Interactions of pea leaf weevil (Coleoptera: Curculionidae) with its primary and secondary host plants in Alberta.

By

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Abstract

The pea leaf weevil, *Sitona lineatus* Linnaeus (Coleoptera: Curculionidae), is an invasive, oligophagous herbivore that feeds on a variety of legume (Fabales: Fabaceae) plants. Field peas (*Pisum sativum*) and faba bean (*Vicia faba*) are the primary host plants of the pea leaf weevil. Adult weevils feed on the foliage of the host plants and larvae feed on *Rhizobium leguminosarum* Frank (Rhizobiales: Rhizobiacea) bacteria associated with root nodules. Pea leaf weevil is a major pest of field peas and faba beans and adult and larval damage can cause significant economic losses to these crops. In this study, I investigate the effect of 1. behavioural plasticity of pea leaf weevil, 2. quality of pea leaf weevil host plants in relation to nitrogen content and legume-*Rhizobium* symbiosis, and 3. pea leaf weevil overwintering biology in shaping pea leaf weevil interactions with host plants and 4. applications to integrated pest management (IPM).

Pea leaf weevil adults feed on legumes during two different reproductive states in a one-year life cycle. In the spring, individuals are reproductively active, and in the fall adults are reproductively inactive and feed to prepare for overwintering. The reproductive state of pea leaf weevil can influence host choice because larvae must feed on root nodules of the primary hosts to develop. I investigated how weevil reproductive state modulates host finding behaviour in the pea leaf weevil. The pea leaf weevil preference for primary hosts, field pea and faba bean, is reproductive state-dependent. Pre-reproductive weevils exhibit a preference for faba bean over field peas, while reproductively inactive weevils show no preference between hosts and the preference for faba bean is strongly linked to larval development. My findings suggest that oligophagous insects may show phenotypic plasticity in host preference to maximize fitness benefits

Legumes exhibit species-specific symbiotic associations with *Rhizobium* bacteria that can affect host quality (nitrogen content and host defences), and hence the above-ground and below-ground insect-plant interactions. I investigated the influence of species-specific legume-*Rhizobium* symbioses on pea leaf weevil adult and larval interactions with its primary and potential secondary host plants. The legume-*Rhizobium* association drives pea leaf weevil larval development, but not adult host acceptance. Larvae only survive in nodules associated with *Rhizobium leguminosarum* in field peas and faba bean. Soybean is an occasional host to larvae. Adults feed on most of the tested legume species (field peas, faba bean, alfalfa, lupin, soybean) but not on chickpeas and little feeding occurs on lentils. *Rhizobium* increased nodulation and protected the yield of faba bean while nitrogen amendment did not protect yield. Taken together, these findings suggest that changes to host quality as a result of legume-*Rhizobium* symbioses influence weevil- host plant interactions and have the potential to protect faba bean yield from pea leaf weevil damage.

A better understanding of cold hardiness and factors influencing cold hardiness is key to predict invasive pest populations. I investigated the overwintering survival and cold hardiness of the pea leaf weevil in its expanded range and the influence of overwintering temperature and duration on pea leaf weevil post-overwintering interaction with its host plants. The overwintering survival of pea leaf weevil adults increased with soil temperature and more weevils survived when they overwintered at tree shelterbelts compared to in open alfalfa fields. The lethal temperature threshold for pea leaf weevil in Alberta is -9.38°C. Warmer and longer winter periods increased post-overwintering feeding and oviposition of pea leaf weevil. My study highlights that warm winters would increase overwintering survival and post -overwintering fitness and facilitate pea leaf weevil invasion in the Prairie Provinces of Canada.

Pea leaf weevil-plant interactions vary with weevil physiological state and plant host quality, and are shaped by overwintering temperature and duration. This information on pea leaf weevil-host interactions will contribute to IPM of pea leaf weevil in Alberta. A major goal of IPM is to prevent pest species from reaching the pre-determined threshold level to minimize economic losses. I developed a data-based nominal threshold for pea leaf weevil on faba bean to contribute to the IPM of pea leaf weevil in Alberta.

Preface

A version of Chapter 2 of this thesis is intended for publication as: Wijerathna A, Cárcamo H and Evenden M. State-dependent plasticity drives host acceptance and feeding preference of an oligophagous insect. I contributed to the experimental design and was responsible for data collection, analysis and manuscript composition. Maya Evenden and Hector Cárcamo contributed to the experimental design and planning of the experiments and were involved with the concept formation and manuscript editing.

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Dedication

In memory of my beloved mother, D. Sriyalatha Polathumodera…

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IPM – Integrated Pest Management

HIPVs – Host Induced Plant Volatiles

SA – Salicylic Acid

JA – Jasmonic Acid

ET - Ethylene

SCP – Supercooling Point

LRDC- Lethbridge Research and Development Centre

ANOVA – Analysis of variance

Sym – Symbiosis signaling pathway

Nod – nodulation factor

ET – Economic Threshold

EIL – Economic Injury Level

AIC – Akaike Information Criterion

MCMCglmm – Multivariate Generalised Linear Mixed Models using Markov chain Monte Carlo techniques

Chapter 1 **General Introduction**

Insect-plant interactions

Phytophagous insects depend on plant resources to forage, feed, find mates, court, oviposit and overwinter at some stage of their life. Host plants influence most of the crucial behaviours that affect insect fitness such as feeding, mating and oviposition. Therefore, host selection is key for herbivorous insects, and especially so for those with a restricted host range. Insect-plant interactions are modulated by 1. fitness of the insect herbivore (plasticity, physiology, adaptation, mutations), 2. ecosystem characters (macro/microfauna, plant species, nutrients, soil characters), and 3. climate dynamics (temperature, winter conditions, precipitation) (Fig. 1-1). In order to better understand the population dynamics of insect herbivores and to aid in management of insect herbivores considered to be pests, it is important to understand how these factors shape insect-plant interactions.

Individual traits of an insect herbivore that affect fitness can alter its interactions with host plants. Phenotypic plasticity is one way that organisms maximize their fitness under dynamic environments (Anderson et al. 2013, Lhomme et al. 2018, Maák et al. 2020). The preference-performance hypothesis suggests that female insect herbivores should select host plants for oviposition that maximize offspring fitness (Gripenberg et al. 2010). The selection of a suitable host plant, however, is challenging for herbivorous insects when one or more of the following conditions occur: 1) the information on host quality is complex, 2) minimal information is available to assess host quality, and 3) the cost of information assessment is high. Phytophagous insects overcome these limitations by exhibiting behavioural phenotypic plasticity in host selection (Anton et al. 2007, Wolf et al. 2008, McNamara and Leimar 2010, Anderson et al. 2013, Dingemanse and Wolf 2013, Anderson and Anton 2014, Gadenne et al. 2016, Lemmen-

Lechelt et al. 2018, Silva and Clarke 2020). Behavioural plasticity allows individuals to adjust their response to host plants according to their physiological state and previous experience with host plants.

Ecosystem characters can also modify insect-plant interactions by directly or indirectly affecting bottom-up and top-down effects on host quality. Host quality varies by plant species, and plant nitrogen content is a major component that affects the host plant quality. Nitrogen levels influence diet quality and host plant defensive capacity that can subsequently affect the longevity and fecundity of herbivore insects (Awmack and Leather 2002). Plant-associated nitrogen-fixing microorganisms influence the quality and defensive strategy of the host plant against insect herbivores and, therefore, affect the behaviour and performance of herbivores (Katayama et al. 2010, Thamer et al. 2011, Pineda et al. 2012, 2013, Dean et al. 2014, Pangesti et al. 2015).

Variation in climatic conditions, especially changes in temperature, affect insect-plant interactions. Changes to the length or severity of overwintering conditions can cause a mismatch between host plant phenology and insect development (Menendez 2007, Forrest 2016, Uelmen et al. 2016). Insects in temperate habitats must dedicate energy and resources to successful overwintering. Cold winter conditions deplete energy stores and affect subsequent insect activity and fitness which can influence post-overwintering population dynamics (Marshall and Sinclair 2012, Sinclair 2015).

The study of insect-host plant interactions and factors that modulate these interactions provide a better understanding of insect population dynamics and aids in the development of Integrated Pest Management (IPM) programs of pest species. Integrated pest management is an approach that considers all possible plant protection measures and integrates appropriate measures to lower the impact of pests to an economically acceptable level (Barzman et al. 2015). The keystone element of IPM is “bioeconomics” which connects the biology and ecology of the target pest(s) with potential economic losses (Pedigo 1996). The integration of economics with information on insect-plant interactions that affect the population dynamics of insect herbivores will assist in the development of IPM programs.

Phenotypic plasticity

Phenotypic plasticity is the capacity of individuals (genotypes) to alter phenotypic traits as a function of environmental change (Whitman and Agrawal 2009, Dingemanse and Wolf 2013). Phenotypic plasticity is common in insects and can result in the plasticity of behavioural, physiological, morphological, neuronal and life-history traits (Nylin and Gotthard 1998, Van Dyck and Wiklund 2002, Anton et al. 2007, Anderson and Anton 2014, Lhomme et al. 2018, Manfredini et al. 2019, Gvoždík and Boukal 2021). Individuals that exhibit behavioural plasticity adjust their behaviour to suit the current environmental conditions, although some individuals exhibit behavioural plasticity in the absence of reliable environmental information (Stamps and Bell 2020).

