plants, estimating stem damage prevalence (i.e., recording each canola stem as either damaged or not damaged), can be sufficient for efficient monitoring this type of damage. Furthermore, we found a strong correlation between cotyledon defoliation and stem damage, suggesting that current monitoring strategies based on defoliation estimates will not miss high stem damage. Future research should investigate the relationship of stem damage prevalence with environmental conditions and flea beetle abundance under field conditions to use this information to guide flea beetle management decisions.

Predators can have direct, consumptive impacts on herbivore populations that in turn may release plants from herbivore pressure, which is the measure of successful biological control (Costamagna et al. 2007). Potential predators of flea beetles, however, have seldom been studied, making the determination of different taxa that are capable of consuming flea beetles important groundwork in this field. While flea beetles are extremely abundant, well protected by chemical defenses, and seemingly hard to capture (Li et al. 2024), there have been several studies suggesting predation on flea beetles (Chiverton 1984, Ekbom et al. 2014). We expand these results by showing that *Pardosa spp.* and *P. melanarius* are efficient predators that are capable of significantly reducing the abundances of flea beetles in both Petri dishes and microcosm laboratory experiments. *Harpalus amputatus* and *Pirata* spp. only cause flea beetle mortality in Petri dishes, but *Pirata* spp. was not tested in microcosms, so its potential role remains to be explored in more realistic arenas. These results suggest that some of the common generalist predators found in canola fields can catch and consume flea beetles, therefore future research should expand these results by testing the gut content of field collected predators for flea beetle DNA in canola fields in the Canadian prairies.

The presence of *P. melanarious* and *Pardosa spp.* in microcosms resulted in reduced flea beetle survivorship and canola damage, suggesting that these predators can contribute to reduce flea beetle impacts on this crop. In addition to consumptive effects, predators can have negative non-consumptive impacts on herbivores, by increasing their stress, forcing them to consume less or poor-quality food, and generally decreasing their fitness, which could also cascade down to reduce plant damage (Schmitz et al. 2004, Thaler and Griffin 2008, Costamagna et al. 2013, Gurr

et al. 2017). In our results we observed a reduction of overall cotyledon damage by means of a reduction in the intensity of damage. The prevalence of cotyledon damage was not affected, however. This might be explained by increased activity (i.e. similar prevalence) combined with a reduction of feeding (i.e. lower intensity) due to stress cause by the presence of predators, which have been observed in other Chrysomelid species (Snyder and Wise 2000, Williams et al. 2001). *Pterostichus melanarius* reduced both the prevalence and intensity of stem damage whereas *Pardosa spp.* reduced the prevalence of stem attacks but had no effect on the overall damage levels. A reason for this might be due to a difference in foraging behaviours between the predators. It was observed throughout the predator assays that *P. melanarius* was more active than spider predators during the time that the flea beetles were active. *Pterostichus melanarius* also displayed active patrolling around the bases of stems, plant climbing attempts, and vertical tunneling in the soil at the base of a plant with quick and targeted emergence if the plant was disturbed. This difference in foraging behaviour between predator taxa might account for the differences in damage reduction patterns. When predators are active on and around plants, prey species tend to exhibit vertical plant migration to avoid predation (Magalhães et al. 2002, Lee et al. 2014), which will result in upward movement onto the cotyledons rather than remaining on the stem. Reductions of flea beetle damage in predator treatments were higher at increased plant densities, which suggest that increasing plant density can enhance canola protection by predators. Follow-up studies including a non-consumptive predator treatment should be conducted to tease apart consumptive and non-consumptive effects of predators on flea beetles and their damage to canola.

In summary, my studies show that both flea beetle species, SFB and CFB, increase feeding of canola at higher temperatures. The increase of stem feeding is mainly driven by the prevalence of attacks while the increase of cotyledon feeding is driven by the intensity of damage. I found no differences in feeding preferences or area consumed between the two flea beetle species, suggesting that monitoring their overall abundance is sufficient for management purposes, but if insecticidal seed treatments are used care must be taken to monitor SFB abundance when certain products are used (Tansey 2019). We also found that the generalist predators *P. melanarius* and *Pardosa spp.*, can reduce the abundance of flea beetles and their damage to canola seedlings under controlled conditions, particularly under higher plant densities for *P. melanarius*. Damage reduction can be mostly attributed to direct consumption of flea

beetles, but there is also evidence of non-consumptive effects mediated by predator behaviour that deserve follow-up studies. The ability of *P. melanarius* and *Pardosa spp.* to reduce flea beetle abundance and canola damage in laboratory studies does not automatically relate to their ability to control flea beetle populations in the field, however. Factors in the field that may affect these generalist predators' ability to control flea beetles may include foraging behaviour, pest density, predator density, presence of other suitable prey populations and activity levels (Barney and Pass 1986, Clark et al. 1994). Further studies under field conditions need to be conducted to determine the potential of increasing generalist predator populations in the field, using conservation biological control approaches, in combination with other agronomic techniques, such as increasing seeding rates, to reduce flea beetle damage to canola crops.

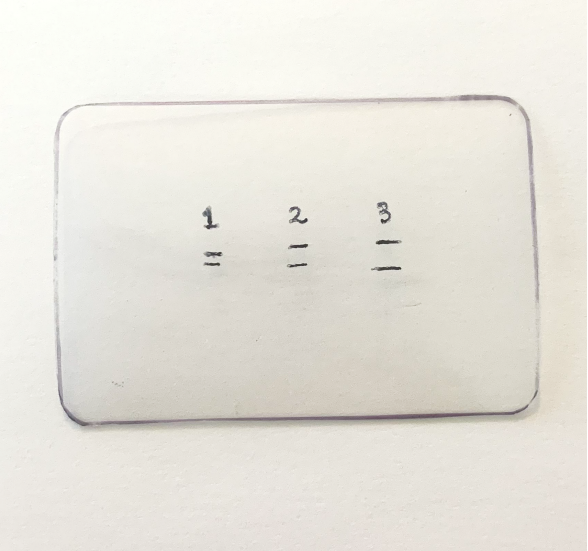


Figure 2.1. Stem estimation tool showing the three size groups (1, 2, or ≥ 3 mm).

Figure 2.2. Microcosm set up consisting of circular plastic pot (950.5 cm^3) and a plastic covering made from the top portion of a 2L plastic pop bottle (23 cm height x 11 cm diameter).

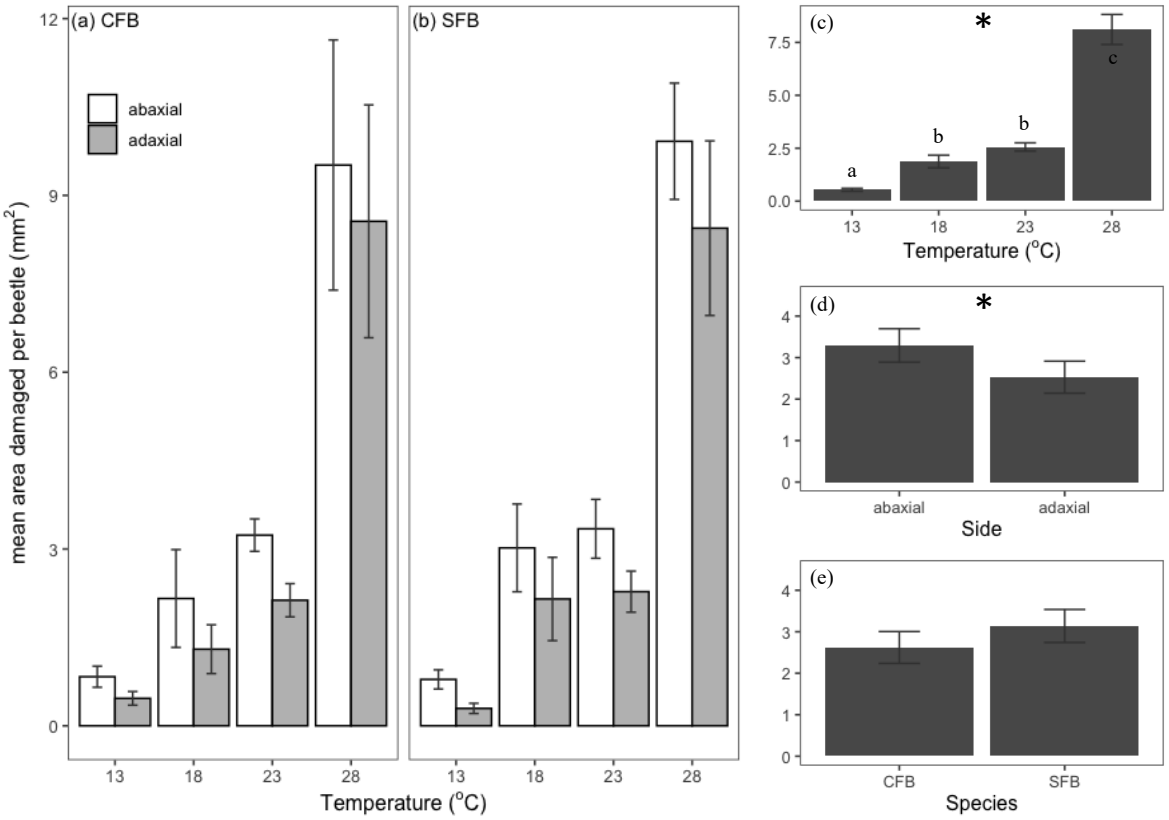


Figure 2.3. Mean canola cotyledon defoliation adjusted per living flea beetle separated by species where (a) CFB = crucifer flea beetle and (b) SFB = striped flea beetle, and main effects of (c) temperature, (d) side of cotyledon (abaxial = under, adaxial = upper) and (e) species. Microcosm study with 5 flea beetles and 2 canola plants in each. Measurements taken after 48 hours. Error bars = ± SEM. Asterisks represent significant main effects. Different letters on bars indicate significant differences between temperatures assessed using estimated marginal means (EMM) adjusted by Bonferroni's method; $\alpha = 0.05$).

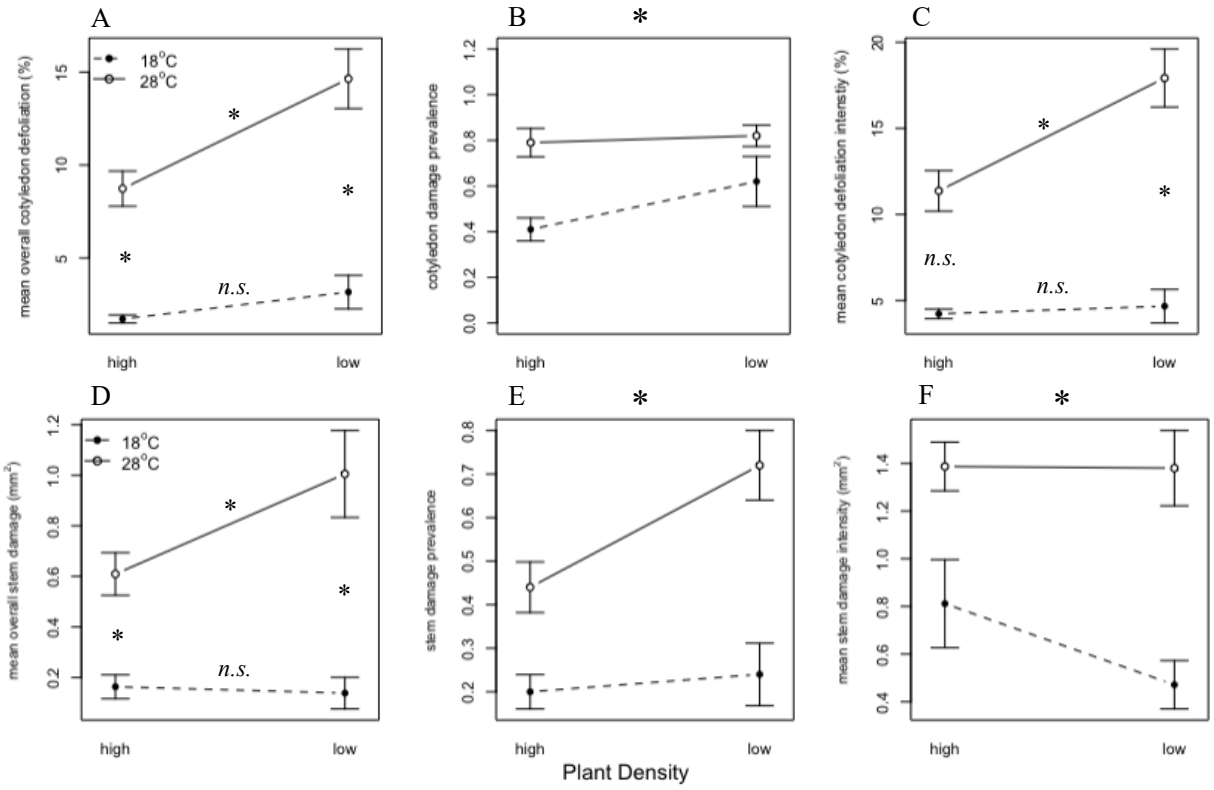


Figure 2.4. Mean cotyledon damage in terms of (A) overall, (B) prevalence, and (C) intensity of damage and mean stem damage in terms of (D) overall, (E) prevalence, and (F) intensity per canola seedling. Treatments consist of either high plant density (10 plants per pot) or low plant density (5 plants per pot) at a temperature of either 18℃ or 28℃. Trials took place over 24 hours with 5 striped flea beetles per pot. n = 10 per treatment combination. Error bars = + SEM. Asterisks on or between the lines indicate significant differences using EMM ($p < 0.05$); asterisks above the plot indicate temperature as a significant main effect ($\alpha = 0.05$), non-significant relationships indicated with n.s.

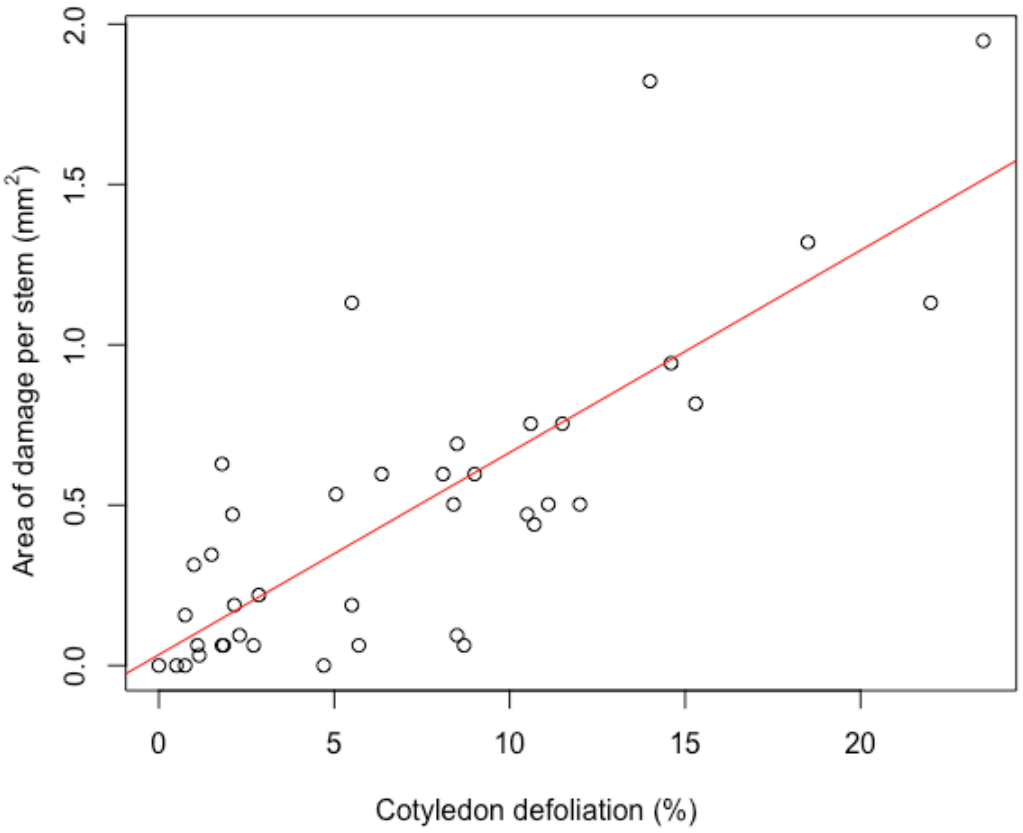


Figure 2.5. Regression of estimated pit size per plant (mm^2) compared to the defoliation of the upper side of the cotyledons per plant (n=40). Plants per pot were either 5 or 10 plants with 5 *Phyllotreta striolata* individuals for 24 hours.