In an ecological context, insect herbivores can exhibit plasticity in response to the nutritional, chemical and physical characters of the host plant environment. Plasticity, however, is expected to occur when a fitness advantage outweighs the costs associated with those strategies (Whitman and Agrawal 2009, Han and Brooks 2014). Plasticity is costly because plastic individuals must spend time and energy sampling environmental cues (DeWitt et al. 1998). Therefore, optimal plasticity is based on the degree of environmental variation, sampling cost and the number of available cues (DeWitt et al. 1998, Auld et al. 2010, Mathot et al. 2012).

Individuals at a given time point under the same environmental conditions can also exhibit variation in behavioural responses (adaptive plasticity) (Wolf et al. 2008). This plastic behaviour could be the result of individual state, spatial and temporal variation in environmental conditions in combination with competition for resources, direct social interactions, or context-dependent plasticity (Anton et al. 2007, Evenden and Gries 2008, Wolf et al. 2008, McNamara and Leimar 2010, Anderson et al. 2013, Dingemanse and Wolf 2013, Anderson and Anton 2014, Gadenne et al. 2016, Lemmen-Lechelt et al. 2018, Silva and Clarke 2020).

State-dependent behavioural plasticity

There is temporal and spatial variation in the diversity and abundance of host plants available for insect herbivores. Insects must recognize and respond to the most suitable host plants at the right stage of their life cycle (Mechaber et al. 2002, Damien et al. 2019). Insect herbivores often use chemical cues (semiochemicals) to locate preferred hosts for feeding or oviposition. Insect physiological state can influence the behavioural responses to these cues and alter insect response to potential host plants (Anton et al. 2007, Gadenne et al. 2016, Lemmen-Lechelt et al. 2018).

Many insect herbivores undergo physiological changes in the adult stage during reproductive maturation and mating. Changes in reproductive physiology can result in drastic changes in the olfactory-mediated behaviour of insects. Insect herbivores in different reproductive states have variable responses to volatile chemicals released from host plants (Mechaber et al. 2002, Anton et al. 2007, Lemmen-Lechelt et al. 2018). Mated female moths are more sensitive to and attracted by the volatile compounds released by host plants compared to unmated females (Masante-Roca et al. 2007, Saveer et al. 2012, Lemmen-Lechelt et al. 2018).

Response by the pepper weevil (*Anthonomus eugenii* Cano (Coleoptera: Curculionidae)) to host plant volatiles released from pepper (*Capsicum annuum* L. (Solanaceae)) varies with the reproductive state of the female weevils (Addesso et al. 2011). The mated females exhibit no preference for the volatiles from plants fed on by males, while unmated females prefer volatiles from plants fed on by males over plants with no feeding activity. Reproductively mature female pepper weevils can discriminate host volatiles from those of non-hosts plants, but younger females that are not reproductively mature do not discriminate between plants (Addesso and McAuslane 2009). Mated cigarette beetle (*Lasioderma serricorne* F. (Coleoptera: Anobiidae)) females are more responsive to host volatiles compared to unmated females (Mahroof and Phillips 2007). Mating decreases the responsiveness to a host kairomone in the Colorado potato beetle (*Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) (Dickens 2007).

The plasticity of behavioural response to plant volatiles based on the physiological state of insect herbivores is modulated by an interplay among hormones, neuropeptides and biogenic amines, acting at the peripheral and/ or central insect nervous system (Gadenne et al. 2016). For example, antennal sensitivity for host-plant volatiles increases after mating in the female noctuid moth, *Spodoptera littoralis* (Lepidoptera: Noctuidae) (Saveer et al. 2012). Up and down regulation of various olfactory genes increases after mating in female *Drosophila suzukii*, and mated females are attracted to fruit volatiles for oviposition after mating (Revadi et al. 2015, Crava et al. 2019). In general, the mechanisms driving plasticity in olfactory response at the antennal sensillar level are not known (Anton and Rössler 2021).

Experience-based behavioural plasticity

Behavioural plasticity is beneficial in variable environments when a single phenotype is not optimal (Mery and Burns 2010). Herbivorous insects can store reliable information from certain life experiences and alter behaviours to adapt to current environmental conditions, based on this stored information. This experienced-based plasticity allows individuals to make rapid responses to host plant cues under specific environmental conditions (Bruce and Pickett 2011, Coyle et al. 2011). For example, Black vine weevil (*Otiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae)) females prefer to oviposit on plants on which they previously fed, regardless of the plant nutritional content (Coyle et al. 2011).

Insects can acquire host-plant experience in both larval and adult stages of their life cycle (Anton and Rössler 2021). The experience gathered from the natal habitat during the early developmental stages can influence larval or adult host preference (Hopkin's host-selection principle) (Barron 2001). Also, experience acquired during the adult stage may cause long-lasting adaptive olfactory behavioural plasticity that persists over several generations (Davis and Stamps 2004). Experience-based plasticity is beneficial for insect herbivores that need to make fast and accurate decisions in host plant selection, especially when larval fitness differs among host plants. Prior experience, however, can also lead insect herbivores to select less suitable plants, which can place them at a fitness disadvantage (Liu et al. 2008, Jones and Agrawal 2019).

Benefits of behavioural plasticity in host finding

Behavioural plasticity allows insect herbivores to alter their phenotype to adaptively match a changing environment. This plasticity can result in an increase of niche breath, and geographic range through changes in dispersal and host colonization (Gibert et al. 2019).

Plasticity can prevent species extinction and create adaptive genetic changes that facilitate evolution (Whitman and Ananthakrishnan 2009).

An understanding of phenotypic plasticity in host selection by invasive insect herbivores can help forecast the establishment and spread of new species and the consequences of environmental disruption in the expanded range (Torchyk and Jeschke 2018). Differentially plastic responses to host plants may alter ecosystem interactions (Berg and Ellers 2010). The preference of insect herbivores for a particular host plant may affect plant community structure and the interactions between below-ground and above-ground communities. The variation in crop and herbivore performance and phenotypically plastic insect-host interactions can impact insect pest management strategies (Agrawal 2005, Whitman and Ananthakrishnan 2009).

Host plant quality

Host plant quality refers to plant traits that positively or negatively affect insect herbivores. Among many components that contribute to host plant quality, host nitrogen content greatly influences the performance and reproductive strategies of insect herbivores and insect-plant interactions. The "plant vigor hypothesis" proposes that more vigorous (nutritious) plants are more beneficial to the insect herbivores that feed on them (Price 1994). Nitrogen levels influence diet quality and plant defensive capability and can affect the survival and fitness of insect herbivores (Awmack and Leather 2002). The quality of the host plant can impact the lifetime fecundity of insects, especially those that produce and mature eggs continuously throughout the reproductive stage of their life cycle (Leather 2018, Cowie et al. 2019, Hopkins and Ekbom 1999). Host nitrogen content affects annual bluegrass weevil (*Listronotus maculicollis* Kirby (Coleoptera: Curculionidae)) host preference, as females prefer nitrogen-rich plants for feeding but lay more eggs on plants with moderate nitrogen levels (Simard et al. 2021). The European root weevil (*Mogulones cruciger* Herbst (Coleoptera: Curculionidae)) has higher fecundity after feeding on nitrogen-rich plants (Hezewijk et al. 2008). Insect herbivores use nitrogen content as an indicator to discern plant host quality and adjust their feeding strategies. Prior experience on a low-nitrogen diet can trigger prolonged consumption and slower growth of *Salvinia momultiplicalis* (Lepidoptera: Pyralidae) larvae on low-*vs.* high-nitrogen diets (Taylor 1989). The response of insect herbivores to host plant nitrogen level, however, varies by feeding guild and species (Awmack and Leather 2002).

In plants that use nitrogen-based defense compounds, nitrogen content affects synthesis of constitutive and induced host defensive secondary compounds. The synthesis of plant-produced nitrogen-containing defensive compounds, such as alkaloids and non-protein amino acids (Awmack and Leather 2002), varies with nitrogen availability to the plant (Dudt and Shure 1994, Stout et al. 1998, Lou and Baldwin 2004, Chen et al. 2008). These defensive compounds can directly or indirectly affect insect herbivores (Chen et al. 2008, Couture et al. 2010).

Host plant quality: legume-Rhizobium symbiosis

Despite the importance of nitrogen in shaping insect-plant interactions, nitrogen availability can be patchy and limited in the soil. Plants have evolved different mechanisms to deal with these nitrogen deficiencies, including: reprogramming nitrogen and carbon metabolism, altering biomass allocation between shoots and roots, and increasing root branching (Lou and Baldwin 2004, Scheible et al. 2004). Some plants, especially legumes (Fabaceae) have adapted to live in soil with low nitrogen content by evolving mutualistic associations with nitrogen-fixing bacteria. Over 15,000 plant species from nearly 12 families have symbiotic associations with nitrogen-fixing bacteria (Sprent and Sprent 1990). The economically important plant family, the Fabaceae, has symbiotic associations with nitrogen-fixing bacteria in the genus *Rhizobium*, commonly referred to as rhizobia (Biddle 2017).