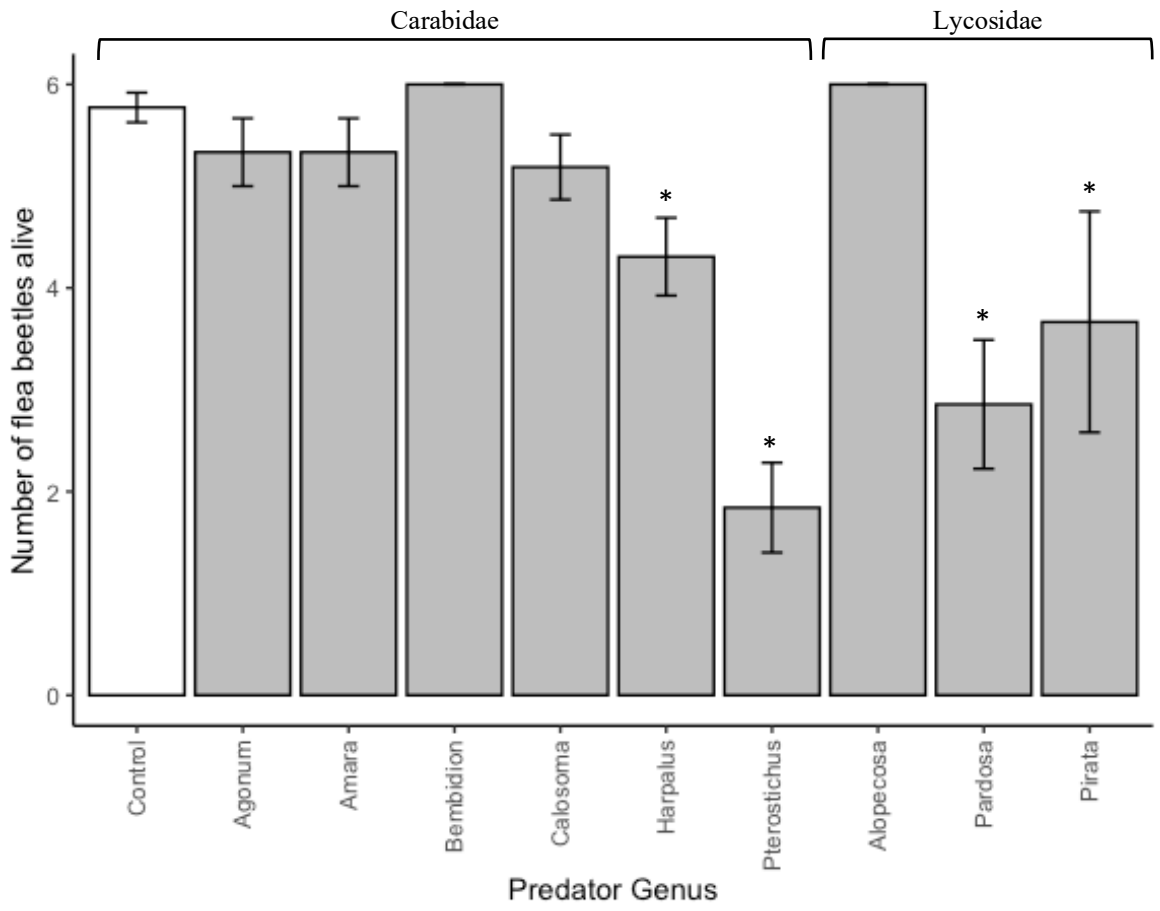


Figure 2.6. Mean number of flea beetles alive after 48 hours with a variety of predator genera from the families Carabidae and Lycosidae. Each replicate was 1 Petri dish (14 cm diameter) with 3 individuals of each flea beetle species (*P. cruciferae* and *P. striolata*), 2 canola plants at the cotyledon stage and 1 respective predator. Control = predator-free flea beetle treatment. Error bars = $\pm$ SEM. Asterisks represent a significant difference in contrast to the control group (EMM; $\alpha = 0.05$).

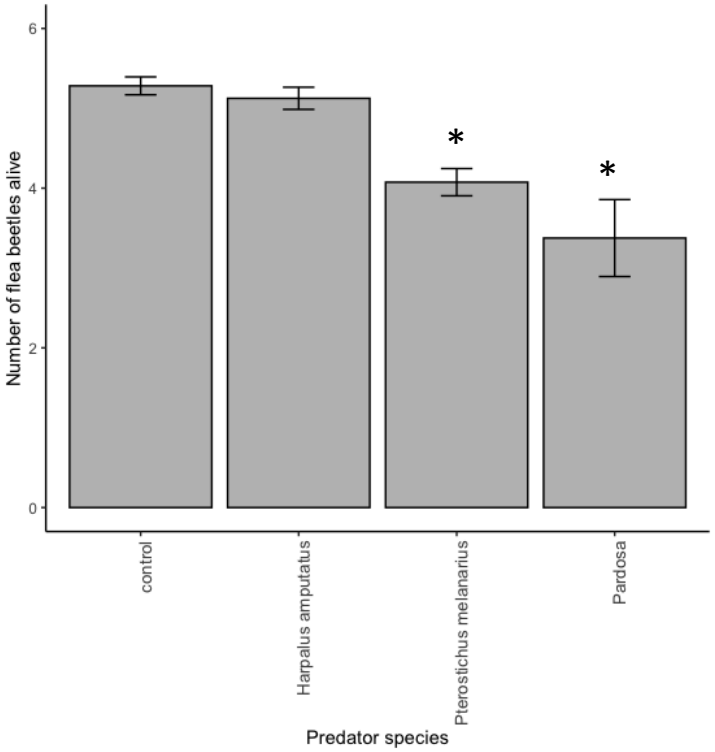


Figure 2.7. Mean number of flea beetles alive after 48-hour trials with a variety of predator species. Each microcosm contained 3 individuals of each flea beetle species (*P. cruciferae* and *P. striolata*) and 3 canola plants at the cotyledon stage. Control = predator-free flea beetle treatment. Error bars = $\pm$ SEM. Asterisks represent a significant difference in contrast to the control group (EMM; $\alpha = 0.05$).

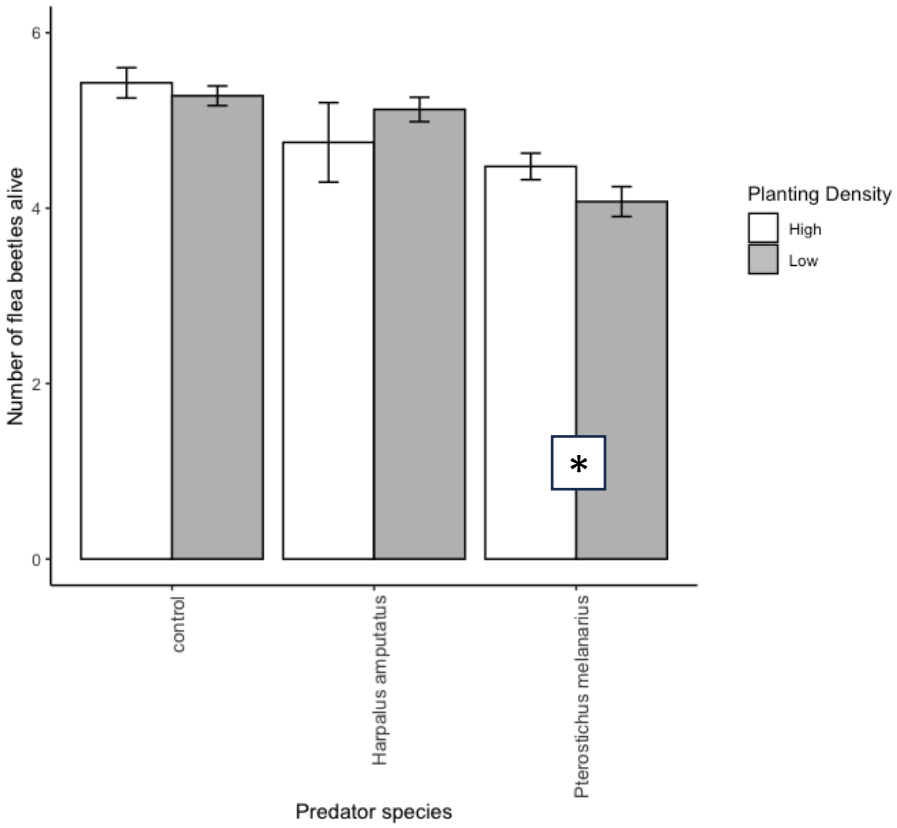


Figure 2.8. Mean number of flea beetles alive after 48-hour trial with different predator species based on plant density. Each microcosm contained 3 individuals of each flea beetle species (*P. cruciferae* and *P. striolata*) and either 6 (high) or 3 (low) canola plants at the cotyledon stage. Control = predator-free flea beetle treatment. Error bars = $\pm$ SEM. Asterisks in squares represent a significant difference between predator species main effect in comparison to the control group (EMM; $\alpha = 0.05$).

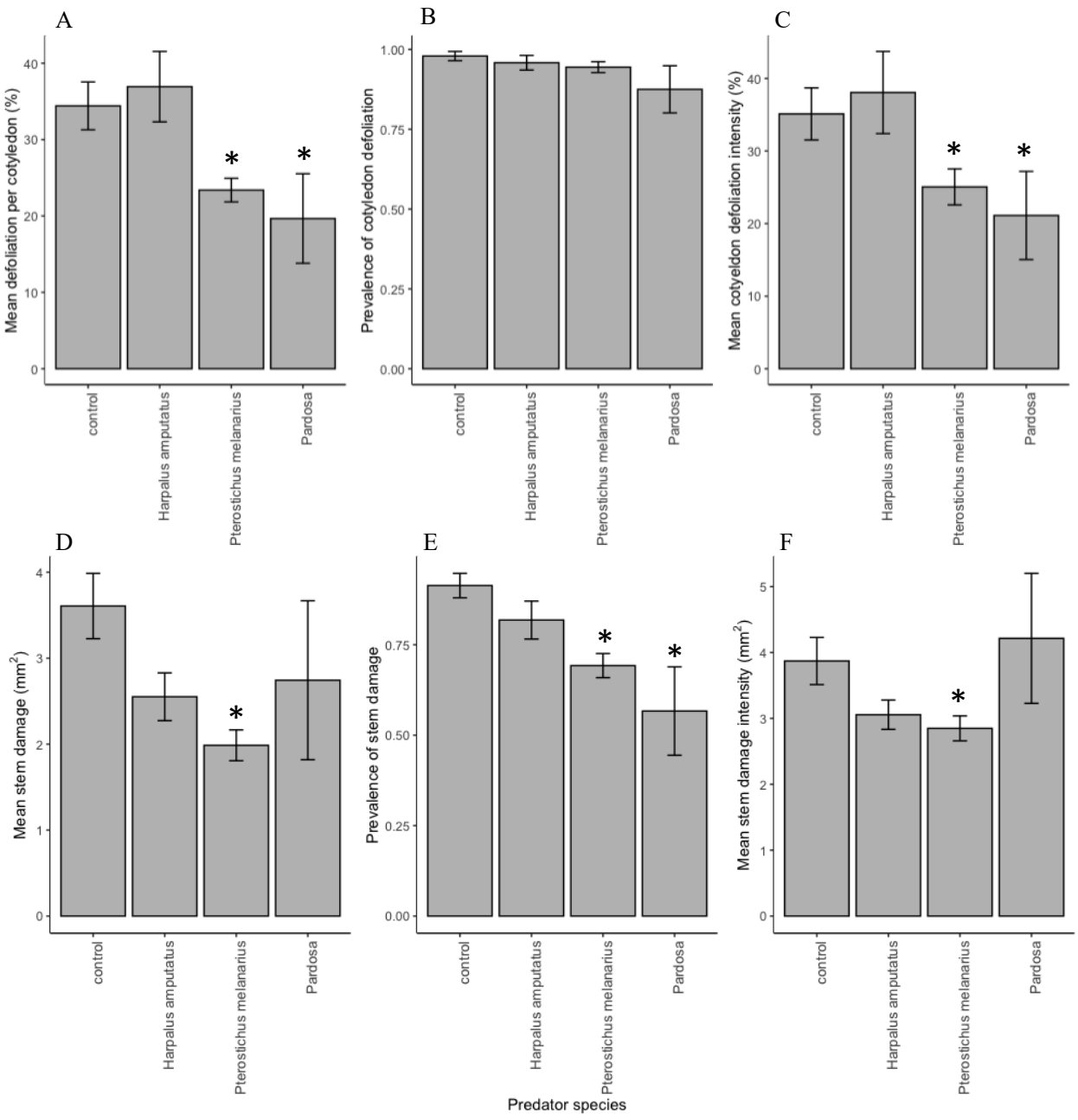


Figure 2.9. Mean cotyledon damage in terms of overall (A), prevalence (B) and intensity (C) of damage and mean stem damage in terms of overall (D), prevalence (E) and intensity (F) per canola seedling based on predator treatments. Control = predator-free flea beetle treatment. Each microcosm contained 3 individuals of each flea beetle species (*P. cruciferae* and *P. striolata*) and 3 canola plants at the cotyledon stage for 48 hours. Error bars = $\pm$ SEM. Asterisks represent a significant difference between predators in comparison to the control (EMM; $\alpha = 0.05$).

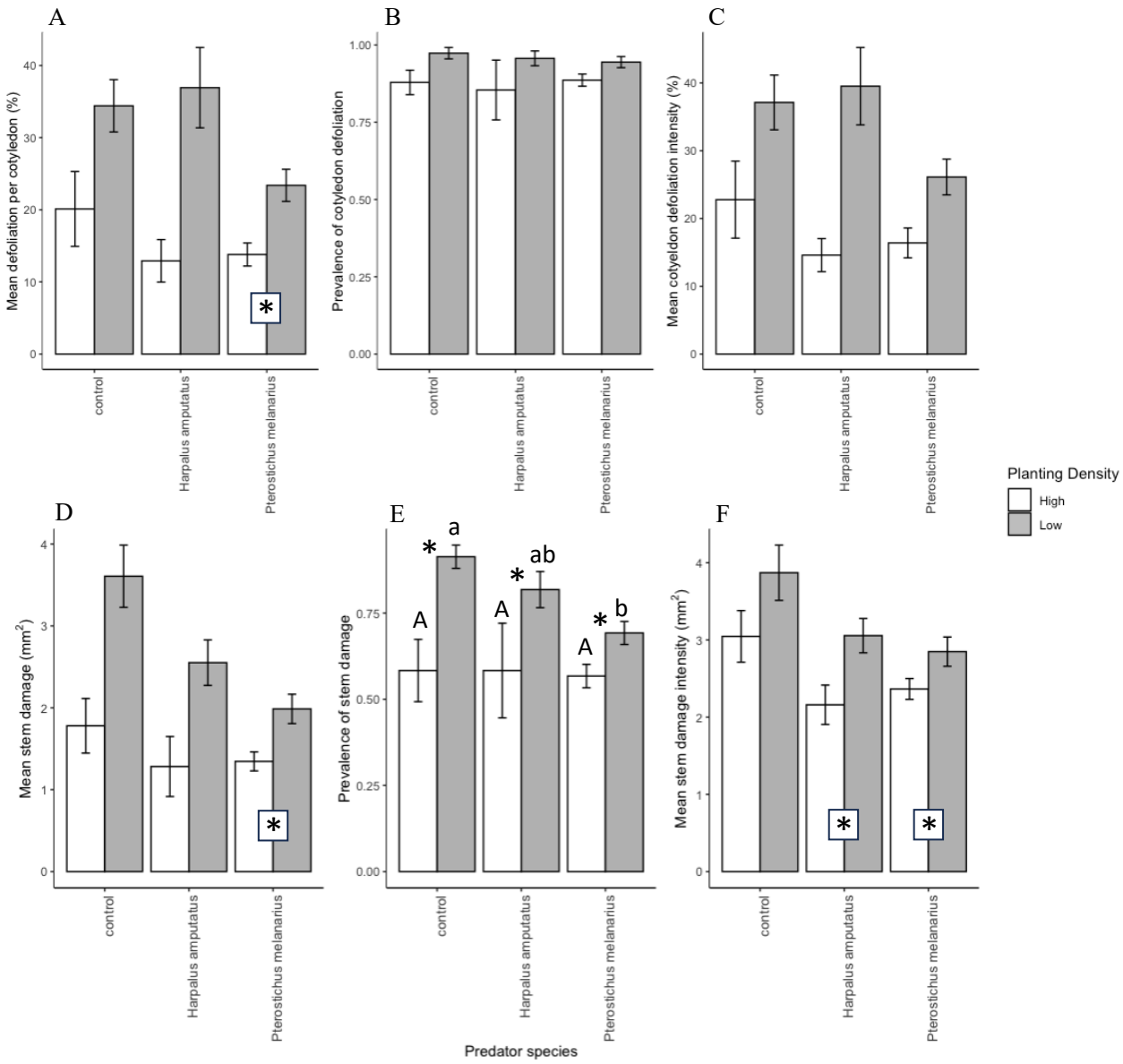
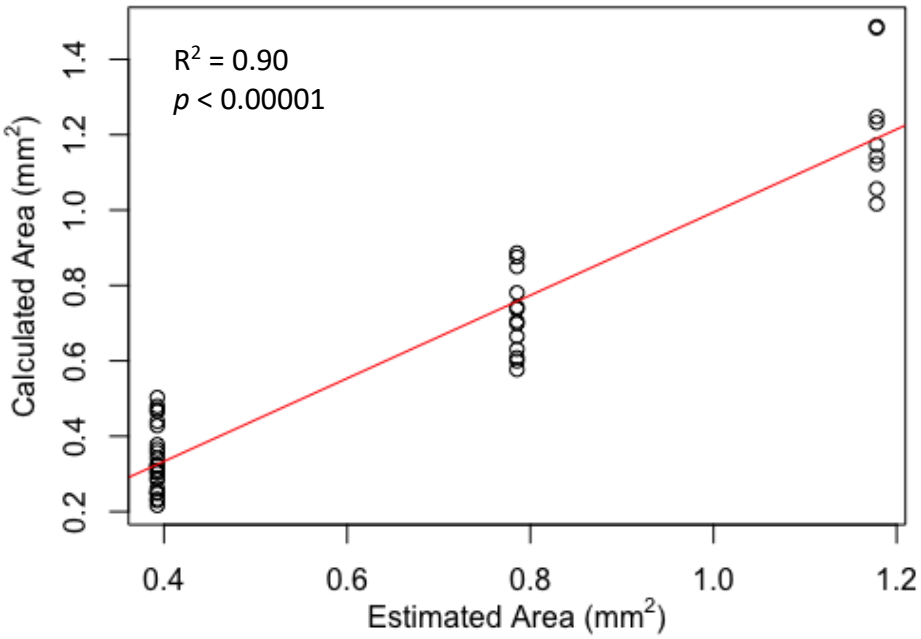


Figure 2.10. Mean cotyledon damage in terms of overall (A), prevalence (B) and intensity (C) of damage and mean stem damage in terms of overall (D), prevalence (E) and intensity (F) per canola seedling based on planting density and predator treatments. Control = predator-free flea beetle treatment. Each microcosm contained 3 individuals of each flea beetle species (*P. cruciferae* and *P. striolata*) and either 6 (high) or 3 (low) canola plants at the cotyledon stage for 48 hours. Error bars = ± SEM. Asterisks in squares represent a significant difference between predators in comparison to the control group (EMM; $\alpha = 0.05$). Significant effect in plant density found in figures A, B, C, D, and F but not presented. Capital letters above the bars

show significant differences within low plant density and lowercase letters show significant differences within high plant density (EMM slicing interaction analysis, $p < 0.05$).



Appendix I. Correlation between estimated and measured pit areas (n = 45). Y = 1.10X – 0.11. The estimated area was estimated by the oval equation with the use of a measuring tool to estimate the length of each pit to the closest mm and a constant width of 0.5 mm. The measured area was obtained using the pixel size converted to the exact area of each pit in the photo editing software GIMP.

Preface to Chapter 3

In Chapter 2, I explored the different ways that biotic and abiotic factors affect the feeding behaviour of *Phyllotreta* flea beetles in canola seedlings. I found that *Pterostichus melanarius* and *Pardosa sp.* as predators can reduce the abundances of flea beetles in a microcosm. I also found that *P. melanarius* results in reduced cotyledon and stem damage by flea beetles. In addition, I found that an increased temperature and a lower planting density result in more flea beetle-mediated damage to both the stems and the cotyledons. Contrary to my hypothesis, I did not observe a difference in feeding preference or defoliation rates between *P. cruciferae* and *P. striolata* at any temperature. Lastly, I found that the novel stem damage estimation technique is an accurate way to measure flea beetle stem damage in canola seedlings. Identifying predators of flea beetles, improving damage estimation on canola stems and understanding multiple factors affecting flea beetle feeding are all necessary for improved field monitoring for the protection of canola crops and improved pest management. My objectives for Chapter 3 were to determine how different seeding rates of canola and application rates of foliar insecticide will affect the density of flea beetles, plant damage, and yield of canola in a field study. In small plot experiments across the Canadian prairies over four years, I associated different insecticide application methods and seeding rates to canola defoliation and in-field flea beetle populations. Understanding how different pest management techniques will affect the outcome of canola crops as well as the effect they have on flea beetle presence and feeding will allow for us to improve our management techniques as we focus on an integrated method to manage flea beetles in canola.

CHAPTER 3. COMBINED EFFECTS OF PLANT DENSITY AND CHEMICAL MANAGEMENT STRATEGIES ON FLEA BEETLE ABUNDANCES, PLANT DEFOLIATION AND YIELD OF CANOLA ACROSS CANADIAN PRAIRIES

3.0 Abstract

Both the crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (CFB), and the striped flea beetle, *Phyllotreta striolata* (Fabricius) (SFB) (Coleoptera: Chrysomelidae), are devastating pests in canola (*Brassica napus* (L.)) in North America. Currently, the main tool used by to farmers in North America to control flea beetles is to prophylactically apply insecticides as seed treatments, which results in a high number of fields treated with insecticides under non-damaging levels of flea beetles. Moreover, this method of control often fails to suppress damage, resulting in the need to apply foliar sprays of insecticides or replant the fields. We investigated the effect of increasing canola density as an alternative method to reduce flea beetle defoliation and insecticide use in canola. We conducted fifteen replicated field trials in four regions of the Canadian prairies testing the effects of three planting densities combined with two flea beetle management treatments (seed treatment and foliar spray) and two controls (flea beetle free treatment and untreated control) from 2018-2021. Although we found an increased number of flea beetles as plant density increased, as predicted by the resource concentration hypothesis, this was offset by a dilution at the plant level, and defoliation levels did not increase at high plant densities. In addition, using seed treatments as a management strategy produced similar results to using only foliar spray at the 25% injury threshold when examining flea beetles per plant and total yield. More importantly, yield increased with increased plant density regardless of flea beetle abundance, management treatment, and region. We conclude that increasing plant density as an alternative or complementary management strategy to chemical control is effective to protect canola yield.

3.1 Introduction

Flea beetles (Coleoptera: Chrysomelidae) are one of the most devastating pests of canola across North America as they are responsible for more than 10% of canola yield losses each year (Lamb and Turnock 1982, Knodel 2017). The two common species found across the Canadian prairies are the crucifer flea beetle, *Phyllotreta cruciferae* (Goeze), and the striped flea beetle, *P. striolata* (Fabricus). Peak emergence of overwintered flea beetles happens early in the growing season, which coincides with the vulnerable seedling stage of the canola causing great

defoliation to the growing crop and reducing yields significantly (Lamb and Turnock 1982). Feeding damage from *Phyllotreta* species has been estimated to cause approximately $300 million USD in losses in North America each year (Knodel and Olson 2002). Due to the high economic losses these pests can cause, management practices are vital to protect the growing canola plants.

The current management practices for flea beetles in North America focuses mainly on the use of systemic insecticide-coated seeds in over 90% of canola crops in the Canadian prairies (Soroka et al. 2008). Chronic neonicotinoid use can lead to unintended negative effects on natural enemies and pollinators (Desneux et al. 2007, Douglas et al. 2015, Knodel 2017) and other organisms in the environment (Morrissey et al. 2015). The use of neonicotinoid insecticides as canola seed treatments has been banned in the EU (European Commission 2019). This act has put pressure on Canada to introduce their own bans and even though there are partial bans in some provinces (Ellis 2018), the government eventually decided to hold off on banning neonicotinoids as an insecticide (Government of Canada 2020). There is still pressure to either reduce the use of this chemical or to ban the use of it to decrease ecological damage (Klingelhöfer et al. 2022), however, so discovering alternative methods for flea beetle management is vital.

The efficacy of some neonicotinoid active ingredients to control the different flea beetle species attacking canola in Canada differ. Tansey et al. (2008) has reported higher tolerance of SFB to the two-most common seed coating neonicotinoids used in Canada (thiamethoxam and clothianidin) compared to CFB regardless of the amount of crowding on the plants. CFB responds differently to these two insecticides as a seed treatment in their host plant by a reduction in feeding and a higher mortality when compared to SFB (Tansey et al. 2008). SFB feeding was only able to be reduced by high application rates of the neonicotinoid compounds, whereas CFB has demonstrated to be more sensitive particularly when thiamethoxam interacts with overwintering stressors on the species resulting in mortalities nearing 100% (Tansey et al. 2008, 2009). The increase on the relative abundance of SFB observed recently in some regions of the Canadian prairies could be explained at least in part by its lower susceptibility to some of the neonicotinoid seed treatments (Soroka et al. 2018). There is a need to find alternative management strategies for canola in the Canadian prairies due to the environmental impacts that

neonicotinoids have on non-target organisms in conjunction with the lower efficacy for controlling SFB.

In addition to insecticide-coated seeds, flea beetle management also relies on foliar insecticides applied to the canola to target feeding adults. Foliar insecticide should be applied when the defoliation reaches or exceeds 25% as an average injury level across the field to increase the yield while minimizing the number of pesticide applications (Ekbom 2010, Tangtrakulwanich et al. 2014). In Sweden, Lundin (2020) found that an injury level of 25% may be too high, especially with the inexpensive control method of foliar pyrethroid spraying. Lundin's calculated economic injury level, using various crop prices and insecticidal treatments, ranged from 8 – 16% (2020). Even with the higher 25% injury threshold in place, foliar insecticide treatments are likely applied more excessively than what is needed, which also could result in unwanted detrimental effects to other beneficial insects (Dosdall and Stevenson 2005), and the development of pest resistance (Højland et al. 2015).

Integrated pest management (IPM) strategies are needed to reduce the dependence canola growers have on chemical control. Effective and sustainable control of pest populations in an agriculture system may stem from the management of landscape composition, including manipulating edge density or availability of host plants (Boetzl et al. 2023). These bottom-up manipulations can promote a system to disrupt pest life cycles and reduce pest survival, change pest behaviour, and affect pest interactions with their host plants (Han et al. 2022). Bottom-up effects are considered important factors to enhance IPM and often includes common cultural control methods such as tillage, cover crops and floral strips. Other cultural control methods such as increasing the seeding rate have been successful in previous studies to reduce flea beetle defoliation per plant in canola but are thought to only be truly successful in conjunction with chemical control (Dosdall et al. 1999, Dosdall and Stevenson 2005). Managing flea beetle pressure with the use of cultural control methods such as increasing the seeding rate has been explained by the dilution effect, where there will be less damage to each plant with a higher plant density due to a greater amount of canola tissue to flea beetle ratio (Dosdall and Stevenson 2005, Knodel 2017). By contrast, the resource concentration hypothesis predicts that there would be a greater abundance of flea beetles in more dense patches of host plants (Hambäck and Englund 2005), which could result in a similar or higher number of flea beetles per plant than in low density plant patches. The effect of plant density on flea beetle abundance and their relationship

with defoliation and canola yield have not been previously examined over multiple years and regions across the Canadian prairies.

This study aims to investigate the effects of bottom-up manipulation on flea beetle interactions with their host plant and fill our knowledge gaps on the combined effects of established management practices with different seeding rates. Specifically, we tested how flea beetle management practices such as foliar spray at the 25% defoliation level and seed-coated neonicotinoid treatments in conjunction with three seeding rates affect (1) flea beetle aggregation at the patch level, (2) flea beetle pressure at the plant level, (3) defoliation levels, and (4) canola yield. We predict that (1) flea beetle abundances will be greater while (2) flea beetle pressure per plant will be lower in plots with a higher plant density compared to a lower density. We also predicted that due to the lower flea beetle pressure per plant, (3) there will be lower defoliation levels in plots with a higher plant density and in turn, (4) a higher yield in those plots compared to plots with a lower plant density.

3.2 Methods

3.2.1. Study Sites and agronomic practices

Small-plot canola trials took place, which consisted of two sites with 48 plots each were set up in four regions in western Canada: (i) Peace River, Alberta (PR) (n = 132 plots), (ii) Lethbridge, Alberta (LB) (n = 120 plots), (iii) Carman, Manitoba (MB) (n = 264 plots), and (iv) Saskatoon, Saskatchewan (SK) (n = 144 plots) in a randomized complete block design. The trials were completed in 2018, 2019, 2020 (LB) and 2021 (MB, PR, and SK). All study sites except Lethbridge had to postpone the trials from 2020 to 2021 due to differing COVID 19 limitations. The Saskatoon location has no data from 2018 and only includes 2019 and 2021 due to methodology constraints. There were 12 unique treatments randomized in four blocks resulting in four replicates per treatment. The seeding rates used were either a low seeding rate (3.5 kg/ha), optimum seeding rate (7 kg/ha), and a high seeding rate (14 kg/ha). At each plant density, there were the following insecticide treatments: (i) control plots with fungicide only treated seeds (i.e. control) (n = 180 plots), (ii) seed treatment with insecticide and fungicide treated seeds (i.e. seed) (n = 180 plots), (iii) foliar insecticide spray at 25% defoliation with fungicide only treated seeds (i.e. foliar) (n = 180 plots) and (iv) weekly foliar insecticide spray with fungicide only treated seeds to achieve low levels of flea beetle defoliation (i.e. FB-free) (n = 120 plots). The

treatment “FB-free” is added to estimate the maximum canola yield achieved by zero to very low levels of flea beetle defoliation. The Lethbridge site completed the FB-free treatment in 2019 only. The seed cultivar used was SY 4135 (mid-season Roundup Ready® hybrid canola, germination 99%; Syngenta). Fungicide only treatments (control plots) contained Vibrant Flexi at 600 mL/ 100 kg (difenoconazole, metalaxyl-M, sedaxane, fludixonil; Syngenta). In 2018, fungicide plus insecticide treated seeds contained Visivio (sulfoxaflor, thiamethoxam, difenoconazole, metalaxyl-M, fludioxonil, and sedaxane; Syngenta) and Fortenza (cyantraniliprole; Syngenta). In all other years, fungicide plus insecticide treatments contained Helix Vibrance at 1500 mL/ 100 kg (thiamethoxam, difenoconazole, metalaxyl-M, fludioxonil, sedaxane; Syngenta), Rascendo at 400 mL/ 100 kg (sulfoxaflor; Syngenta), and Fortenza at 500 mL/ 100 kg (cyantraniliprole; Syngenta). The foliar insecticide spray used for both foliar and FB-free treatments was Decis 5 EC (deltamethrin - 50 g/L; Bayer CropScience) at 24.3 mL/ha for all trials, except for Saskatchewan 2019 which used Matador 120 EC (lambda-cyhalothrin - 120 g/L; Syngenta) at 34 mL/ac.

3.2.2. Sampling measures

Each plot contained one sticky card (18 x 14 cm; Alpha Scent Inc.) placed in the middle to monitor flea beetle populations that was replaced weekly until the canola reached the four true-leaf stage (2.4; 2-3 weeks). Plant density was measured by counting the number of canola plants in three permanent 1-m linear transects at four separate times: (i) 50% canola emergence, (ii) cotyledon stage (1.0), (iii), two true-leaf stage (2.2) and (iv) before harvest. The average number of plants per 1-m linear transect was used to compare plant survivorship between treatments.

Flea beetle defoliation was assessed two times a week on 20 plants per plot by visual percent damage assessment until the 2.4 stage. The defoliation was assessed for the plants’ two cotyledons and two true leaves, with each cotyledon or leaf assessed individually. The first ten plants were assessed in a randomly selected row in the front of each plot, and another ten consecutive plants in another randomly selected row in the back of the plot. In 2020, due to COVID 19 restrictions, canola ratings were completed by assessing photographs of randomly selected rows for each plot in Lethbridge, AB. The visual ratings used either the 0-10 scale (Palaniswamy et al. 1992) where 0 = no defoliation and 10 = entirely defoliated (Lethbridge, AB) or an adapted version using percent damage where 0% = no defoliation and 100% = entirely

defoliated (MB, SK, and AB Peace River). Plant phenology was recorded according to Harper and Berkenkamp (1975) so that assessments and insecticide applications could be ceased at the 2.4 stage. The seeding dates and insecticide spray dates for each trial are summarized in Table 3.1.