The fixation of nitrogen by *Rhizobium* can affect plant interactions with herbivores and pathogens by altering the nutritional quality or production of plant defensive compounds (Katayama et al. 2010, Thamer et al. 2011, Pineda et al. 2012, 2013, Dean et al. 2014, Pangesti et al. 2015). Plants produce herbivore-induced plant volatiles (HIPVs) in response to insect herbivory (Jensen et al. 2002, Dicke and Baldwin 2010, McCormick et al. 2012). The production of HIPVs in plants is triggered by elicitors in insect saliva (Howe and Jander 2008) and regulated by herbivore-induced phytohormones, such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Kessler and Baldwin 2002, Van Poecke and Dicke 2002, Pieterse et al. 2012). Symbiotic *Rhizobium* have evolved to modulate plant hormone levels (Martínez-Abarca et al. 1998, Van der Ent et al. 2009) and these changes influence interactions with herbivores (Kempel et al. 2009, Thamer et al. 2011).

Nitrogen is a limiting factor for root-dwelling insect herbivores, and they have adapted to the low nitrogen levels in plant roots by feeding on root nodules that house symbiotic *Rhizobium* (Mattson 1980, Johnson and Rasmann 2015). Feeding on nitrogen-rich root nodules increases herbivore fitness and protects herbivores that feed within nodules from predators and extreme conditions (Byers and Kendall 1982, Goldson et al. 1985, Quinn and Hower 1986, Wolfson 1987, Gerard 2001, Barnett and Johnson 2013). Nodule feeding by below-ground herbivores reduces nodulation and can alter plant nitrogen content (George 1962, Allen and Allen 1981, Vankosky et al. 2011). Legume-*Rhizobium* associations are species-specific, and these specific interactions may affect host plant quality in unique patterns. Therefore, these symbioses may shape the insect-host plant interactions through bottom-up effects and affect host use by insect herbivores.

Overwintering survival and cold hardiness

Insects are ectothermic organisms that regulate body temperature to survive adverse climatic conditions. Insects exhibit a range of behavioural and physiological mechanisms to adapt to cold winters, including: dispersal to favourable habitats, entering a diapause or quiescent state, and physiological mechanisms to overcome cold and freezing (Danks 1996, Toepfer et al. 2000, Menu and Desouhant 2002, Matsuo 2006, Denlinger 2008, Marshall and Sinclair 2012). Insects can overcome freezing and cold using strategies of “freeze-tolerance” or “freeze-avoidance” (Salt 1961, Danks 1996). Freeze-tolerant insects can withstand extracellular ice formation, while freeze avoidance is achieved by a lowering of the supercooling point (SCP) of the insect body fluid.

Insect-plant interactions are impacted by the overwintering survival and cold hardiness of insect herbivores, along with the climatic conditions during winter. Global warming as a result of climate change has already altered the phenology of overwintering in many insect species (Menendez 2007, Forrest 2016, Uelmen et al. 2016). Warm winter temperatures can shorten the overwintering period of insects and result in the depletion of stored nutrients due to increased metabolic activity that could lead to early emergence from the overwintering state (Denlinger 1972, Cárcamo et al. 2009, Sinclair 2015). Alternatively, warm winter temperatures might delay or terminate emergence from overwintering if a chilling cue is required to terminate diapause (Fielding 2008). Both eventualities could lead to a mismatch in host-plant synchrony with insect herbivores that rely on the specific host plant.

Cold hardiness is an important adaptation that facilitates range expansion into colder areas that have not been previously occupied (Steenman et al. 2014). Range expansion will allow herbivores to encounter new host plants and alter feeding patterns and plant-insect interactions. The size of the post-overwintering population may vary depending on temperature during the winter and the duration of exposure to winter conditions (Bale and Hayward 2010). Post-overwinter dispersal might also vary with overwinter temperature due to direct effects on insect body condition that alters subsequent fitness (Marshall and Sinclair 2012, Sinclair 2015, Wiman et al. 2015, Schenk et al. 2018), or changes in insect population density post-winter (Cárcamo et al. 2009, Sorvari et al. 2011, Marshall and Sinclair 2015). Insect dispersal capacity, in part, determines the invasive nature of insect range expansion (Lombaert et al. 2014, Renault et al. 2018).

Insect-host plant interactions: Implications for pest management

Integrated Pest Management integrates numerous management tactics to prevent economic crop losses caused by pest damage (Poston and Pedigo 1983). A major goal of IPM in agriculture is to prevent pest populations from reaching an economic injury level (EIL) on the target crop plant (Stern et al 1959, Pedigo et al. 1986). The economic injury level is the lowest population density that will cause economic damage on the target plant at the time of management. To prevent pest population density from reaching the EIL, control measures should be applied at a lower pest population density; the economic threshold (ET) (Stern et al 1959, Pedigo et al. 1986). Economic injury levels and ETs are important components of IPM and are useful for decision-making for management action, including pesticide application (Stern et al. 1959, Pedigo et al. 1986, Sharma et al. 2011, Batistela et al. 2012, Ramsden et al. 2017). A nominal threshold serves the same purpose as ETs in IPM programs, and can be used when pest population density is unknown (Poston and Pedigo 1986, Tangtrakulwanich et al. 2014).

The integrations of pest biology and ecology with economic aspects of the managed agroecosystem allow for the development of EILs and economic thresholds. In order to make these connections it is important to understand the forces driving pest population dynamics. Variation in insect-pest interactions between the target pest and the crop to be protected can occur as a result of insect traits that impact fitness, host plant quality and climatic fluctuations that influence pest populations. A better understanding of host plant-insect herbivore interactions would assist in the implementation of the EIL metric and subsequent management tactics.

Pea leaf weevil-legume interactions

The pea leaf weevil, *Sitona lineatus* Linnaeus (Coleoptera: Curculionidae), is an invasive agricultural pest of field peas (*Pisum sativum*) and faba bean (*Vicia faba*) (Jackson 1920) in several regions of the world. Pea leaf weevil is native to Europe and North Africa and has become an invasive pest in North America (Vankosky et al. 2009). Since the 1930s, pea leaf weevil has become established in the northwestern regions of the United States of America (USA) including Washington, Oregon, northern California and northwestern Idaho (Hubert and Reehe 1961, Hoebeke and Wheeler 1985). Pea leaf weevils were first recorded in northeastern USA in 1984 (Hoebeke and Wheeler 1985) and in Florida in 2002 (Bloem et al. 2002). Recently, pea leaf weevil has been recorded in North Dakota, USA (Pea leaf weevil detected in western N.D, 2017). The pea leaf weevil was first recorded in southern Alberta, Canada in 1997 in field peas (Vankosky et al. 2009). Pea leaf weevil is expanding its range in North America to include the Prairie Provinces of Canada (Hubert and Reehe 1961, Hoebeke and Wheeler 1985, Bloem et al. 2002, Cárcamo et al. 2018, Gavloski 2019). Canada is among the world's largest pulse producers (https://pulses.org/what-are-pulses/where-do-pulses-grow). Understanding pea leaf weevil interactions with its primary host plants in its expanded range is important for the prediction of its population dynamics, invasion to new areas, and the management of populations that cause economic damage.

Pea leaf weevil adults are oligophagous on legumes (Fabales: Fabaceae) and feed on a variety of leguminous plants, but field pea and faba bean are the primary host plants and are required for reproduction (Jackson 1920, El-Dessouksi 1971, Nielsen 1990). Larvae mainly feed within the root nodules of the primary hosts. Feeding by pea leaf weevil larvae on root nodules and adults on foliage can cause yield losses in both field peas and faba bean (El-Dessouksi 1971, Nielsen 1990). Feeding damage results in reduced seed and soil nitrogen content in field peas (Doré and Meynard 1995, Corre-Hellou and Crozat 2005), and reduces the production of pods in faba bean (El-Dessouksi 1971).

Pea leaf weevil undergoes a univoltine life cycle in its expanded range on the Canadian Prairies (Fig. 1-2). Adult weevils occur in two different physiological states in the one-year life cycle. Reproductively inactive adults overwinter in field margins and shelterbelts containing perennial legumes (Jackson 1920, Schotzko and O'Keeffe 1988, Murray and Clements 1992, Landon et al. 1995). In the spring, adults emerge from overwintering in a reproductively immature state, and feed initially on secondary host plants, such as alfalfa (*Medicago sativa*), before dispersing in search of primary host plants (Hamon et al. 1987). Location of suitable primary host plants is crucial for spring dispersing weevils because they have to feed on field pea or faba bean to become reproductively active (Jackson 1920, Landon et al. 1995).