Weed control of the plots consisted of hand weeding as needed. Canola was inspected for damage from other pests or animals such as aphids and deer. Herbicide (glyphosate (540 g/L) at 0.33 L/ac) was to be applied when needed at a maximum of twice per site before the 2.6 stage (6 true leaves). Canada thistle was present at both Lethbridge sites, so the glyphosate treatment was tank-mixed with Lontrel 360 at 113 mL/ac and applied once after the 2.2 stage (2 true leaves).

At maturity, the plots were swathed and harvested. The seed was cleaned, and the total seed weights were measured for each plot. The final yield for each plot was estimated as a measure of kg/ha based on the total seed weight per plot.

3.2.3. Data analysis

The variables of interest were plant number, total flea beetle abundance, flea beetle abundance per plant, defoliation (%) per plant, and yield. The total flea beetle (FB) abundance is a sum of the counted SFB and CFB individuals per sticky card per day to adjust for the varying trapping durations. The two species were combined since they both show the same general trends in their response to the treatments (Appendix II and III) and the defoliation caused cannot be attributed to a specific species, but rather combined effect of both species. A measure of FB per plant was determined by weighting the total number of FB per sticky card by the mean number of canola plants in a 1-m linear transect per plot as a proxy for the flea beetle pressure on each plant. Defoliation per plant is an average of the defoliation found on the two cotyledons and the first two true leaves (Soroka and Grenkow 2013) of 20 randomly chosen plants per plot twice a week. Canola yield was estimated as kg/ha according to the measured seed weight and plot size, and then standardized at 8.5% moisture.

All variables were analyzed using generalized linear mixed effects models and Type III ANOVA with block and plot as random effects ("lmerTest" and "car" packages in R (Kuznetsova et al. 2017, Fox and Weisberg 2019)). The independent variables used were region, insecticide treatment, plant density, and their two- and three-way interactions. Year and plot size were both used as covariates. All variables besides yield were transformed to meet the ANOVA assumptions as follows: square root transformation (defoliation) and log transformation (plant

number, total FB abundance and FB per plant). To minimize the unwanted variability from plot size, environmental conditions, and any other variability associated with each trial, the data were centred and scaled per trial for all variables examined. The final models were chosen using a manual backward selection procedure starting with triple interaction terms between the independent variables and the non-significant interaction terms with the highest *p* values were removed one by one until the final model was retained (Table 3.2). All individual explanatory variables were kept in the final model. All post-hoc multiple test comparisons were done using estimated marginal means with the Šidák correction for all significant interactions using the "emmeans" package in R (Lenth 2023). Unscaled values averaged at the treatment level are shown in the Appendix.

3.3 Results

3.3.1 Plant number

On average, seeding rates successfully manipulated plant density (PD), with an average (mean ± SE) plant number per metre of 30.3 ± 0.7, 16.5 ± 0.4, and 9.5 ± 0.3 on the high, optimum, and low plant density treatments, respectively. When examining the mean numbers of plants per linear metre transect, a three-way interaction was found between region, FB management, and plant density (Table 3.3). All treatments per region showed a significant increase in plant number as plant density increased except for the seed treatment in SK where low and optimum plant densities resulted in a similar number of plants, which is likely driving the interaction (Appendix II). There were a few differences among some FB management treatments in all regions but PR, but not in a consistent way that may have affected our results.

3.3.2 Flea beetle abundance

In general, increasing PD resulted in a higher abundance of flea beetles, and on average low PD plots had 88.3 ± 6.1 FB/ sticky card (72.4 ± 5.7 CFB, 17.4 ± 1.5 SFB), optimum PD plots had 111.5 ± 7.2 FB (89.2 ± 6.3 CFB, 22.5 ± 1.9 SFB), and high PD plots had 135.8 ± 9.4 FB (107.9 ± 7.8 CFB, 28.5 ± 2.7 SFB). The treatment combination with the lowest FB abundance was low PD with foliar treatment (81.8 ± 11.3) and the greatest abundance was high PD control (162.7 ± 20.5). Raw means and SE for each PD, FB management treatment and region are provided in Appendix V. In general, within FB management treatments, a higher PD

consistently increased FB abundances, but a two-way interaction between FB management and PD treatments (Table 3.2) resulted from seed treatments having only moderate increases of flea beetles with increased PD compared to other treatments (Fig. 3.1a).

On a per plant basis, we observed the opposite pattern, with a reduction in FB per plant with PD increase (Fig. 3.1b). Low PD plots had 11.4 ± 0.9 FB/sticky trap/plant (9.0 ± 0.8 and 2.5 ± 0.3; for CFB and SFB, respectively), optimum PD plots had 8.6 ± 0.6 FB/sticky trap/plant (6.9 ± 0.6 and, 1.7 ± 0.2 for CFB and SFB, respectively), and high PD plots had 5.4 ± 0.4 FB/sticky trap/plant (4.4 ± 0.4 and 1.1 ± 0 for CFB and SFB, respectively). The treatment with the lowest FB/sticky trap/plant was high PD with seed treatment (4.4 ± 0.6) and the greatest abundance was low PD with seed treatment (12.4 ± 1.8). Raw means and SE for each PD, FB management treatment and region are provided in Appendix VI. Consistent with total FB abundance, the significant interaction between PD and FB management (Table 3.2) was due to a steeper decrease in FB abundance per plant observed in the seed treatment at with increased PD compared to other FB management treatments (Fig. 1b). A second two-way interaction was found between PD and region (Table 3.2). Significant reductions in FB abundance per plant with increase PD were found among all PD treatments in Peace River and Manitoba, but low and optimum PD in Lethbridge and optimum and high PD in Saskatchewan showed only a non-significant trend of reduction (Fig. 3.1c).

3.3.3 Defoliation

Despite effects of PD on FB total abundance and abundance per plant, there was no consistent pattern of defoliation associated with PD and FB management treatments. On average, low PD plots had 20.6 ± 0.9 % defoliation, optimum PD plots had 20.6 ± 0.9 % defoliation, and high PD plots had 18.9 ± 0.9 % defoliation. The treatment with the lowest defoliation was high PD with seed treatment (10.3 ± 0.7 %) and the greatest defoliation was optimum PD control (29.8 ± 2.0 %). Raw means and SE for each PD, FB management treatment and region are provided in Appendix VII. Three significant interactions were found (Table 3.2): FB management and PD (Fig. 3.2a), region and PD (Fig. 3.2b), and region and FB management (Fig. 3.2c). Defoliation was higher in control and foliar treatment treatments compared with the seed and FB free treatments at all PD (Fig. 3.2a) and regions (Fig. 3.2c). Defoliation levels were similar within FB management treatments except for lower defoliation at high PD within the seed treatment (Fig. 3.2a). The interaction between region and PD was probably due to

defoliation in Saskatchewan and Manitoba decreasing with PD increases contrasting with the opposite pattern observed for Lethbridge and not differing in Peace River (Fig. 3.2b).

3.3.4 Yield

Yield was significantly increased by PD across all FB management treatments and regions (Table 3.2, Fig 3.3a). On average, low PD plots had a yield of 1321 ± 79.0 kg/ha, optimum PD plots had 1535.5 ± 86.9 kg/ha, and high PD plots had 1588.7 ± 85.6 kg/ha. Raw means and SE for each PD, FB management treatment and region are provided in Appendix VIII. The yield was significantly affected by an interaction between region and insecticide treatment (Table 3.2). All regions showed different trends when considering which FB management results in a higher yield (Fig. 3.3b). Peace River shows no differences in yield across any management strategy, while other regions show a yield increase compared to the control plots with any management action (Lethbridge), foliar and seed treatment (Manitoba) or the FB free treatment (Manitoba and Saskatchewan) (Fig. 3.3b). Within management treatments, regions did not have significant effects (Fig. 3.3b).

3.4 Discussion

Increased canola density in experimental plots resulted in an overall increase in yield and a decrease in flea beetle abundance per plant, offsetting a higher abundance of flea beetles observed at higher canola densities. This general trend was consistent among the different regions, flea beetle species assemblages, and flea beetle management treatments and suggest that plant density is a viable method to manage flea beetles in the Canadian prairies.

The results show that the flea beetles were more abundant in plots with a higher canola density when compared to more sparse plots, supporting the resource concentration hypothesis (Root 1973). Comparing pure or dense stands of collards with rows of collars in diverse plant stands mixed, Root (1973) observed that specialist herbivores, including CFB, were found at higher abundance in pure than in diverse stands of collard plants. Further studies manipulating collard patch sizes suggested that increasing fragmentation and reducing patch size also reduce flea beetle abundance (Kareiva 1982, Banks 1998). Flea beetles are likely to aggregate and immigrate into *Brassica*-dense areas and move from small patches to large patches based on the increase in volatiles that *Brassica* plants produce (Kareiva 1985, Fletcher 2006), and are also more likely to emigrate from smaller patches (Kareiva 1985). Recently, studies conducted at

landscape scales in Sweden also indicated that spatial isolation of canola fields decrease flea abundance and defoliation (Boetzl et al. 2023). Altogether these results indicate that aggregation and retention of flea beetles will increase at higher plant densities, although we demonstrate that these increased density does not translate into increase defoliation and is offset by the higher yield observed at higher plant densities.

Canola plants in high density plots experienced lower flea beetle pressure on a per plant basis, counteracting the aggregation of flea beetles and resulting in an overall dilution effect of increased plant density. Previous research manipulating plant density in Alberta, Canada, and in Sweden also suggested dilution effects on canola based on a reduction of defoliation at increased plant densities under a variety of conditions, including canola species, different seed treatments, planting dates (Dosdall et al. 1999, Lundin et al. 2020), row spacing, and tillage regimes (Dosdall and Stevenson 2005); however, most studies did not quantify flea beetle abundance. Low plant density plots may be attractive to flea beetles due to the increase in colour contrast (Dosdall et al. 1999) and increased warmth emitted from a greater amount of exposed soil (Ulmer and Dosdall 2006), which combined with a dilution effect may explain why low plant densities have more defoliation (Dosdall and Stevenson 2005). Dosdall et al. (1999) report that increasing seeding rates to 12.5 kg ha^{-1} in untreated plots reduced canola damage to similar levels as lower seeding rates treated with seed insecticides, suggesting that increasing plant density can contribute to manage flea beetles without prophylactic applications of insecticides in seeds. Here, we expand previous results by demonstrating dilution effects due to a reduction on flea beetle density per plant in multiple trials across a wide region and under two flea beetle management regimes.

Interestingly, the defoliation levels of this study do not clearly show a dilution effect the same way the flea beetles per plant did. Increased plant density reduced defoliation only in the seed treatment but not in the other flea beetle management treatment. Regionally, plant density reduced defoliation in Manitoba and Saskatchewan, but it showed the reverse trend in Lethbridge and, under lower defoliation levels, showed no effect on Peace River. This interaction may be partially explained by the different number of trials conducted per region: Manitoba has at least double the number of plots (n = 264), as the other three regions (LB: n = 120; PR: n = 132; SK: n = 144 plots). This suggests that in the regions with higher replication and defoliation, increased plant density showed the expected pattern of reduced defoliation. The lack of significant

differences between treatments in Peace River specifically could also be explained by the low numbers of flea beetles captured per plot combined with the large plot size (Appendix V). Along with this, the relative abundance of CFB and SFB varied across the Canadian prairies. In our study, CFB was found in greater numbers than SFB, except for the Peace River region where the numbers were approaching zero and there were also relatively few SFB (Appendix IV), which supports previous reports (Soroka et al. 2018). In summary, these results combined with previous research suggest that increasing plant density results in dilution effects that reduce flea beetle pressure per plant, which in turn may reduce defoliation under certain conditions. Further studies are needed to establish the factors governing flea beetle damage under field conditions.

The results demonstrate that our defoliation levels are consistent with previous findings in the Canadian prairie provinces. Dosdall et al (1999) showed mean flea beetle defoliation on *B. napus* ranging from 5 – 27% defoliation. Similarly, Cárcamo et al (2008) found that flea beetle defoliation in Alberta ranged from 0 – 36% while Dosdall and Stevenson (2005) reported 9.9% as being their highest levels of defoliation across their sites in Alberta. This research shows mean defoliation ranging from as little as 5.4% in Peace River (2019) to 53.3% in Manitoba (2019), with less than 1/4 of the plots exceeding the 25% economic threshold throughout the entire study. Interestingly, previous results combined with the new data from small plots conducted in multiple regions across western Canada and across four years, show that typical flea beetle defoliation levels fall below the 25% threshold damage, suggesting that the current widespread use of seed treatments may not be necessary in most canola fields.

As expected, flea beetle management treatments affected flea beetle defoliation, with either seed or FB-free treatments being the most effective at reducing the defoliation when compared to the control depending on the region or plant density. In terms of management regimes, seed-coated treatments are already the main strategy used across the Canadian prairies (Soroka et al. 2008, Cornelsen et al. 2024). These results confirm previous reports on the effectiveness of using neonicotinoid-coated seeds to reduce defoliation by flea beetles (Antwi et al. 2007, Knodel et al. 2008, Reddy et al. 2014). The reduction of defoliation observed at higher PD in the seed treatment may be due to the increase in insecticide product in the whole plot, as there were more seeds (and therefore insecticide) at higher PD. However, the exact mechanism of this effect remains to be investigated. Despite their effectiveness, the overuse of neonicotinoids may lead to unintended and detrimental effects in terms of ineffectiveness

towards striped flea beetles due to tolerance (Tansey et al. 2008), pest resurgence (Reddy et al. 2014), and impacts on non-target organisms (Desneux et al. 2007), so alternative methods of control should be considered to reduce the current reliance on this control method. Finally, although the foliar spray treatment at the 25% defoliation threshold generally resulted in slightly less defoliation reduction than the seed treatment, it always reduced defoliation compared to controls. The advantage of this method is that it results in insecticide applications only in response to economic populations of flea beetles, reducing unnecessary prophylactic applications of systemic insecticides in seeds (Tangtrakulwanich et al. 2014). However, this method requires more active scouting of canola fields to ensure timely application of control measures when needed, which is not always practical for growers (Mitchell and Conley 2017).

Another important caveat to note is that the high plant density treatment used in the study is unrealistic to use in an agricultural setting. While the chosen treatments were beneficial in showing clear trends in data, the seed rates would be far too costly for Canadian canola growers to implement. In addition, the economic benefit from an increased yield would not outweigh the high cost of the seed at 14 kg/ha. Even so, the benefits found from an increased plant density are even shown as the seed rate increased from 3.5 to 7.0 kg/ha, which is more realistic. Future research with lowered seed rates should be done to test if similar patterns are found.

Increased plant density resulted in higher canola yield, independently of flea beetle abundance, species composition, region, or flea beetle management strategy over a wide region of western Canada during the four years of this study. Increasing plant density is compatible with the management of other insect pests in canola (Dosdall et al. 1999, Dosdall and Stevenson 2005) and other techniques that may further reduce flea beetle defoliation, including no-till, row spacing, larger seed sizes, varying planting date and ratios of insecticide-treated and untreated seeds (Dosdall et al. 1999, Dosdall and Stevenson 2005, Cárcamo et al. 2008, Soroka et al. 2008, Lundin et al. 2018, Lundin 2019, Lundin et al. 2020). Combination of these techniques with the use of insecticides only as foliar sprays in response to threshold populations of flea beetles can provide a more sustainable method to manage flea beetle populations without relying on prophylactic treatments of seeds with systemic insecticides. More research is needed to develop prediction models of flea beetle occurrence and efficient scouting techniques that may allow growers to sustainably manage flea beetles in canola.