Pea leaf weevil antennae respond electrophysiologically to volatile organic compounds released by primary host plants (Blight et al. 1984, Landon et al. 1997). Leguminous plants have similar volatile organic compound profiles but the proportions of individual chemical constituents that occur in the blend vary with plant species (Landon 1997). Both primary hosts of the pea leaf weevil produce the same major host plant volatiles (*Z*)-3-hexen-1-yl acetate and (*Z*)-3-hexen-1-ol (Blight and Wadhams 1987, Landon 1997). Linalool is also emitted by faba bean (Blight et al. 1984) while, 2-hexanal is emitted by field pea (Landon 1997). Pea leaf weevil adults in the different reproductive states exhibit variation in behavioural and electrophysiological response to pea plant volatiles (Landon 1997). Response to host volatile cues measured in the laboratory is greatest during dispersal periods (Landon 1997), but synthetic copies of host plant volatiles do not attract pea leaf weevil in the field (Blight et al. 1984, 1991, Evenden et al. 2016, St Onge et al. 2018). The response of adult pea leaf weevils to semiochemicals is plastic (Landon 1997), but it is not known whether differences in reproductive state influence host acceptance and feeding preference.

Reproductively active male pea leaf weevils produce an aggregation pheromone, 4-methyl-3,5-heptanedione, which attracts both males and females (Blight et al. 1991, Evenden 2018). Faba bean host volatiles synergize adult weevil response to the aggregation pheromone, in the fall but not in the spring (Evenden et al. 2016, St Onge et al. 2018). It is not known whether host selection decisions in the spring are crucial for pheromone production by males, as the effect of host plants on pheromone production in this species has not been studied to date. Pheromone-mediated aggregation leads to mating encounters, followed by oviposition in soil cavities close to the primary host plant (Jackson 1920).

Developing larvae feed on *Rhizobium leguminosarum* Frank (Rhizobiales: Rhizobiacea) bacteria associated with root nodules of primary host plants (Hamon et al. 1987). Host selection by pea leaf weevil adults is crucial for larval development because larvae develop in root nodules and larval success is dependent on the availability and density of root nodules. Although pea leaf weevil adults feed on a variety of legume plants, larval feeding has only been recorded on field peas, faba bean and to a lesser extent on chickpeas (Jackson 1920, Fisher and O'Keeffe 1979a, 1979b, Fisher and O'Keeffe 1979, Hoebeke and Wheeler 1985, Hamon et al. 1987, Williams et al. 1991, Murray and Clements 1992). Legumes have species-specific interactions with symbiotic *Rhizobium* bacteria, and it is not known whether there is an influence of host-specific *Rhizobium* strains on adult host preference and larval development of the pea leaf weevil. *Rhizobium* inoculation suppresses root nodulation on field peas (Vankosky et al. 2011). It is necessary to understand the effect of the *Rhizobium*-legume symbiosis on host-plant interactions of the pea leaf weevil.

After pupation in the soil, new generation adults feed on any late maturing Fabaceae before dispersing to overwintering sites often in perennial legumes (Jackson 1920, Fisher and O'Keeffe 1979, Hamon et al. 1987, Murray and Clements 1992). In the fall, adult weevils are reproductively inactive and lack a developed reproductive tract (Schotzko and O'Keeffe 1986). Less is known, however, about the overwintering biology of the weevil, which when combined with estimates of population density taken in the fall (St. Onge et al. 2018), is crucial for pest management decisions prior to spring planting. No research has been conducted to date on the effects of overwintering conditions on post-overwintering host-plant interactions and future research is required to understand pea leaf weevil range expansion with the warming climate.

Thesis objectives

In this thesis, I identify knowledge gaps in pea leaf weevil-host plant interactions in its expanding range in the Prairie Provinces of Canada. I investigate how insect fitness (plasticity), ecosystem characters (plant species and host quality) and climatic dynamics (overwintering temperature, duration and microhabitats) influence pea leaf weevil interactions with its host plants. In Chapter 2, I investigate the state-dependent phenotypic plasticity of pea leaf weevil host acceptance and host preference. A series of no-choice and choice laboratory experiments assesses the influence of pea leaf weevil reproductive state on host acceptance and feeding preference between its primary host plants, field pea and faba bean, and the secondary host plant, alfalfa. I further evaluate the effect of primary host plants on larval development in a no-choice experiment. In Chapter 3, I investigate the influences of host quality on pea leaf weevil-plant interactions. I manipulate host quality through treatments that alter the legume-*Rhizobium* symbiosis and nitrogen content of the host plant. In a no-choice experiment, I investigate the effect of species-specific legume-*Rhizobium* associations on pea leaf weevil adult host acceptance and larval development using seven different potential host plants. I also investigate the effects of *Rhizobium* and nitrogen on adult pea leaf weevil host acceptance, feeding preference, oviposition and larval development on faba bean using a series of field and laboratory experiments. In Chapter 4, I investigate the overwintering survival and cold hardiness of pea leaf weevil in its expanded range. A field study evaluates the overwintering survival of adult pea leaf weevil in three different sites in the expanded range and the influence of overwintering microhabitat on overwintering survival. I determine the supercooling point of overwintered weevils as a measure of cold hardiness. I investigate the influence of hormones on cold hardiness and the effects of overwintering conditions on pea leaf weevil post-overwintering feeding and oviposition in the proximity of host plants. In Chapter 5, I develop a nominal threshold for pea leaf weevil on faba bean in a three-year field study which will serve as the foundation of an IPM approach to pea leaf weevil management in its expanding range.

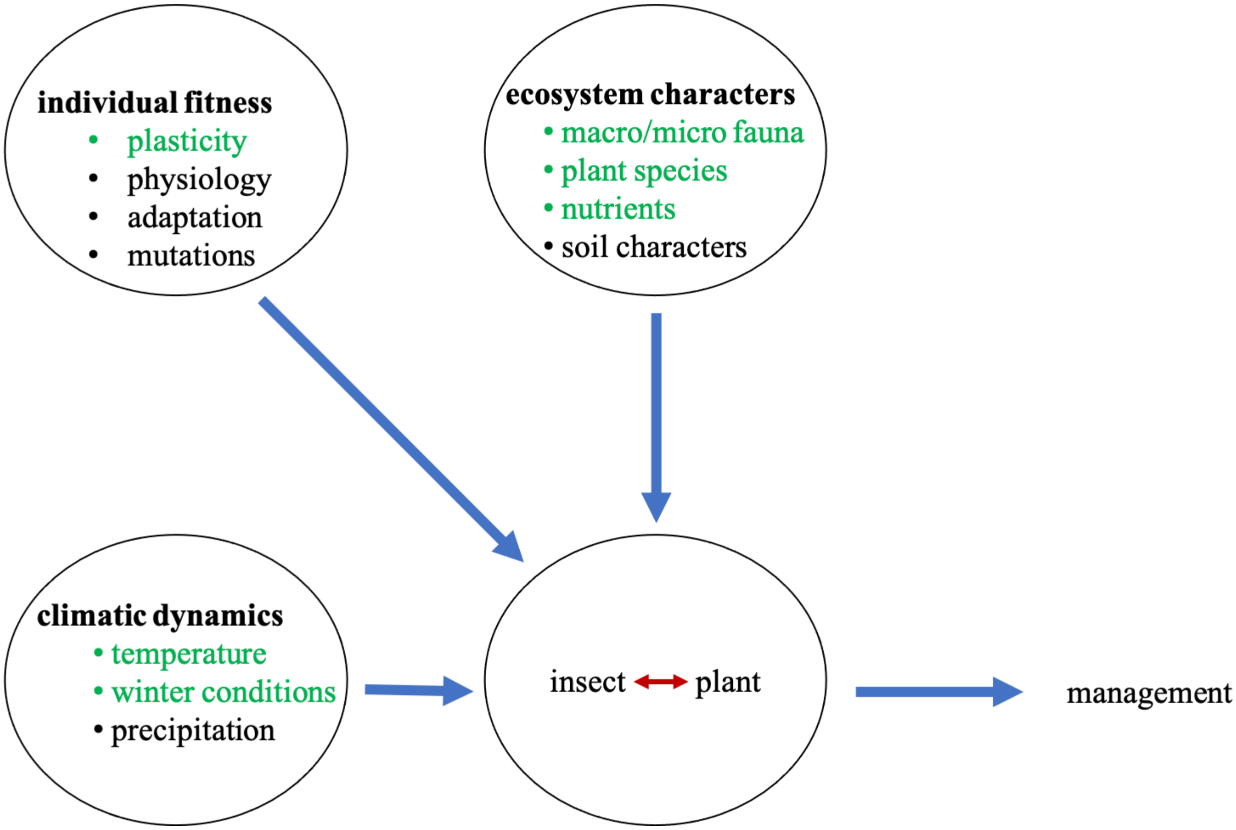

Figure 1-1. A schematic diagram showing three major factors that modulate insect-plant interactions. Insect-plant interactions are modulated by, the fitness of the insect herbivore, ecosystem characters, and climate dynamics. A better understanding of the influence of these factors on insect-plant interactions and, hence the population dynamics of insect herbivores, could aid in the development of Integrated Pest Management (IPM) program of the target insect pest(s). The factors of interest in this thesis are highlighted in green.