Table 3.1. Details for canola study sites used to determine the effects of flea beetle management strategies and plant density treatments on flea beetle populations, defoliation, and canola yield

Year	Province	Site	Plot Size (m)	Seeded Date	Times Sprayed Foliar	Dates Sprayed Foliar	Flea Beetle-free Control
2018	AB	Lethbridge	2.9 x 8	13-May-18	2	29-May-18 04-Jun-18	no
	AB	Peace River	3.6 x 10	19-May-18	2	05-Jun-18 09-Jun-18	no
	MB	Carman 1	1.5 x 5	11-May-18	2	28-May-18 04-Jun-18	no
	MB	Carman 2	1.5 x 5	11-May-18	2	28-May-18 04-Jun-18	no
2019	AB	Lethbridge	2.9 x 8	11-May-19	3	21-May-19 05-Jun-19 12-Jun-19	yes
	AB	Peace River	3.6 x 10	11-May-19	2	31-May-19 05-Jun-19	yes
	SK	Saskatoon 1	1.5 x 6.1	11-May-19	4	05-Jun-19 06-Jun-19 08-Jun-19 19-Jun-19	yes
	SK	Saskatoon 2	1.5 x 6.1	06-Jun-19	2	19-Jun-19 04-Jul-19	yes
	MB	Carman 1	1.5 x 5	14-May-19	4	30-May-19 04-Jun-19 06-Jun-19 14-Jun-19	yes
	MB	Carman 2	1.5 x 5	14-May-19	4	30-May-19 04-Jun-19 10-Jun-19 14-Jun-19	yes
2020	AB	Lethbridge	2.9 x 8	12-May-20	3	04-Jun-20 11-Jun-20 17-Jun-20	no
2021	AB	Peace River	3.6 x 10	11-May-21	1	10-Jun-21	yes[†]
	SK	Saskatoon	1.5 x 6.1	31-May-21	3	07-Jun-21 15-Jun-21 18-Jun-21	yes

MB	Carman 1	1.5 x 5	13-May-21	3	03-Jun-21 10-Jun-21 17-Jun-21	yes
MB	Carman 2	1.5 x 5	31-May-21	0ǂ	NA	yes

† Flea beetle-free control treatment only sprayed once due to COVID field limitations

ǂ Foliar treatment plots never reached 20-25% injury damage threshold

Table 3.2. Final GLMM models showing retained parameters after a manual backward selection procedure. Blank cells indicate that parameter was removed from the final model. Significant parameters are bolded. FB Mgtmt. = flea beetle management treatment; PD = plant density.

		Plant Number		FB per plot (SC)		FB per plant (m)		Defoliation		Yield	
Model parameter	**DF**	**X^2**	***p***	**X^2**	***p***	**X^2**	***p***	**X^2**	***p***	**X^2**	***p***
(Intercept)	1	8.04	0.005	1.09	0.297	0.65	0.420	0.129	0.719	0.486	0.486
Region	3	1.84	0.607	0.04	0.998	0.97	0.808	10.43	**0.015**	1.12	0.772
FB Mgmt.	3	11.56	**0.009**	52.91	**<0.001**	23.66	**<0.001**	147.78	**<0.001**	12.16	**0.006**
PD	2	96.83	**<0.001**	66.74	**<0.001**	11.64	**0.003**	11.02	0.004	44.79	**<0.001**
Plot Area	1	0.34	0.558	<0.01	0.951	0.30	0.586	2.92	0.087	0.57	0.451
Year	2	0.13	0.939	3.88	0.275	1.15	0.564	2.38	0.498	0.59	0.899
Region x FB Mgmt.	9	23.46	**0.005**					52.47	**<0.001**	26.15	**0.002**
Region x PD	6	10.29	0.113			10.17	0.118	32.64	**<0.001**		
FB Mgmt x PD	6	5.14	0.527	20.92	**0.002**	16.57	**0.011**	25.19	**<0.001**		
Region x FB Mgmt. x PD	18	49.53	**<0.001**								

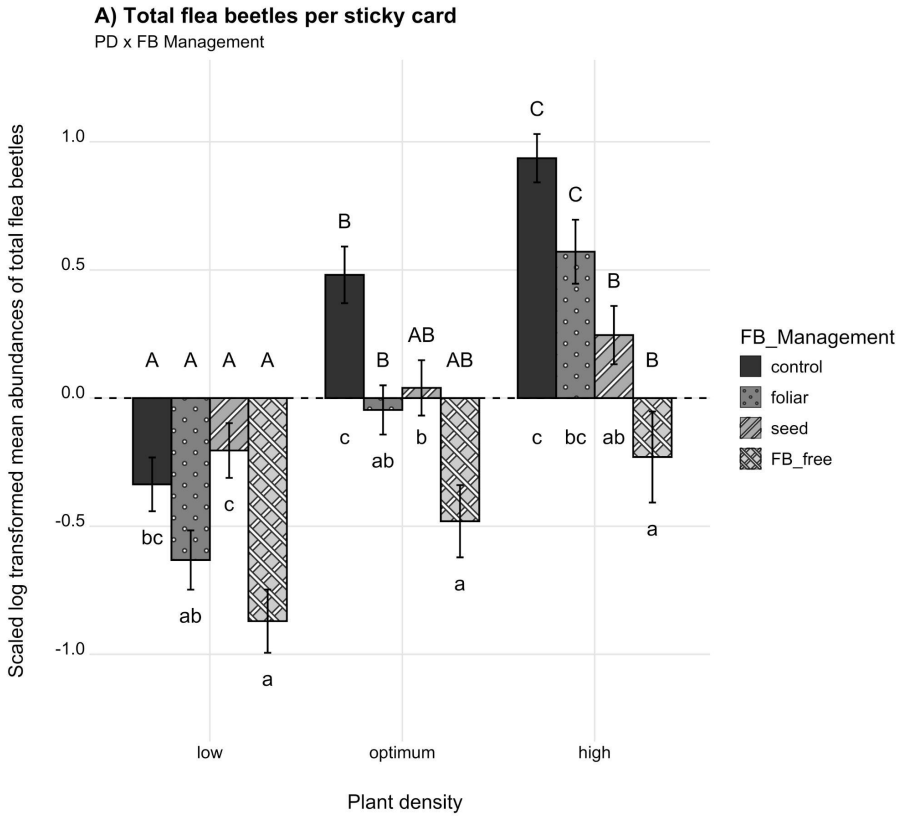
Table 3.3. Mean (± SE) plant number per linear metre transect averaged per plot among 3 years, 3 plant densities, and 4 FB management treatments

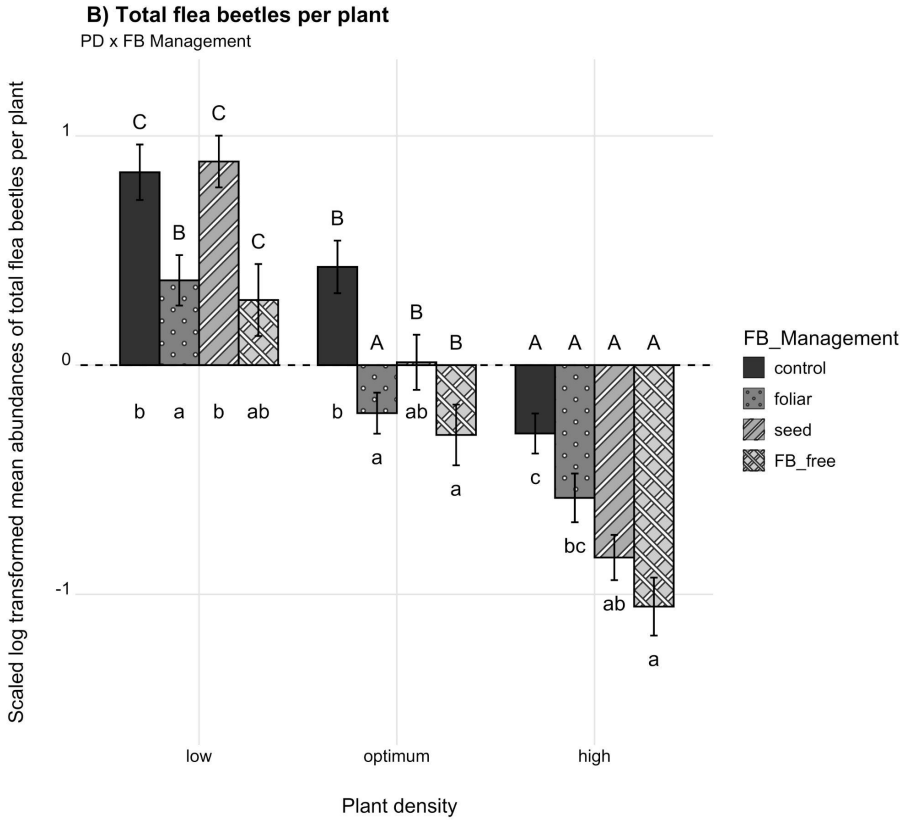
Region	FB Management	Plant Density	Plant Number			EMmeans group† (Sidak, α = 0.05)
LBǂ	control	low	7.9	±	1.1	a
		optimum	10.6	±	1.1	b
		high	20.0	±	1.5	c
	FB-free	low	8.8	±	1.3	a
		optimum	13.0	±	0.6	b
		high	23.9	±	1.1	c
	foliar	low	7.8	±	0.6	a
		optimum	12.3	±	1.0	b
		high	23.2	±	2.4	c
	seed	low	8.9	±	0.6	a
		optimum	12.9	±	0.7	b
		high	22.4	±	1.8	c
PR	control	low	6.0	±	0.3	a
		optimum	11.4	±	1.0	b
		high	23.0	±	1.1	c
	FB-free	low	5.6	±	0.6	a
		optimum	12.5	±	1.3	b
		high	22.6	±	2.6	c
	foliar	low	6.6	±	0.6	a
		optimum	12.1	±	0.7	b
		high	23.1	±	1.5	c
	seed	low	5.9	±	0.7	a
		optimum	11.9	±	1.2	b
		high	19.7	±	2.0	c
MB	control	low	10.4	±	1.1	a
		optimum	15.3	±	0.8	b
		high	31.6	±	1.8	c

	FB-free	low	9.9	±	1.0	a
		optimum	15.6	±	1.0	b
		high	27.6	±	1.8	c
	foliar	low	11.1	±	0.8	a
		optimum	18.0	±	0.9	b
		high	32.7	±	1.7	c
	seed	low	8.6	±	0.5	a
		optimum	18.3	±	1.2	b
		high	33.6	±	2.0	c
SK	control	low	10.9	±	1.5	a
		optimum	24.7	±	1.9	b
		high	42.2	±	3.0	c
	FB-free	low	14.3	±	1.9	a
		optimum	29.1	±	0.9	b
		high	53.6	±	5.5	c
	foliar	low	14.5	±	2.1	a
		optimum	25.2	±	1.5	b
		high	39.7	±	2.2	c
	seed	low	13.7	±	2.1	a
		optimum	15.8	±	1.6	a
		high	36.0	±	1.9	b

† pairwise comparisons made using estimated marginal means (EMM) using Sidak correction, comparing between plant densities within each region x FB management treatment combination

ǂ LB = Lethbridge, Alberta; PR = Peace River, Alberta; MB = Carman, Manitoba; SK = Saskatoon, Saskatchewan


A) Total flea beetles per sticky card
PD x FB Management
Scaled log transformed mean abundances of total flea beetles
1.0
0.5
0.0
-0.5
-1.0
A A A A
bc ab c a
B B AB AB
c ab b a
C C B B
c bc ab a
low
optimum
high
Plant density
FB_Management
control
foliar
seed
FB_free


B) Total flea beetles per plant
PD x FB Management
Scaled log transformed mean abundances of total flea beetles per plant
1
0
-1
C B C C
b a b ab
B A B B
b a ab a
A A A A
c bc ab a
low
optimum
high
Plant density
FB_Management
control
foliar
seed
FB_free

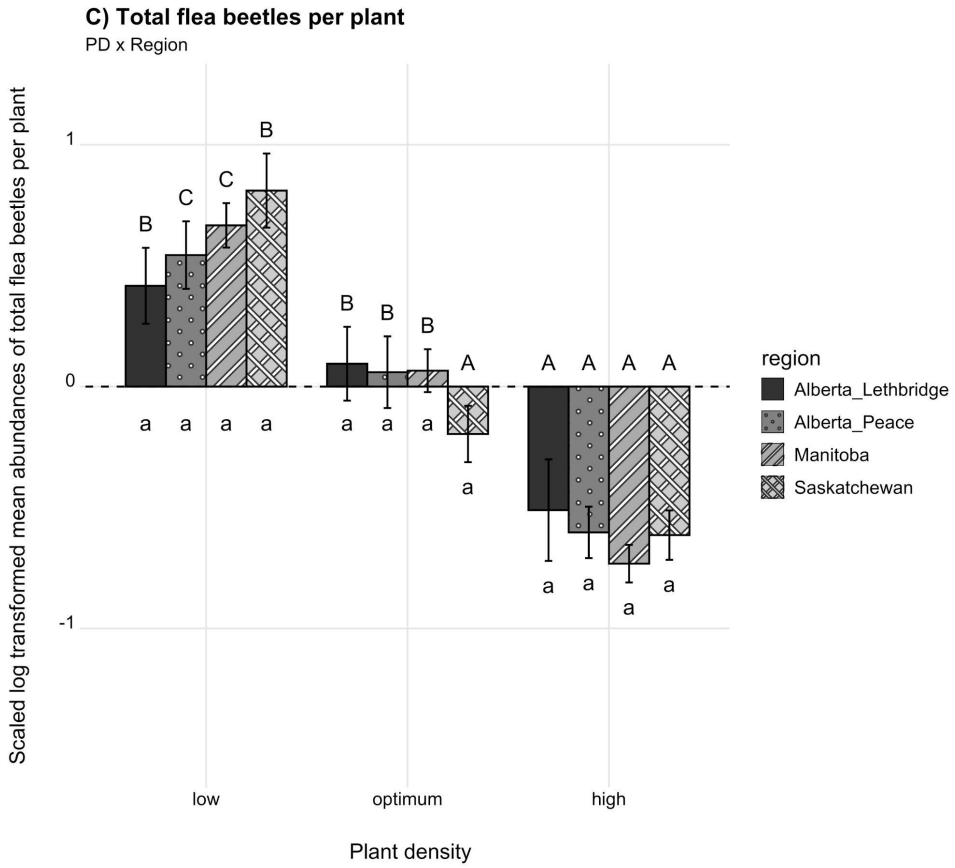
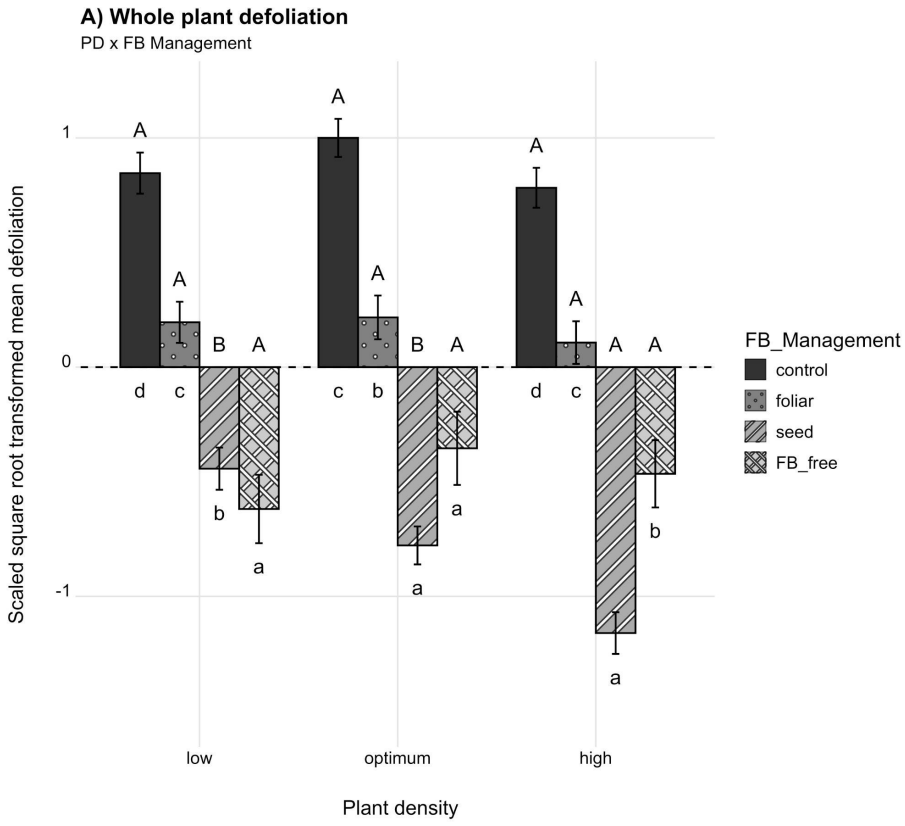
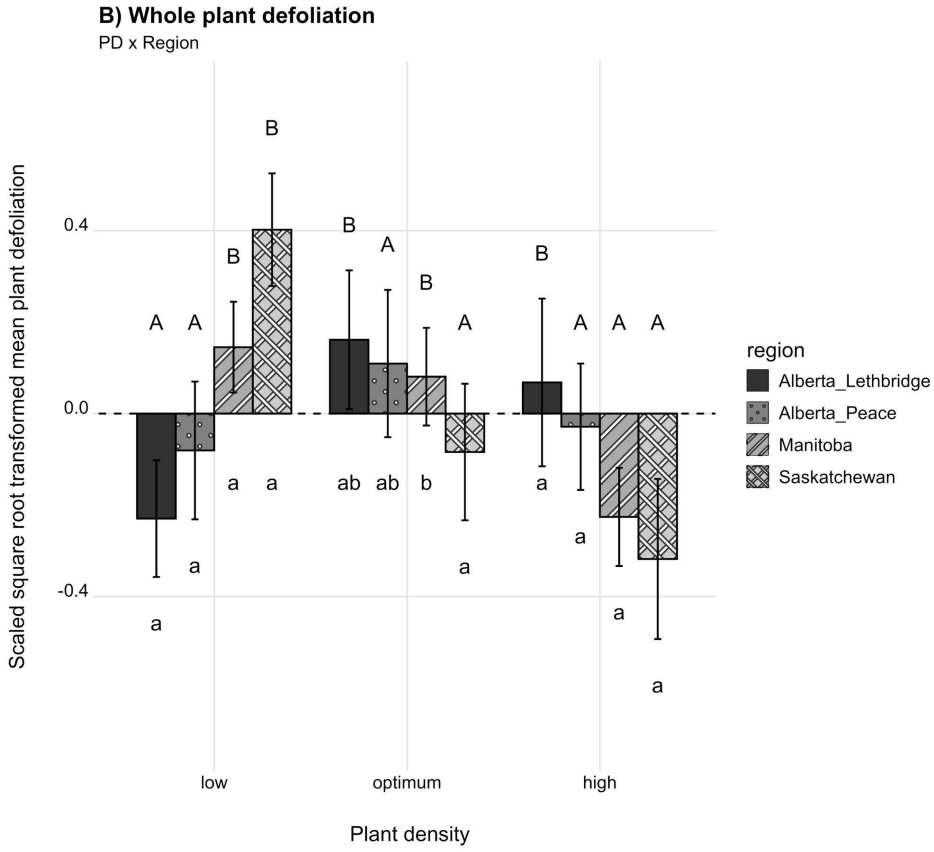


Figure 3.1. Mean (± SE) scaled log transformed abundances of (A) total FB/sticky card (CFB + SFB) based on PD and FB management treatment for all regions combined, (B) total FB/sticky trap/plant based on PD and FB management treatment and (C) total FB/sticky trap/plant based on FB management treatment for each region. Capital letters represent differences among PD treatments within each FB management treatment/ region. Lower-case letters represent differences among FB management treatments/ region within each PD treatment (EMM, $\alpha = 0.05$).


A) Whole plant defoliation
PD x FB Management
Scaled square root transformed mean defoliation
1
0
-1
low
optimum
high
Plant density
FB_Management
control
foliar
seed
FB_free


B) Whole plant defoliation
PD x Region
Scaled square root transformed mean plant defoliation
0.4
0.0
-0.4
low
optimum
high
Plant density
region
Alberta_Lethbridge
Alberta_Peace
Manitoba
Saskatchewan

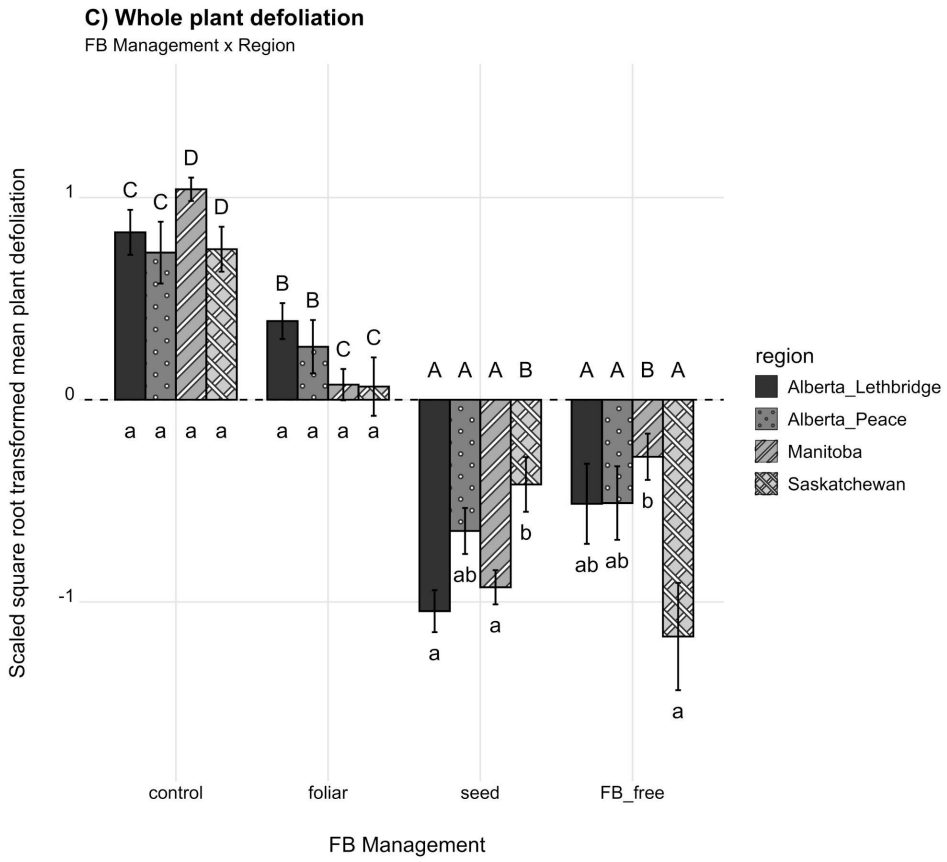


Figure 3.2. Mean (± SE) scaled square root transformed percentages of defoliation per plot based on (A) PD and FB management treatment for all regions combined, (B) PD for each region, and (C) FB management treatment for each region. Capital letters represent differences among (A,B) PD treatments or (C) FB management treatment within each (A) FB management treatment or (B,C) region. Lower-case letters represent differences among (A) FB management treatments or (B,C) region within each (A,B) PD treatment or (C) FB management treatment (EMM, $\alpha = 0.05$).

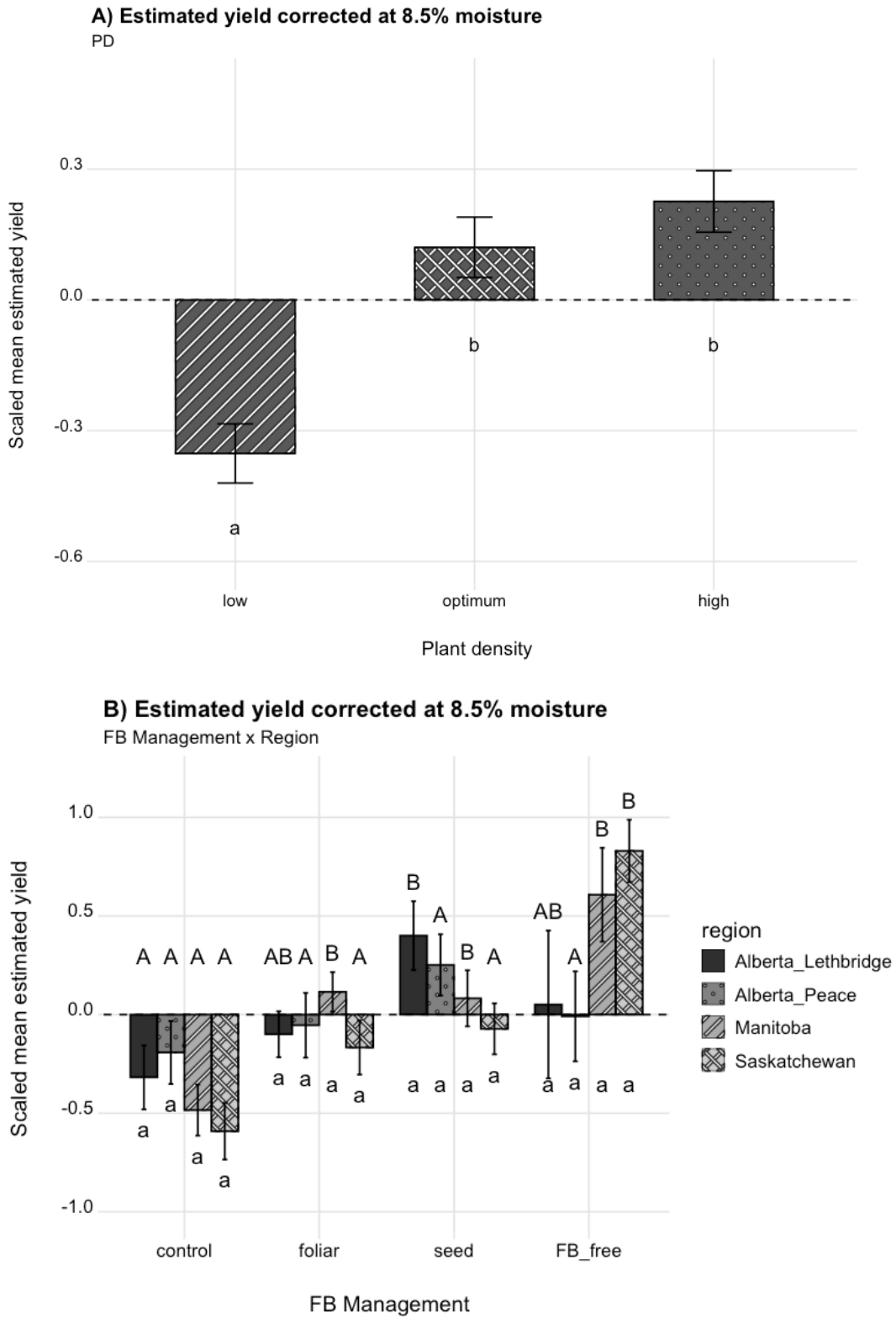
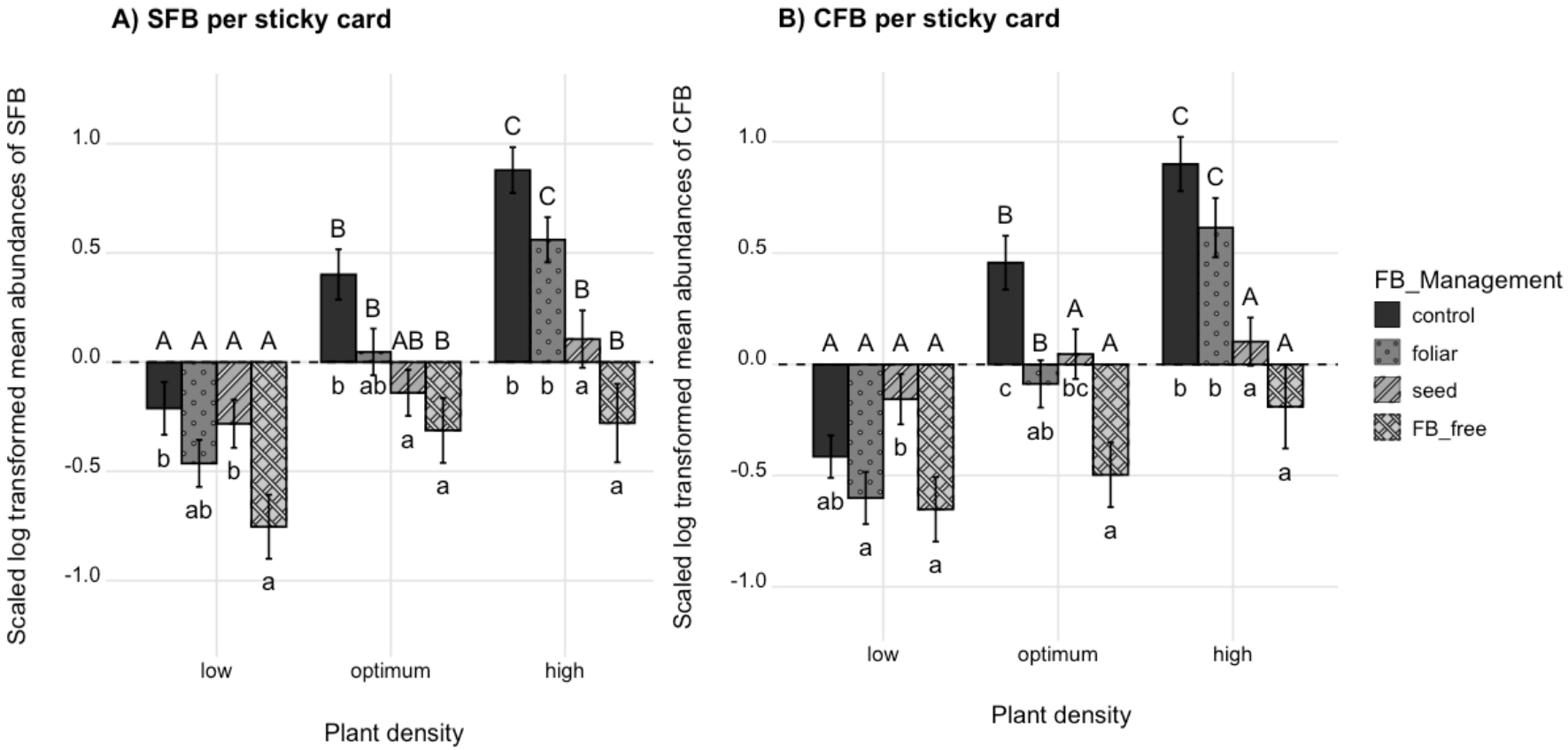


Figure 3.3. Mean (± SE) scaled yield per plot based on (A) PD and (B) FB management treatment for each region. Yield is estimated and corrected at 8.5% moisture. Capital letters

represent differences among FB management treatment within each region. Lower-case letters represent differences among regions within each FB management treatment (EMM, $\alpha = 0.05$).



Appendix II. Mean (+ SE) scaled log transformed abundances of (A) total SFB/sticky card and (B) total CFB/sticky card based on PD and FB management treatment for all regions combined. Capital letters represent differences among PD treatments within each FB management treatment/ region. Lower-case letters represent differences among FB management treatments/ region within each PD treatment (EMM, $\alpha = 0.05$).

Appendix III. Additional GLMM models showing retained parameters after a manual backward selection procedure for SFB and CFB per sticky card (per plot). Blank cells indicate that parameter was removed from the final model. Significant parameters are bolded. FB Mgtmt. = flea beetle management treatment; PD = plant density.