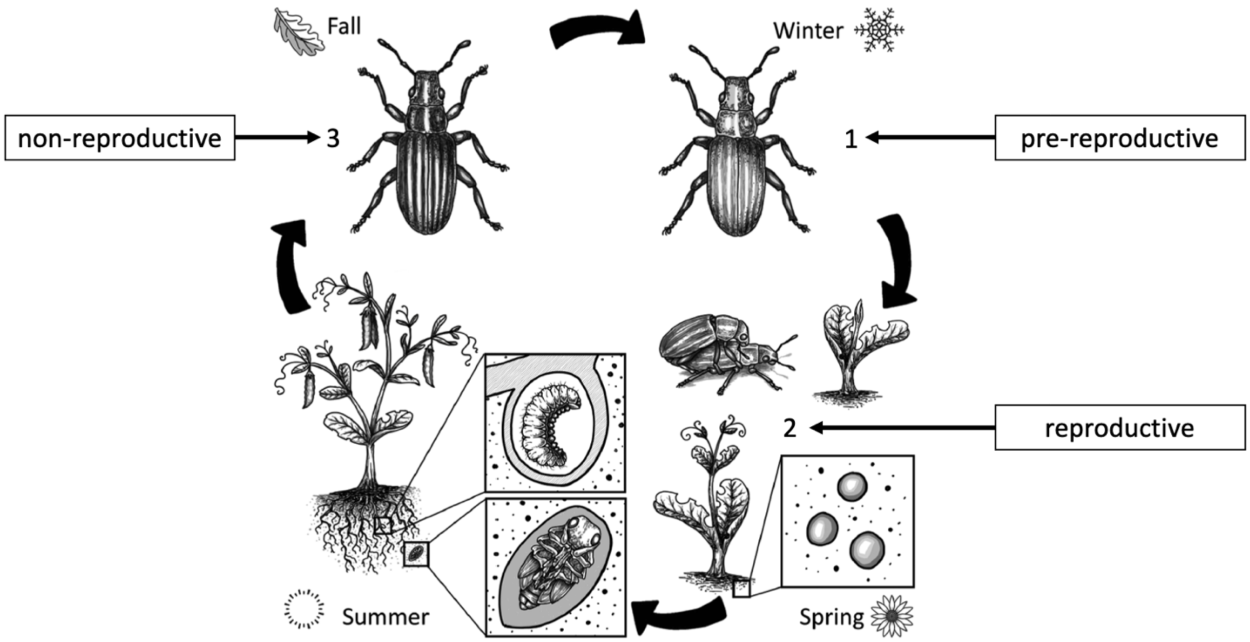

Figure 1-2. Pea leaf weevil life cycle in relation to season, plant phenology and reproductive stage. Pea leaf weevils overwinter as adults and emerge in early spring. These emerging pre-reproductive weevils (1) feed on available secondary host plants before dispersing in search of primary host plants (field pea and faba bean). After finding primary host plants adults feed on foliage, mate and reproductive females (2) lay eggs in the soil crevices near the plant. Larvae develop inside root nodules of host plants feeding on root nodule associated with *Rhizobium* bacteria. Larvae pupate in the soil, newly eclosed adults feed on remaining primary and secondary host plants. These adults are non-reproductive (3) and disperse in search of overwintering habitats at the end of the fall. Figure adapted from Evenden 2018.

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Chapter 2 **State-dependent plasticity drives host acceptance and feeding preference of pea leaf weevil (Coleoptera: Curculionidae)**

Abstract

Herbivores often respond to cues from host plants when searching for a suitable host for feeding or oviposition. State-dependent response to this information can help herbivores utilize resources when they are physiologically capable. This is particularly important for herbivores with a specialized diet that need to access specific host plants at certain phenological stages. The pea leaf weevil, *Sitona lineatus,* is an oligophagous insect herbivore that feeds on leguminous host plants. Adult weevils are in different reproductive states in the one-year life cycle. Pre-reproductive adults emerge from overwintering sites in the spring and feed on secondary legume plants before dispersing to primary host plants field pea and faba bean, where they feed and become reproductive. Adults in the fall are reproductively inactive and feed on both primary and secondary legume plants. In this chapter, I investigate how reproductive state modulates host finding behaviour in the pea leaf weevil. Pea leaf weevil adults were exposed to field pea and faba bean seedlings in a series of choice and no-choice tests and counted the feeding notches on leaves after five days. The experiments were repeated introducing secondary host plant, alfalfa. I found that pea leaf weevil preference for the primary hosts, field pea and faba bean, is state-dependent. Pre-reproductive weevils exhibit a preference for faba bean over field peas, while reproductively inactive weevils show no preference between hosts. The state-dependent plasticity in host preference disappears when weevils are presented with both primary hosts and a secondary host, alfalfa. This study suggests that oligophagous insects may show phenotypic plasticity in host preference to maximize fitness benefits.

Introduction

Behavioural phenotypic plasticity is the ability of one genotype to produce multiple behavioural phenotypes in response to changing environmental conditions (Mery and Burns 2010). For behavioural plasticity to occur, organisms usually develop associations between information from their environment and corresponding behavioural response(s), although organisms can also exhibit plastic behaviours in the absence of reliable information (Stamps and Bell 2020). Behavioural phenotypic plasticity is one way that organisms maximize fitness under dynamic environments (Anderson et al. 2013, Lhomme et al. 2018, Maák et al. 2020). Host plant choice is crucial for specialist herbivores and behavioural plasticity in host plant choice promotes resource use in complex environments with temporal and spatial variation of suitable host plants (Anderson and Anton 2014, Lhomme et al. 2020). Behavioural plasticity permits herbivores to colonize new habitats (Riffell et al. 2008), and avoid unsuitable or non-host plants (Mader et al. 2012, Lhomme et al. 2018, Lhomme et al. 2020). Selection of a suitable host is particularly critical for oligophagous or monophagous herbivores when offspring fitness is determined by the host plant characteristics. In herbivorous insects, the preference-performance hypothesis suggests that female insect herbivores should oviposit on, in or near host plants that maximize the fitness of their offspring (Thompson 1988). Host choice for high-quality plants is more apparent in oligophagous than polyphagous insect herbivores and offspring survival is higher on preferred host plants (Gripenberg et al. 2010).

Insect herbivores often use chemical cues (semiochemicals) to locate preferred hosts for feeding or oviposition. The physiological state of herbivorous insects (Anton et al. 2007, Evenden and Gries 2008, Gadenne et al. 2016, Lemmen-Lechelt et al. 2018) and previous experience with the host plant (Anderson et al. 2013, Anderson and Anton 2014, Silva and

Clarke 2020) can influence the response to these semiochemicals. There is a cost associated with adjusting an innate preference for a host, and the cost of decision-making increases with increasing complexity of the environment (Bernays 2001, Mery and Burns 2010). Plastic behaviours can differ with physiological state to maximize fitness. Insect herbivores should be most acute to host plant cues when they are physiologically prepared to use the plant for feeding or oviposition (Mechaber et al. 2002, Lemmen-Lechelt et al. 2018, Damien et al. 2019). Physiological state-dependent plasticity can minimize the mismatch between conditions in the environment and insect development or reproduction because host plant choice decisions are made based on the current state of the insect herbivore.

Prior experience with plant volatiles can alter the strength and type of response by insect herbivores to plant-produced cues (Bruce and Pickett 2011, Anderson and Anton 2014). Insect herbivores can learn host volatile chemical signatures through neuromodulation (Busto et al. 2010, Riffell et al. 2012) and exhibit context-dependent neuronal plasticity to modulate host location (Gadenne et al. 2016). Although experience-based plasticity may be beneficial in coping with a changing environment, it can also be costly (Dukas 2008). Experience with alternative hosts may lead insect herbivores to choose poor quality host plants and put them at a fitness disadvantage (Liu et al. 2008, Jones and Agrawal 2019). Prior experience of larval or adult insect herbivores with the host plant can lead to modulation of subsequent host selection behaviours (Anderson and Anton 2014) that influences feeding and oviposition behaviours and host plant acceptance (Zhang et al. 2007). Most insect herbivores, however, show a hierarchical response to host plants based on response to unique blends of ubiquitous host volatile compounds (Bruce and Pickett 2011).

Host plant selection is a complex process for insect herbivores, in which behavioural responses result from the integration of intrinsic (innate preference, behavioural plasticity, experience) and extrinsic (host plants, natural enemies) factors (Carrasco et al. 2015). The intrinsic and learned behaviours co-exist and might result in complex behavioural responses for host location. The underlying neural pathways, between state-dependent innate and learned behaviours are shared and contribute to plastic host location behaviours (Kadow 2019). Despite the integration of these neural pathways, the state-dependent and experience-dependent plasticity in host selection are mostly studied separately. In this study, I investigate state-dependent plasticity of host selection and how experience and the presence of a secondary host affect host selection in an oligophagous insect.

The pea leaf weevil, *Sitona lineatus* Linnaeus (Coleoptera: Curculionidae), is an invasive agricultural pest of field peas (*Pisum sativum*) and faba bean (*Vicia faba*) (Jackson 1920) in several regions of the world. It is oligophagous on legumes (Fabales: Fabaceae) and feeds on a variety of leguminous plants. Field pea and faba bean are the primary host plants of pea leaf weevil (Jackson 1920, El-Dessouksi 1971, Nielsen 1990), as larvae mainly survive in the root nodules of these hosts. Pea leaf weevil is an ideal candidate to study state-dependent plasticity in host location, as there are two different reproductive states in the adult stage of the one-year life cycle. Pea leaf weevil adults overwinter in a reproductively inactive state in field margins and tree shelters containing perennial legumes (Jackson 1920, Schotzko and O'Keeffe 1988, Murray and Clements 1992, Landon et al. 1995). In the spring, adults emerge from overwintering sites in a reproductively immature state and feed initially on secondary host plants, such as alfalfa (*Medicago sativa*), before dispersing in search of primary host plants (Hamon et al. 1987). Location of a suitable host is crucial for spring dispersing weevils because they have to feed on field pea and faba bean to become reproductively active (Jackson 1920, Landon et al. 1995). Adults feed on host foliage in characteristic "U" shaped notches along leaf margins (Jackson 1920). Male pea leaf weevils produce an aggregation pheromone, 4-methyl-3,5-heptanedione, which attracts both males and females (Blight et al. 1991, Evenden 2018). It is not known whether host selection decisions in the spring are crucial for pheromone production by males, as the effect of host plants on pheromone production in this species has not been studied to date. After mating, females deposit eggs in soil cavities close to the primary host plant (Jackson 1920). Developing larvae feed on *Rhizobium leguminosarum* Frank (Rhizobiales: Rhizobiacea) bacteria associated with root nodules of primary host plants (Hamon et al. 1987). Host selection by pea leaf weevil adults is also crucial for larval development because larvae develop in root nodules and larval success is dependent on the availability and density of root nodules. Larvae feed and pupate in the soil, and adults eclose after ~3 weeks (Jackson 1920). Post-eclosion, new generation adults are oligophages and feed on any late maturing Fabaceae before dispersing to overwintering sites such as forage alfalfa fields (Jackson 1920, Fisher and O'Keeffe 1979, Hamon et al. 1987, Murray and Clements 1992). Adult weevils in the fall differ in reproductive state from overwintered spring weevils as they are not reproductively active and do not have a developed reproductive tract (Schotzko and O'Keeffe 1986).

The response of adult pea leaf weevils to semiochemicals is plastic (Landon 1997). As spring and fall adult weevils are in different reproductive states (Evenden 2018), this plasticity may affect weevil host acceptance and feeding preference. Leguminous plants produce similar volatile compounds but in different proportions (Landon 1997). Both primary hosts of the pea leaf weevil produce the same major host plant volatiles (*Z*)-3-hexen-1-yl acetate and (*Z*)-3-hexen-1-ol (Blight and Wadhams 1987, Landon 1997). Linalool is only emitted by faba bean

(Blight et al. 1984) while, 2-hexanal is only emitted by field pea (Landon 1997). Pea leaf weevil exhibit variation in behavioural and electrophysiological response to pea plant volatiles in laboratory bioassays during the two different reproductive stages (Landon 1997). Response to host volatile cues measured in the laboratory is greatest during dispersal periods (Landon 1997), but synthetic copies of host plant volatiles do not attract pea leaf weevil in the field (Blight and Wadhams 1987, Blight et al. 1991, Evenden et al. 2016, St Onge et al. 2018). Male pea leaf weevils produce aggregation pheromone when they are reproductively active in the spring (Blight et al. 1991), but weevils of both sexes respond to the aggregation pheromone in both spring (Blight and Wadhams 1987, Nielsen 1993, Evenden et al. 2016) and fall activity periods (Blight et al. 1991, Evenden et al. 2016, St Onge et al. 2018). Faba bean host volatiles synergize adult weevil response to the aggregation pheromone, during fall, but not in spring (Evenden et al. 2016, St Onge et al. 2018).

Because host location is heavily reliant on semiochemicals in herbivorous insects, they should have a strong behavioural response to the appropriate blend of host volatiles (Bruce and Pickett 2011). Therefore, it is crucial for pea leaf weevil adults to respond to the right blend of host volatiles during each reproductive state. In this chapter, I test the hypothesis that adult pea leaf weevil reproductive state influences host acceptance and feeding preference among primary and secondary leguminous hosts. I further test the hypothesis that host acceptance and preference influence offspring development in each host plant. I predict that pea leaf weevil adults will exhibit plastic host selection behaviours that differ when they are pre-reproductive in spring and reproductively inactive in the fall. I predict that pre-reproductive weevils will choose the host plant that maximizes larval development and that the availability of secondary host plants will influence these plastic host selection behaviours.

Materials and Methods

Insects

Pea leaf weevil adults were collected on legume crops using sweep nets at fields close to the Agriculture and Agri-food Canada, Lethbridge Research and Development Center (LRDC), Lethbridge, Alberta, Canada (49.7004°N - 112.7632°W). Collected weevils were fed on alfalfa *ad libitum* in mesh cages (47.5cm X 47.5cm X 93cm) before each experiment (23°C, 16:8 L:D). Conical flasks (0.5l) containing cotton swabs were used to provide water. Weevils were starved for 24 hours and weighed to the nearest 0.01 mg (Mettler Toledo, XS105, Columbus, OH) before the experiments.

Plants

Plants were grown under controlled conditions (21°C, 16:8 L:D) in low nitrogen soil inoculated with the corresponding *Rhizobium* inoculant at seeding. Faba bean (Zero tannin, CDC Snow Drop) and field pea (Smirnoff AAC, Lacombe) seeds were treated with Nodulator inoculant (1.222 kg/ 1000 kg seeds) (Nodulator® FB Peat, 2008027A, BASF Canada, Ontario, Canada) and alfalfa (AC, Blue Jay) seeds were treated with Dormal inoculant (0.108g /5.98g seeds) (850057A, Becker Underwood, MO, USA). Plants were watered typically two-three times a week.

Pea leaf weevil host acceptance and feeding preference between primary hosts

This study was conducted in a greenhouse during the spring and fall of 2017. I conducted a series of no-choice and choice experiments with weevils in different reproductive states to test the hypothesis that adult weevil reproductive state influences feeding preference (choice tests) and host acceptance (no-choice tests) between the primary host plants, field pea and faba bean. Two plants of the same species or one plant of each species were planted in individual pots (diameter =15.24cm). Pots containing plants were caged individually using a mesh sleeve and four male or female weevils were introduced to plants at the 2nd unfolded leaf stage (Staging guide faba bean, no date) (n=10/sex) following a completely randomized design. The control pots did not receive any weevils. The experiments were conducted over five days under controlled conditions (21°C, 16:8 L:D). After five days of feeding, weevils were recovered from the plants and the number of feeding notches on foliage was counted. I also measured fresh and dry leaf weight and the C:N ratio of the leaves (Carlo Erba NA 2100 Elemental Analyser, Carlo Erba Strumentazione, Milan, Italy). I conducted this study with pre-reproductive weevils collected in the spring (May) and with reproductively inactive weevils collected in late summer (August). For both activity periods weevils were collected from alfalfa fields.

Pea leaf weevil host acceptance and feeding preference between primary hosts in the presence of secondary host

This study tested the hypothesis that adult weevil reproductive state influences host acceptance and feeding preference between primary hosts when a secondary host, alfalfa, is present. Plants were individually grown in pots (diameter = 10.16cm). Three pots, each containing one plant of the same species or three different species, were placed in a white plastic container (7.6l) with space between the pots filled with soil. Plastic containers containing the three plants were caged and the study was repeated following the same steps as outlined above. These choice and no-choice experiments were repeated three times using pre-reproductive weevils in the spring (May) (collected on alfalfa), reproductive weevils (June) (collected on faba bean) and fall- dispersing non-reproductive weevils (August) (collected on faba bean) in 2018.

The influence of pea leaf weevil primary host acceptance and feeding preference on offspring development

I conducted a final greenhouse study to determine whether preference and host acceptance influences larval development on different phenological stages of the primary host plants. Pre-reproductive pea leaf weevils were collected in the spring and fed *ad libitum* with alfalfa before the experiment. Two males and two females were introduced into a Petri dish (90mm) with a wet filter paper (Whatman #1) and a faba bean leaf to facilitate oviposition (n=20 Petri dishes). Eggs were collected daily and stored at 10°C prior to the experiment. Field pea and faba bean were grown individually in pots (diameter =15.24cm) and fifty eggs were placed at the base of each plant at either the 2nd or 5th unfolded leaf stage (n=10 plants/host/phenological stage). The soil around plants was sprayed with distilled water twice a day until egg hatch was considered complete (2 weeks). The control pots did not receive eggs but were caged in the same manner. Plants were harvested at the early flower stage and roots of each plant were washed and the number of larvae, the number of damaged root nodules, and the number of root nodules per plant were counted.

Data analysis

I analysed data using R version 3.6.3 (R Core Development Team 2018). Initial models contained all explanatory variables and interactions. Full and reduced models were tested using ANOVA hypothesis testing ($p<0.05$) until the most parsimonious model remained. Model fit was tested using qq-plots and normality of the residuals were checked using the Shapiro Wilk test. The models were checked for homogeneity of variance using Levene's test. Mixed models were conducted using the lme4 library (Bates et al. 2015). A Tukey's post hoc test was used to test the separation of means for each model using lsmeans package (Russell 2016).