		SFB per plot (SC)		CFB per plot (SC)	
Model parameter	**DF**	**X^2**	***p***	**X^2**	***p***
(Intercept)	1	0.61	0.433	0.87	0.349
Region	3	2.16	0.540	0.00	1.000
FB Mgmt.	3	50.83	**<0.001**	46.81	**<0.001**
PD	2	20.00	**<0.001**	64.67	**<0.001**
Plot Area	1	0.00	1.000	<0.01	0.989
Year	2	2.34	0.506	2.28	0.517
Region x FB Mgmt.	9				
Region x PD	6	14.35	**0.026**		
FB Mgmt x PD	6	15.46	**0.017**	31.56	**<0.001**
Region x FB Mgmt. x PD	18				

Appendix IV. Mean (± SE) flea beetles per sticky card (per plot) based on species, region and year collected

Year	FB species	LB[†]	PR	MB	SK
2018	SFB*	30.1 ± 4.8	8.5 ± 0.5	13.9 ± 1.0	NA
	CFB	169.9 ± 22.4	0.9 ± 0.2	67.8 ± 4.3	
2019	SFB	25.8 ± 0.9	26.2 ± 2.9	65.3 ± 5.7	27.1 ± 2.3
	CFB	256.6 ± 9.1	0.0 ± 0.0	150.6 ± 8.1	13.4 ± 1.4
2020/2021[ǂ]	SFB	0.5 ± 0.1	4.0 ± 0.3	6.2 ± 0.3	10.2 ± 0.6
	CFB	112.2 ± 7.1	0.0 ± 0.0	160.8 ± 8.1	15.1 ± 1.5

[†]LB = Lethbridge, Alberta; PR = Peace River, Alberta; MB = Carman, Manitoba; SK = Saskatoon, Saskatchewan
*SFB = striped flea beetle; CFB = crucifer flea beetle
[ǂ] 2020: Lethbridge (AB); 2021, Peace River (AB), MB, SK – different years due to different COVID-19 limitations

Appendix V. Mean (± SE) flea beetles per sticky card (per plot) based on collection date among 3 years, 3 plant densities, and 4 FB management treatments

Year	Plant density	FB Management	LB[†]	PR	MB	SK
2018	Low	Control	83.6 ± 15.5	7.2 ± 1.1	52.9 ± 6.8	
		Foliar	86.8 ± 24.3	5.4 ± 0.7	56.5 ± 8.0	
		Seed	75.8 ± 4.7	7.8 ± 0.3	61.5 ± 6.6	NA*
	Optimum	Control	211.7 ± 56.9	11.0 ± 1.7	96.4 ± 16.3	
		Foliar	235.3 ± 48.1	7.4 ± 0.7	75.3 ± 10.9	
		Seed	106.5 ± 12.3	8.9 ± 1.3	57.5 ± 4.4	NA
	High	Control	452.0 ± 73.3	14.6 ± 1.7	141.9 ± 17.2	
		Foliar	447.2 ± 44.2	9.9 ± 2.7	126.1 ± 19.8	
		Seed	98.3 ± 13.2	12.3 ± 2.8	67.2 ± 7.3	NA
FB totals 2018			**199.7 ± 27.0**	**9.4 ± 0.7**	**81.7 ± 5.3**	-
2019	Low	Control	246.4 ± 15.4	22.0 ± 5.8	192.1 ± 37.4	34.3 ± 9.5
		Foliar	241.3 ± 10.1	22.3 ± 1.2	148.0 ± 32.8	27.4 ± 5.4
		Seed	307.6 ± 5.1	14.4 ± 2.7	152.8 ± 27.6	47.4 ± 13.8
		FB-Free	217.6 ± 8.2	16.8 ± 3.4	144.9 ± 26.2	26.1 ± 8.5
	Optimum	Control	352.4 ± 23.1	55.1 ± 31.9	278.7 ± 38.3	49.0 ± 15.0
		Foliar	222.1 ± 19.3	26.8 ± 1.7	211.8 ± 42.6	46.5 ± 13.5
		Seed	318.5 ± 22.9	17.0 ± 2.5	209.7 ± 39.4	31.2 ± 8.3
		FB-Free	228.9 ± 22.9	26.5 ± 7.6	169.3 ± 26.5	30.9 ± 10.8
	High	Control	368.7 ± 42.2	31.6 ± 2.8	331.4 ± 60.5	65.1 ± 18.4
		Foliar	300.0 ± 36.4	34.3 ± 3.7	260.6 ± 44.1	56.9 ± 16.1
		Seed	280.7 ± 28.7	23.0 ± 3.1	204.6 ± 42.1	42.8 ± 11.8
		FB-Free	305.8 ± 38.3	24.8 ± 1.9	287.2 ± 74.0	28.9 ± 10.2
FB totals 2019			**282.5 ± 9.6**	**26.2 ± 2.9**	**215.9 ± 13.2**	**40.5 ± 3.6**
2020/2021[ǂ]	Low	Control	74.9 ± 8.8	3.6 ± 0.7	179.6 ± 28.3	18.9 ± 4.0

	Foliar	71.9	±	19.2	2.1	±	0.6	156.9	±	38.6	19.4	± 3.4
	Seed	125.4	±	21.5	1.9	±	0.2	176.3	±	32.0	21.9	± 1.8
	FB-Free	NA			1.3	±	0.3	111.3	±	23.1	24.4	± 4.4
Optimum	Control	118.7	±	10.4	3.8	±	0.5	182.0	±	27.1	23.8	± 1.3
	Foliar	105.5	±	7.1	2.7	±	0.5	142.5	±	19.2	22.6	± 4.7
	Seed	157.6	±	10.4	4.1	±	0.7	205.6	±	27.0	23.1	± 3.5
	FB-Free	NA			4.8	±	0.6	125.0	±	17.7	14.8	± 3.2
High	Control	101.7	±	5.6	6.6	±	1.3	175.4	±	18.7	38.5	± 10.1
	Foliar	71.5	±	20.5	7.0	±	1.4	170.5	±	25.8	39.5	± 5.8
	Seed	135.6	±	30.4	6.6	±	1.6	222.7	±	23.8	26.5	± 3.7
	FB-Free	NA			3.1	±	0.6	114.6	±	17.6	30.9	± 15.6
FB totals 2020/2021		**107.0**	**±**	**6.9**	**4.0**	**±**	**0.3**	**163.5**	**±**	**7.8**	**25.3**	**± 1.9**

[†]LB = Lethbridge, Alberta; PR = Peace River, Alberta; MB = Carman, Manitoba; SK = Saskatoon, Saskatchewan

* NAs indicate data missingness due to inability to access fields, collect data, or lack of personnel due to either COVID-19 limitations or other reasons preventing proper protocol

[ǂ] 2020: Lethbridge (AB); 2021, Peace River (AB), MB, SK – different years due to different COVID-19 limitations

Appendix VI. Mean (± SE) flea beetles per plant based on plot among 3 years, 3 plant densities, and 4 FB management treatments

Year	Plant density	FB Management	LB†	PR	MB	SK
2018	Low	Control	12.0 ± 1.7	1.1 ± 0.2	4.8 ± 0.6	
		Foliar	11.5 ± 3.3	0.9 ± 0.1	4.7 ± 0.6	
		Seed	8.5 ± 0.7	1.2 ± 0.1	5.7 ± 0.7	NA*
	Optimum	Control	15.5 ± 4.0	1.1 ± 0.2	5.1 ± 0.9	
		Foliar	16.0 ± 3.0	0.6 ± 0.1	3.3 ± 0.5	
		Seed	7.0 ± 0.6	0.6 ± 0.1	2.6 ± 0.2	NA
	High	Control	18.6 ± 2.5	0.7 ± 0.1	3.8 ± 0.6	
		Foliar	16.2 ± 2.8	0.4 ± 0.1	3.2 ± 0.5	
		Seed	4.1 ± 1.0	0.6 ± 0.1	1.7 ± 0.2	NA
2019	Low	Control	27.1 ± 3.7	4.3 ± 1.0	37.0 ± 10.1	6.3 ± 2.2
		Foliar	28.5 ± 3.0	3.0 ± 0.5	21.1 ± 6.4	5.6 ± 2.4
		Seed	46.8 ± 3.4	1.9 ± 0.1	20.4 ± 4.0	5.3 ± 1.8
		FB-Free	26.9 ± 4.9	2.7 ± 0.6	20.1 ± 4.0	NA
	Optimum	Control	28.9 ± 1.4	4.0 ± 2.4	24.4 ± 3.7	2.5 ± 0.8
		Foliar	16.3 ± 1.0	2.4 ± 0.3	15.4 ± 3.6	2.0 ± 0.6
		Seed	29.2 ± 3.8	1.1 ± 0.1	17.2 ± 4.8	3.4 ± 1.4
		FB-Free	17.9 ± 2.4	1.8 ± 0.4	12.8 ± 2.3	NA
	High	Control	17.1 ± 1.2	1.3 ± 0.1	14.1 ± 3.0	1.8 ± 0.5
		Foliar	13.0 ± 3.7	1.6 ± 0.3	10.8 ± 3.1	1.5 ± 0.4
		Seed	16.2 ± 2.2	0.9 ± 0.1	9.2 ± 1.9	1.3 ± 0.4
		FB-Free	13.0 ± 2.1	1.1 ± 0.2	11.8 ± 3.5	NA
2020/2021ǂ	Low	Control	13.7 ± 3.4	0.6 ± 0.1	13.7 ± 1.4	1.5 ± 0.4
		Foliar	11.7 ± 4.1	0.4 ± 0.0	11.3 ± 1.5	1.1 ± 0.2
		Seed	11.3 ± 1.3	0.6 ± 0.1	25.3 ± 4.0	1.8 ± 0.2
		FB-Free	NA	0.3 ± 0.1	9.0 ± 1.1	1.9 ± 0.5

Optimum	Control	22.1 ± 4.3	0.4 ± 0.1	11.9 ± 1.2	0.9 ± 0.1
	Foliar	15.1 ± 5.3	0.2 ± 0.0	8.4 ± 0.9	0.9 ± 0.2
	Seed	12.9 ± 0.7	0.6 ± 0.1	12.3 ± 1.9	1.4 ± 0.3
	FB-Free	NA	0.5 ± 0.1	7.5 ± 1.0	0.5 ± 0.1
High	Control	7.2 ± 0.3	0.3 ± 0.0	5.8 ± 0.5	0.8 ± 0.2
	Foliar	5.4 ± 2.2	0.3 ± 0.0	5.4 ± 0.5	0.9 ± 0.2
	Seed	5.6 ± 0.6	0.5 ± 0.1	6.1 ± 0.8	0.8 ± 0.1
	FB-Free	NA	0.2 ± 0.1	3.8 ± 0.4	0.5 ± 0.2

[†]LB = Lethbridge, Alberta; PR = Peace River, Alberta; MB = Carman, Manitoba; SK = Saskatoon, Saskatchewan

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[ǂ] 2020: Lethbridge (AB); 2021, Peace River (AB), MB, SK – different years due to different COVID-19 limitations

Appendix VII. Mean (± SE) defoliation (whole plant) (%) per plot among 3 years, 3 plant densities, and 4 FB management treatments

Year	Plant density	FB Management	LB[†]	PR	MB	SK
2018	Low	Control	24.5 ± 0.8	18.8 ± 1.8	35.7 ± 1.6	
		Foliar	21.5 ± 2.4	15.6 ± 1.8	22.1 ± 0.9	
		Seed	12.3 ± 1.2	13.6 ± 1.9	16.3 ± 0.9	NA*
	Optimum	Control	30.5 ± 5.5	20.3 ± 2.6	36.2 ± 1.4	
		Foliar	28.2 ± 5.2	17.7 ± 2.1	21.8 ± 0.9	
		Seed	9.9 ± 1.1	11.0 ± 2.3	11.7 ± 1.1	NA
	High	Control	32.0 ± 3.7	19.2 ± 2.2	31.6 ± 1.5	
		Foliar	27.8 ± 2.1	19.4 ± 2.9	21.0 ± 1.4	
		Seed	9.4 ± 1.7	10.3 ± 1.3	10.2 ± 1.3	NA
2019	Low	Control	17.8 ± 3.0	26.6 ± 3.3	53.3 ± 2.9	20.6 ± 5.3
		Foliar	13.9 ± 1.3	25.1 ± 3.3	25.9 ± 4.0	17.0 ± 3.9
		Seed	12.9 ± 1.9	7.8 ± 2.2	21.5 ± 4.0	16.2 ± 3.9
		FB-Free	9.9 ± 1.1	5.4 ± 0.8	29.0 ± 4.0	NA
	Optimum	Control	22.6 ± 1.8	22.7 ± 3.2	52.9 ± 3.0	20.4 ± 5.3
		Foliar	18.5 ± 1.0	18.9 ± 2.9	31.5 ± 3.9	14.1 ± 4.1
		Seed	12.3 ± 1.4	11.1 ± 2.5	17.4 ± 2.6	11.8 ± 2.4
		FB-Free	13.7 ± 1.6	7.6 ± 1.2	26.9 ± 4.6	NA
	High	Control	22.7 ± 3.0	20.7 ± 4.1	46.8 ± 4.9	16.0 ± 4.1

		Foliar	19.0 ± 1.2	13.9 ± 2.0	27.3 ± 2.9	12.5 ± 3.3
		Seed	9.5 ± 1.8	10.3 ± 2.9	16.4 ± 3.5	9.9 ± 2.7
		FB-Free	14.7 ± 1.4	9.9 ± 1.4	31.4 ± 4.2	NA
2020/2021[ǂ]	Low	Control	34.8 ± 1.6	8.5 ± 1.0	29.2 ± 7.6	22.1 ± 1.2
		Foliar	32.0 ± 2.0	8.6 ± 1.0	23.4 ± 6.0	19.5 ± 1.4
		Seed	18.5 ± 4.5	8.3 ± 0.5	11.9 ± 1.3	15.3 ± 1.2
		FB-Free	NA	6.5 ± 1.2	19.6 ± 6.2	10.3 ± 2.5
	Optimum	Control	40.1 ± 2.0	11.4 ± 2.5	28.7 ± 7.4	22.7 ± 1.3
		Foliar	36.7 ± 1.6	8.2 ± 1.5	23.6 ± 5.5	16.0 ± 1.4
		Seed	18.5 ± 1.4	7.6 ± 0.4	9.6 ± 1.6	12.2 ± 1.0
		FB-Free	NA	10.7 ± 0.9	21.2 ± 6.3	6.0 ± 1.2
	High	Control	43.9 ± 4.4	11.3 ± 1.8	25.7 ± 6.8	20.7 ± 2.6
		Foliar	33.1 ± 4.3	8.8 ± 0.5	18.9 ± 4.5	17.3 ± 1.4
		Seed	13.6 ± 1.5	6.7 ± 0.6	6.0 ± 0.9	10.1 ± 1.0
		FB-Free	NA	8.1 ± 0.6	16.1 ± 5.1	7.8 ± 2.4

[†]LB = Lethbridge, Alberta; PR = Peace River, Alberta; MB = Carman, Manitoba; SK = Saskatoon, Saskatchewan

* NAs indicate data missingness due to inability to access fields, collect data, or lack of personnel due to either COVID-19 limitations or other reasons preventing proper protocol

ǂ 2020: Lethbridge (AB); 2021, Peace River (AB), MB, SK – different years due to different COVID-19 limitations

Appendix VIII. Mean (± SE) yield (corrected at 8.5% moisture) (kg ha^{-1}) per plot among 3 years, 3 plant densities, and 4 FB management treatments

Year	Plant density	FB Management	LB†	PR	MB	SK
2018	Low	Control	661.4 ± 125.7	2985.1 ± 165.6	1698.4 ± 154.1	
		Foliar	811.6 ± 71.2	2544.1 ± 450.7	2158.3 ± 210.1	
		Seed	920.9 ± 26.7	3295.9 ± 167.7	2250.5 ± 220.3	NA*
	Optimum	Control	856.9 ± 38.1	3326.3 ± 303.6	2013.3 ± 186.7	
		Foliar	922.4 ± 54.2	3480.7 ± 122.0	2134.0 ± 210.9	
		Seed	1004.4 ± 13.1	3630.8 ± 163.1	2405.8 ± 242.8	NA
	High	Control	955.6 ± 32.4	3463.7 ± 323.2	2188.2 ± 255.6	
		Foliar	996.9 ± 21.0	3747.0 ± 119.4	2507.8 ± 247.8	
		Seed	976.5 ± 29.6	3792.8 ± 318.0	2090.2 ± 163.1	NA
2019	Low	Control	652.5 ± 76.9	2596.1 ± 366.7	215.8 ± 40.8	1978.2 ± 385.3
		Foliar	831.2 ± 176.3	3354.8 ± 293.5	870.0 ± 66.7	3424.6 ± 141.4
		Seed	725.0 ± 84.1	3123.2 ± 150.3	819.1 ± 98.1	2111.9 ± 345.1
		FB-Free	699.7 ± 88.1	3629.9 ± 300.9	774.3 ± 49.3	NA
	Optimum	Control	685.3 ± 94.8	3287.7 ± 276.8	378.7 ± 81.8	2891.5 ± 232.3
		Foliar	780.6 ± 75.0	3388.1 ± 396.7	895.1 ± 68.2	3913.6 ± 215.4
		Seed	736.8 ± 109.6	3304.7 ± 384.4	882.5 ± 61.3	3161.9 ± 160.3
		FB-Free	685.3 ± 58.9	3857.2 ± 259.6	804.1 ± 65.1	NA
	High	Control	817.7 ± 116.2	3801.9 ± 157.0	608.1 ± 61.3	2668.2 ± 171.8

		Foliar	595.7	±	48.8	3625.5	±	355.8	1010.5	±	63.2	3321.5	±	195.7
		Seed	708.0	±	34.1	3368.0	±	137.7	840.8	±	70.2	2986.0	±	136.1
		FB-Free	802.4	±	87.9	3849.0	±	316.2	973.9	±	52.5	NA		
2020/2021[ƒ]	Low	Control	106.9	±	29.0	783.9	±	223.3	106.8	±	32.0	567.6	±	68.5
		Foliar	99.7	±	11.1	628.8	±	173.7	285.4	±	112.6	748.7	±	58.6
		Seed	230.1	±	37.0	1049.5	±	162.9	192.9	±	74.4	753.6	±	64.4
		FB-Free	NA			441.7	±	124.1	162.5	±	87.4	698.0	±	78.3
	Optimum	Control	163.5	±	7.9	855.6	±	193.7	140.0	±	57.5	722.2	±	93.4
		Foliar	217.9	±	45.8	1139.6	±	332.1	378.8	±	94.5	1212.5	±	105.1
		Seed	392.6	±	31.8	973.5	±	210.5	290.5	±	101.6	884.0	±	65.6
		FB-Free	NA			969.8	±	249.6	231.2	±	125.7	700.4	±	52.7
	High	Control	231.1	±	37.1	738.3	±	122.9	211.6	±	136.6	741.5	±	60.1
		Foliar	341.2	±	10.5	630.7	±	151.8	487.4	±	115.8	1268.0	±	76.7
		Seed	457.5	±	35.9	781.2	±	176.9	357.6	±	159.7	900.9	±	53.4
		FB-Free	NA			870.7	±	173.6	439.1	±	194.3	954.0	±	109.2