The mean number of adult feeding notches on plants in no-choice and choice tests was analysed using separate general mixed effect models. Weevil reproductive stage, sex and type of host were treated as fixed factors in the models, and cage was treated as a random factor. The foliage C:N ratios were analysed using linear models in which type of host was the independent variable. The mean number of individuals that emerged per plant and the mean number of nodules per plant at the two different plant phenological stages were analysed using separate generalized mixed effect models following the Poisson error distribution. The host plant species and phenological stage were the fixed factors and cage was treated as a random factor in each model. The percentage of nodules fed on by weevil larvae on each host plant at the two different plant phenological stages was analysed in a linear model with host plant and phenological stage as fixed factors.

Results

Pea leaf weevil host acceptance and feeding preference between primary hosts

An interaction between weevil reproductive state and sex affected the mean number of feeding notches in the no-choice test that measured host acceptance (χ^2=7.39, df=1, P= 0.006) (Fig. 2-1A & 2-1B). Pre-reproductive females fed more on both host plants compared (Fig. 2-1A) to reproductively inactive weevils of both sexes (Fig. 2-1B) (Tukey's post hoc, P<0.05). Pre-reproductive males fed more on faba bean compared to field peas. Pre-reproductive females fed more than males on field peas but not on faba bean. Reproductively inactive male and female weevils fed a similar amount on both hosts. Overall, pre-reproductive weevils fed more in the host acceptance tests than reproductively inactive weevils.

In the choice test between peas and faba bean, the mean number of feeding notches was affected by an interaction between weevil reproductive state and sex (χ^2=4.83, df=1, P= 0.03)

(Fig. 2-1C & 2-1D). When both primary host plants were available to weevils, both sexes of pre-reproductively weevils fed more compared to the reproductively inactive weevils (Tukey's post hoc, $P<0.05$). Pre-reproductive females (Fig. 2-1C) fed more compared to the males, but there was no difference in feeding between sexes in reproductively inactive weevils (Fig. 2-1D). There was an interaction effect between weevil reproductive state and the host plant (χ^2=4.05, df=1, P=0.04) that affected host preference. Pre-reproductive weevils fed more on faba bean compared to field peas, but reproductively inactive weevils fed equally on both host plants (Tukey's post hoc, $P<0.05$). The C:N ratio was similar between field pea and faba bean plants (F=1.39, df=1, P= 0.25) (Table 2-1).

Pea leaf weevil host acceptance and feeding preference between primary hosts in the presence of a secondary host

An interaction between weevil reproductive state and sex affected the mean number of feeding notches (χ^2=10.60, df=2, P= 0.005) (Fig. 2-2A, 2-2B & 2-2C) in the no-choice test that measured host acceptance. Female pre-reproductive weevils produced more feeding notches on all three hosts compared to males, but feeding was not different between sexes of reproductive and non-reproductive weevils (Tukey's post hoc, $P>0.05$). There were more feeding notches on the secondary host, alfalfa, compared to faba bean and field pea (χ^2=86.80, df=2, $P< 2.2e-16$) in all weevil reproductive states.

A three-way interaction of weevil reproductive state, sex and plant host type affected the mean number of feeding notches (χ^2=9.55, df=4, P= 0.04) in the choice test that measured host preference among the three hosts (Fig. 2-2D, 2-2E & 2-2F). There were more feeding notches on alfalfa produced by weevils in all reproductive states except for the reproductive females, which produced similar number of notches compared to females on primary hosts (Tukey's post hoc,

$P<0.05$). Pre-reproductive and reproductive weevils created a similar number of feeding notches on both faba bean and field pea in the presence of the secondary host, alfalfa. Reproductively inactive females produced more notches on faba bean compared to field pea. The C:N ratio was different among the three crops ($F=60.45$, $df=2$, $P<0.0001$) (Table 2-1), which was highest in alfalfa and lowest in field peas.

The influence of pea leaf weevil host acceptance and feeding preference on offspring development

An interaction between host plant species and plant phenological stage affected the mean number of weevils that developed on and emerged from host plants ($\chi^2=24455$, $df=1$, $P< 2.2e-16$). More adult weevils emerged from faba bean compared to field peas when eggs were added at both 2nd and 5th unfolded leaf stages (Tukey's post hoc, $P<0.05$) (Fig. 2-3). An average of 3.37±0.54 weevils emerged from field peas and 8.55±11.37 emerged from faba bean. An average of 4.82±0.77 weevils emerged when eggs were added at the 2nd unfolded leaf stage and 10.13±1.62 emerged when eggs were added at 5th unfolded leaf stage. The host plant species ($\chi^2=188.62$, $df=1$, $P< 2.2e-16$) and plant phenological stage ($\chi^2=35.23$, $df=1$, $P= 2.9e-09$) affected the mean number of nodules produced by the host. Faba bean produced more nodules than field peas, and, as expected, the number of nodules increased with plant growth stage on both host plants (Tukey's post hoc, $P<0.05$). The percentage of nodule damage did not differ between the host plants ($F=1.42$, $df=1$, $P=0.23$) at either phenological stage ($F=0.05$, $df=1$, $P=0.82$).

Discussion

Here I provide evidence for behavioural plasticity in host location of an oligophagous insect that is modulated by insect reproductive state. I found that pea leaf weevil preference between the primary hosts, field pea and faba bean, is state-dependent. When presented with both primary hosts, pre-reproductive weevils preferred faba bean over field pea. But reproductively inactive weevils fed equally on both primary hosts. Pea leaf weevil is capable of discriminating between leguminous species and between different varieties of field peas (Landon et al. 1995). Pre-reproductive weevils may prefer faba bean over field peas for two major reasons. Pre-reproductive adults may increase their current reproduction (*i.e.* immediate fitness) by choosing a high-quality diet over a low-quality diet, while non-reproductive weevils may increase survival (*i.e.* future fitness gains) by minimizing the cost of the foraging decision-making process (Bernstein and Jervis 2008). According to the Optimal Foraging Theory, organisms make foraging decisions to maximize their fitness at a low energy cost and low mortality (Pyke et al. 1984). Reproductive state of the insect, however, can affect foraging behaviours based on the host nutrient quality (Damien et al. 2019, Le Gall et al. 2021). Pea leaf weevil adults must feed on a primary host plant to become reproductively active; thus, a diet with high nutritional quality may give an advantage in ovarian tubule development (Jackson 1920, Landon et al. 1995). Males may benefit from feeding on a host with high nutritional content for production of the aggregation pheromone. This may explain my results of more feeding notches on faba bean by reproductively active males in both no-choice and choice experiments.

Nutritional quality of the host is known to affect pea leaf weevil host choice (Landon et al. 1995) and non-reproductively active adults discriminate between lupin varieties based on the stipule alkaloid content (Cantot and Papineau 1983). Pea leaf weevil state-dependent plasticity in host selection may be driven by nutrients other than the C:N ratio, because the C:N ratio of the foliage of the two primary hosts was similar in this experiment. The ratio of dietary macronutrients such as proteins, carbohydrates and lipids are important in maximizing an organism's fitness and should be tested in future studies (Harrison et al. 2014, Le Couteur et al. 2016).

According to the preference-performance hypothesis, reproductive adults should select a host that maximizes offspring fitness (Thompson 1988). Faba bean plants produced more nodules at both phenological stages than field peas and supported more larvae as a result. Pea leaf weevil larvae feed on root nodules along with the associated *Rhizobium* bacteria (Johnson and O'Keeffe 1981, Hamon et al. 1987). Preference for faba bean over field peas by pre-reproductive weevils, indeed resulted in more offspring per plant. Interestingly, reproductively inactive weevils did not exhibit this same host preference. Reproductive state affects host choice in many other insects in which reproductively active individuals exhibit an increased sensitivity to host volatiles (Anton et al. 2007, Saveer et al. 2012, Kromann et al. 2015; Gadenne et al. 2016, Lemmen-Lechelt et al. 2018, Crava et al. 2019). A positive preference-performance linkage is important for an insect herbivore in which larvae have low mobility and larval development entirely depends on the female host choice (Price 1994). The modification of innate preference is costly, and it is crucial for insect herbivores to exhibit behavioural plasticity only when it maximizes fitness (Bernays 2001, Mery and Burns 2010), and this may explain the absence of host preference in reproductively inactive weevils.

I show that pre-reproductive weevils feed more on both primary host plants compared to the reproductively inactive weevils. This difference in feeding at two different reproductive stages may relate to the nutrients required during the overwintering and dispersal periods. Adult insects that overwinter in diapause are more sensitive to host cues after diapause termination than during diapause (Lemmen-Lechelt et al. 2018). Overwintering pea leaf weevils are reproductively inactive, but these weevils do not undergo a true diapause. Overwintering weevils resume feeding on a variety of wild legumes throughout the winter when the temperature is high enough to permit foraging (Landon et al. 1995). This overwintering strategy may result in the lower feeding rates of the reproductively inactive weevils observed in this study. The increased feeding by pre-reproductive weevils may relate to the subsequent energy investment in offspring production. Pre-reproductive females fed more on both primary host plants compared to males. Both sexes of reproductively inactive weevils, however, fed equally on both hosts. Male and female insects can respond differently to host volatiles and the number of olfactory receptor neurons sensitive to host volatiles can differ between sexes (Solé et al. 2010, Bruce and Pickett 2011, Lemmen-Lechelt et al. 2018, Wu et al. 2020). Variation in response to host volatiles between sexes in pre-reproductive weevils promotes greater feeding by females than males on both primary hosts. Females are larger than males and may consume more foliage to support their body size (Morley-Senkler et al. 2020). It is not known, however, whether food quality or quantity affect reproductive potential or fitness of pea leaf weevil.