[†]LB = Lethbridge, Alberta; PR = Peace River, Alberta; MB = Carman, Manitoba; SK = Saskatoon, Saskatchewan

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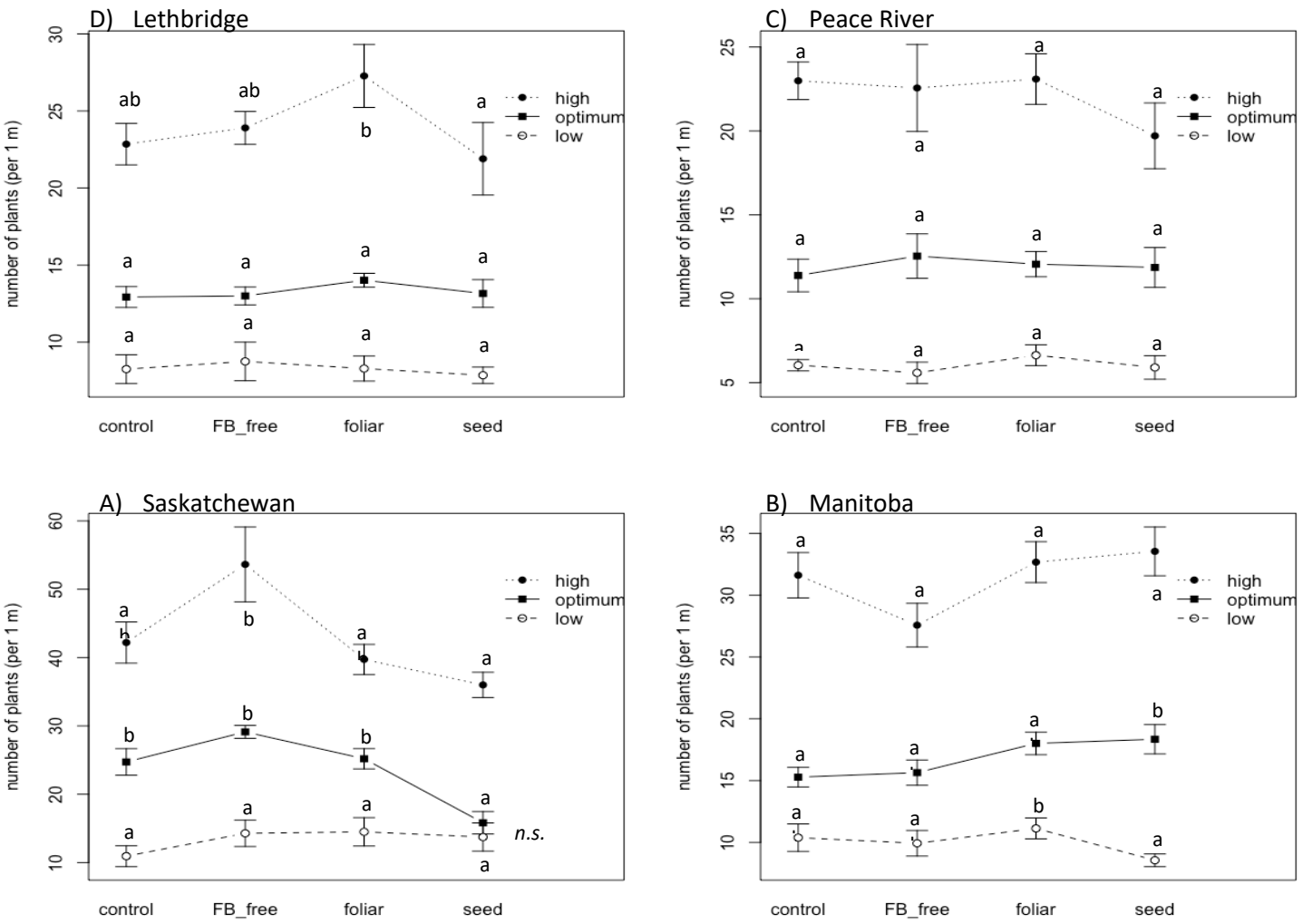
[ƒ] 2020: Lethbridge (AB); 2021, Peace River (AB), MB, SK – different years due to different COVID-19 limitations



Appendix IX. Number of plants per weekly linear metre transect per FB management treatment, and plant density for A) Lethbridge, AB; B) Peace River, AB; C) Saskatoon, SK; and D) Carman, MB. Data taken from 50% plant emergence to growth stage 2.4. Letters indicate significant differences between FB management treatments within each region and PD (EMM, $\alpha = 0.05$). No difference between plant densities is indicated with *n.s.*

CHAPTER 4. CONCLUSIONS AND IMPLICATIONS

4.1 Thesis Summary

In this study, I explored the possibility of generalist predators to supress flea beetle abundances and decrease the defoliation they produce on canola, the effects of temperature and seeding rate on flea beetle feeding behaviour (Chapter 2), as well as the implications of various insecticide application methods and seeding rates on flea beetle populations and defoliation in canola fields (Chapter 3). My objectives throughout this study were: (i) to determine the biotic and abiotic factors affecting the interactions between flea beetles and canola, (ii) determine how different plant densities of canola and application methods of insecticide will affect the density of flea beetles, plant damage, and yield and (iii) gather insights from my findings and provide informed suggestions for Integrated Pest Management (IPM) of flea beetles in canola in conjunction with knowledge from previous literature.

My first objective was met by identifying two predator species that significantly reduce flea beetle abundances in a laboratory setting, *Pterostichus melanarius* and *Pardosa* spp. I determined that the presence of *P. melanarius* reduces the intensity of flea beetle-mediated cotyledon defoliation as well as the intensity and prevalence of flea beetle-mediated stem damage. *Pardosa spp.* similarly resulted in less cotyledon damage caused by flea beetles. I determined that there is no difference in feeding between the flea beetle species, SFB and CFB regardless of temperature. Overall, increased temperature as well as a lower plant density results in more cotyledon and stem damage caused by flea beetles. Finally, I determined a methodology to efficiently and accurately estimate the amount of stem damage caused by flea beetle feeding on canola seedlings. I met my second objective by identifying patterns and interactions between currently used flea beetle management strategies and planting densities of canola and how they affect flea beetle abundances, defoliation, and yield. I determined that in general, increased plant density will increase the flea beetle abundances per plot and decrease the flea beetle pressure per plant across most management regimes showing evidence of both resource concentration hypothesis at the plot level and a dilution effect at the plant level. I found that defoliation did not follow the same trend and was not affected by plant density, indicating other factors are likely mediating damage to canola in addition to flea beetle abundance. Finally, I determined that an increased plant density from 3.5 to 7.0 kg/ha will increase the total yield regardless of the flea

beetle management strategy used and under variable populations of flea beetles naturally occurring in all regions examined across the Canadian prairies. Below, I use the results from my study in conjunction with observations and results from previous literature to make suggestions for Integrated Pest Management, thus meeting my last objective.

4.2 Predators of Flea Beetles

I found that there is a potential for *P. melanarius* and *Pardosa spp.* to be effective predators of *Phyllotreta* flea beetles by either direct consumption, non-consumptive effects, or some combination of the two. Decreasing canola damage via direct consumption may be important in canola crops, but the benefits observed may also be caused by non-consumptive effects. The presence or threat of predation causes prey species to change their behaviour in terms of activity levels and feeding habits (Williams et al. 2001, Magalhães et al. 2002, Lee et al. 2014). When exposed to the risk of predation, seed-eating carabids have demonstrated an increase in seed consumption while decreasing their foraging effort and choosiness (Blubaugh et al. 2017, Charalabidis et al. 2017). Other studies have shown that Chrysomelidae prey species respond differently by decreasing their food consumption when exposed to predators (Snyder and Wise 2000, Williams et al. 2001). Decreasing their host plant consumption might be a response to acute stress as the prey species' resting metabolic rate increases (Thaler et al. 2014). In my research, the presence of *P. melanarius* and *Pardosa spp.* decreased canola damage by flea beetle feeding either on the stem, cotyledons, or both. Both predators reduced the overall abundances of flea beetles so this effect might be caused by direct consumptive effects rather than non-consumptive effects. The success of *P. melanarius* and *Pardosa spp.* to reduce flea beetle abundances and negatively impact their feeding behaviour under laboratory conditions doesn't necessarily translate to field conditions. In the field, there are numerous factors that affect the ability of these generalist predators to be a successful control method for flea beetles in canola crops including predator density, prey density, abundance of alternative prey and predator foraging behaviours (Barney and Pass 1986, Clark et al. 1994).

Increasing or conserving the abundances of generalist predators such as Lycosids and *P. melanarius* communities can be important for biological control to aid in flea beetle management along with other pests due to their polyphagous nature (Symondson et al. 2002). The ability to be polyphagous and switch between prey give generalist predators the ability to keep pest species

suppressed without lowering their abundance to alarming levels which would disrupt the community structure (Symondson et al. 2002). Lycosidae predators including *Pardosa spp.* have been shown to consume flea beetles in canola fields (Ekbom et al. 2014) and other chrysomelid species in wheat fields (Kheirodin et al. 2020) found by molecular DNA analyses. By encouraging Lycosidae presence in crop fields, pest densities including flea beetles were lowered in crops including radish, broccoli, potato and tomato and plant damage decreased (Riechert and Bishop 1990). Our results agree with this finding where Lycosidae predators decreased cotyledon damage and the prevalence of stem damage. Encouraging a higher density and increased immigration of *Pardosa spp.* into canola crops may result in lower canola plant damage at the seedling stage.

Lycosids and carabids both demonstrate the potential to be effective biocontrol agents together in crops such as squash by increasing fruit production with increased immigration (Snyder and Wise 1999). While this study did not test the interactions between *Pardosa spp.* and *P. melanarius* on flea beetle predation, the success they demonstrate predating alone shows the potential that they have as biocontrol agents in canola fields and future research looking into potential interference and relationships is encouraged. It would be beneficial to promote the immigration and conservation of these generalist predators to contribute to pest suppression within canola fields. This can be done by habitat manipulation, such as increasing refugia with straw shelters (Halaj et al. 2000) and mulch (Riechert and Bishop 1990) to increase Lycosidae abundances and adding flowering or grassy strips to promote *P. melanarius* immigration (Lys 1994). The potential for these predators to be conservation biological control agents should drive future research into their effectiveness under field conditions as well as looking into immigration and emigration rates with various habitat manipulation techniques to create adequate predator refugia.

4.3 Assessment of flea beetle damage

Another aspect of Integrated Pest Management that is vital to flea beetle management techniques is scouting and assessing in-field damage. Our research suggests a way to streamline scouting and damage assessments by increase scouting at higher temperatures and assessing prevalence of damage rather than overall damage amounts at lower temperatures. In addition, a new stem damage assessment method was created to provide the opportunity of assessing the

whole plant. This results in a more complete picture of the damage that the flea beetles are causing on canola seedlings. Through the knowledge that stem damage is primarily driven by prevalence of damage, we can efficiently incorporate stem damage into our damage ratings by checking for the prevalence of flea beetle damage on each stem. I found that stem damage can be predicted by the cotyledon damage, but it is unclear if it is a viable way to fully assess stem damage in practice. Therefore, future research should focus on using this new tool to estimate stem damage to determine the need of action thresholds for stem damage.

4.4 Effects of Temperature and Plant Density on Flea Beetles

This study reveals important information on abiotic effects on flea beetle species differences and canola feeding preferences. We now know that there is no difference in how the two species respond to different temperatures in terms of their defoliation amounts. Both flea beetles increase defoliation rates with temperature, which agrees with research on other chrysomelid species (Logan et al. 1985, Soares et al. 2015). This effect is likely due to higher glucosinolate levels in canola crops that occur at higher temperatures, which encourages flea beetle feeding stimulation (Valente Pereira et al. 2002). In our study, flea beetle damage at a variety of temperatures is seen. This knowledge will aid future management and field scouting strategies by increasing the amount of scouting and damage assessments done when the air temperature increases above 20°C and decreasing the amount of times the field is scouted when temperatures are below this level. This will ensure assessments are still being done to check for the presence of flea beetle damage, but the efficiency will be increased by assessing canola according to the air temperature.

With the knowledge that an increased temperature increased flea beetle feeding, this can be applied to research focusing on global warming and how that will affect the future of canola crop health and their pests. Future climate conditions are predicted to increase pest status in flea beetles in Canada and result in more economic loss over a greater area in North America (Olfert et al. 2018). As temperatures continue to rise, other factors such as spring emergence times will be altered potentially lengthening the pest's feeding season (Olfert et al. 2018). Further, the future temperature shift and the associated decrease in water produced smaller stems and leaves in canola (Qaderi et al. 2006). This increase in wilting canola plants will promote flea beetle feeding since feeding is preferred on plants that are already wilted due to the increased

production of the stress hormone abscisic acid, which leads to an increased production of glucosinolates in the canola tissue (Bodnaryk 1994, Xu et al. 2020). An increase in glucosinolates may lead to a greater presence of flea beetles since they rely on it to find their host plants (Nielsen et al. 1979), but may not necessarily increase their feeding rate (Bodnaryk and Palaniswamy 1990). The flea beetles' preference and the increased ability to find water-stressed plants, however, means that more feeding is likely to occur on these plants in the field.

Another reason as to why more feeding is observed at higher temperatures could be due to the increased activity of flea beetles when compared to cooler temperatures (Burgess 1977, Soroka et al. 2005). The more flea beetles move around from plant to plant, the more each plant is likely to see damage. In addition, the feeding rate is likely to increase with warmer temperatures as seen in other chrysomelid species (Noronha and Cloutier 2006) thus promoting an increased prevalence of attack and more tissue consumed at each attack. This is consistent with our findings since a higher temperature increased the prevalence of attack and the overall damage for both stems and cotyledons. This suggests more active flea beetles that are eating plant tissue at a faster rate when temperatures are higher.

4.5 Implications for Insecticide Application Methods and Plant Density in Canola Fields

The strong evidence that increased plant density provides an increased yield independently of the insecticide management strategy used can provide useful insight into spray decisions for canola growers in the Canadian prairies. The success of each flea beetle management strategy used varied, showing either seed or a prophylactic method being the most effective, showing that seed-coated neonicotinoids are effective at reducing defoliation in canola. Moreover, foliar insecticide at 25% damage also reduced defoliation compared to the control, indicating promising management strategies if limited neonicotinoid use is necessary due to unwanted environmental effects (Desneux et al. 2007, Morrissey et al. 2015, Douglas et al. 2015) and decreased efficacy for *P. striolata* due to tolerance (Tansey et al. 2009).

This research provides good evidence of both the resource concentration hypothesis as well as the dilution effect since the trends in the data shows increased plant density increases the flea beetle abundance while decreasing flea beetle damage. While it is clear that flea beetles are being attracted to higher density plots, which is offset by their abundances being diluted at higher host plant densities, it is unclear why the number of flea beetles doesn't directly affect the

amount of defoliation per plant. While it is possible that flea beetle consumption rates have no relationship to their abundances, it is also reasonable to consider the possibility that current monitoring methods are not accurately portraying the abundances of flea beetles. More research into examining methods of monitoring flea beetle abundances and finding a relationship to damage on canola is necessary to make predictions based on observed numbers of flea beetles.

4.6 Conclusion

Phyllotreta flea beetles continue to be one of the most economically devasting pests of canola crops in North America. To have effective pest management strategies, there first needs to be an understanding on biotic and abiotic factors that change the way flea beetles feed on canola. In addition, cultural control methods such as increasing the planting density need to be used in conjunction with chemical control to avoid the chronic overuse of insecticide application in canola. Conserving *P. melanarius* and *Pardosa sp.* populations in canola may be an important step in improving pest management for flea beetles. The presence of these predators decreases plant damage and flea beetle abundances under laboratory conditions, so the next vital step is observing these interactions in field conditions to see if their effects translate. In addition, increasing scouting during warmer temperatures and improving the technique by examining stem damage in conjunction with defoliation will increase our chances for early detection of flea beetles to improve the yield of canola crops.

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