The presence of the secondary host, alfalfa, altered the state-dependent preference of reproductively active weevils between faba bean and field peas. The complexity of the host volatile profile increases with an increase in the type of host plants which further complicates host selection. When the host selection process is complex, it may be more reliable to make host choices based on previous host experience rather than spending energy on neural modifications (Bernays 2001, Mery and Burns 2010). In this study, pea leaf weevil adults were fed on alfalfa before the experiment and the experience on alfalfa may have allowed the adults to prefer alfalfa over the primary host plants, even in a reproductively active state. Insects can base host plant choice on previous experience, which can lead to a restriction in host range (Papaj and Prokopy

1986, Landolt and Molina 1996, Zhang et al. 2007, Riffell et al. 2008, Anderson and Anton 2014). In another study, however, previous feeding experience on host plants did not influence the subsequent host selection decisions in pea leaf weevil (Landon et al. 1995). Alternatively, adults may have made more feeding notches on alfalfa because they have to feed more on alfalfa to get an equal quantity or quality of food compared to the primary hosts. In the three-way host preference experiment, the presence of alfalfa altered the pea leaf weevil state-dependent preference for the two primary hosts. Reproductively active weevils did not exhibit a preference for faba bean over field peas in the presence of alfalfa, but reproductively inactive weevils preferred faba bean over field peas in the presence of alfalfa. This suggests that the effect of the secondary host on preference towards primary hosts varies with insect reproductive state. Secondary hosts may affect individual fitness because host quality affects fecundity of herbivorous insects (Awmack and Leather 2002).

In conclusion, this study suggests that physiological state modifies the host preference of pea leaf weevil in a manner that enhances their fitness through the success of their offspring. Pea leaf weevil may acquire fitness advantages in increased reproduction or survival by state-dependent plasticity. The presence of a secondary host plant, however, influences the state-dependent primary host preference either by experienced-based modulation of innate host responses or due to the differences in quality or quantity of food among host plants. Understanding these relationships between weevil reproductive state and pea leaf weevil host finding behaviour is important because all three host plants are available on the landscape at the time of weevil dispersal. Even though my results show that adult host preference is state-dependent, the underlying mechanisms driving these plastic behaviours in host selection is not known. Plasticity in olfactory response may occur through neural modulation in central and/or peripheral nervous system in insect herbivores (Anton et al. 2007, Lemmen and Evenden 2009). This kind of state-dependent neural plasticity is expected to occur in pea leaf weevil because its behavioural and electrophysiological response to host volatiles is state-dependent (Landon 1997). In specialist herbivore insects, neuro-modulated plasticity to host volatiles should be more acute when it maximizes the fitness, and it may vary with the sex. Future studies are required to understand the underlying mechanisms that drive the behavioural plasticity in host finding at both central and peripheral nervous systems of this invasive insect.

Table 2-1. Foliage C:N ratio of legume plants in two-way and tree-way choice/ no-choice experiments

plant	C:N	F	df	P
two-way choice/no-choice				
faba bean	6.53	1.39	1	0.25
field pea	6.68			
three-way choice/no-choice				
faba bean	7.03	60.45	2	<0.0001
field pea	6.71			
alfalfa	7.51			

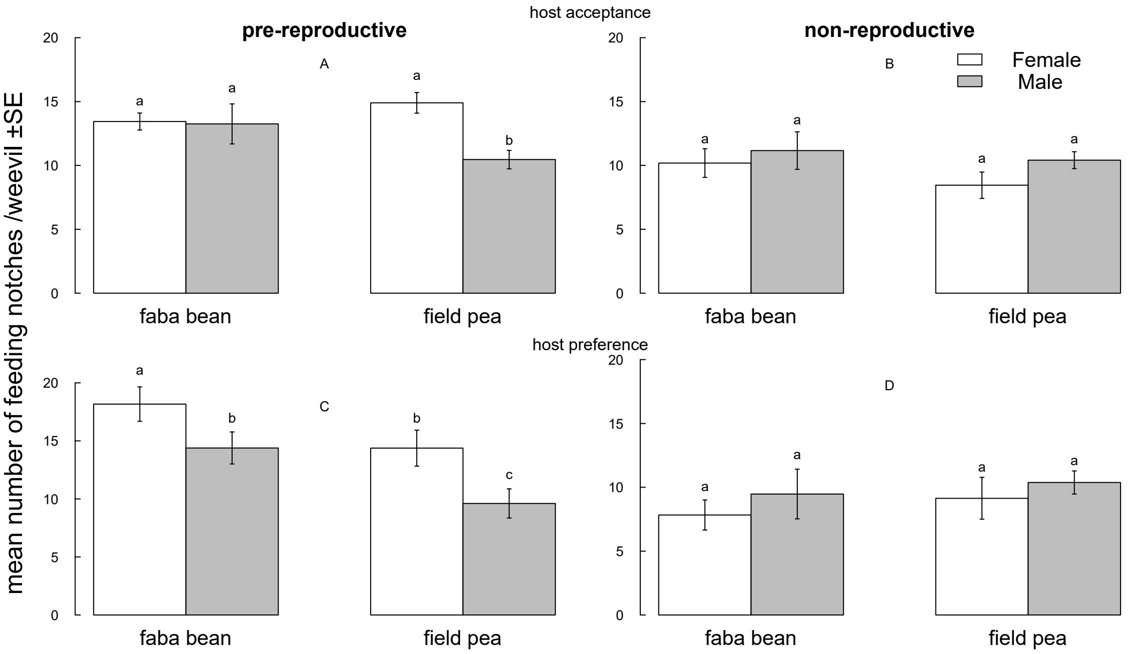

Figure 2-1. Mean number of feeding notches per weevil on faba bean and field pea by spring dispersing pre-reproductive (A & C) and fall dispersing reproductively inactive (B & D) weevils in a no-choice (host acceptance) and choice (host preference) study. Weevils (n=4) were introduced to a cage with two seedlings of the same host plant or one seedling of faba bean and a field pea plant (n=10 pots/treatment). Weevils were allowed to feed on host plants for five days and the feeding notches on foliage were counted. Males and females were tested in separate cages. Means with different letters denote significant differences within each panel (Tukey's post hoc, $P<0.05$)

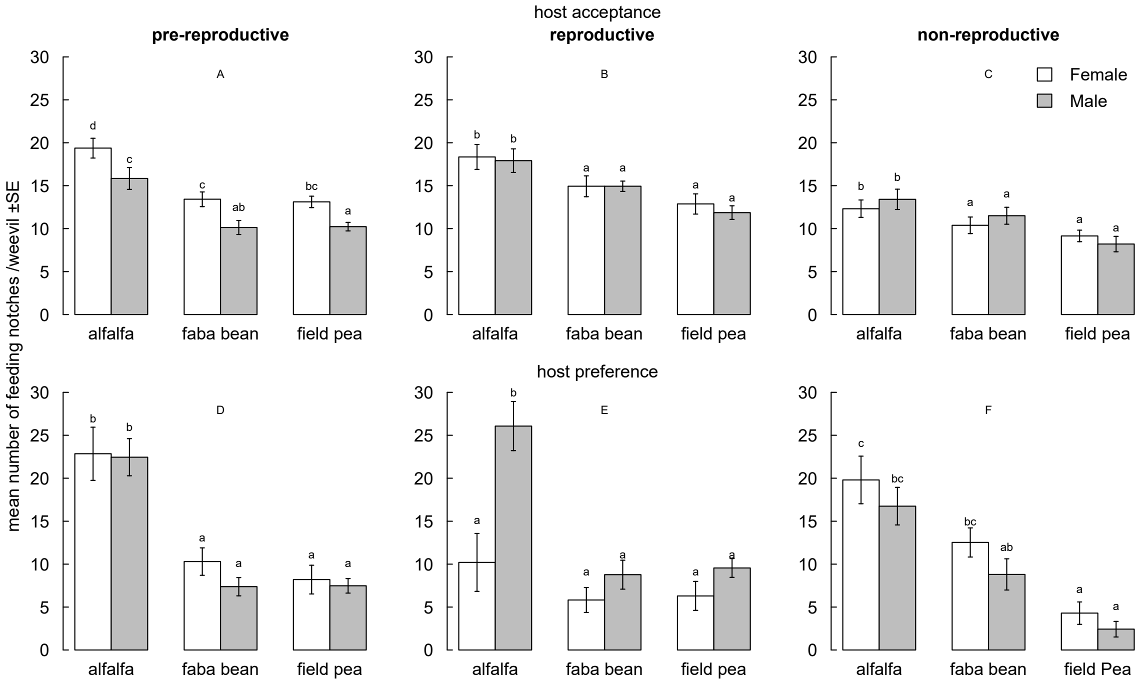

Figure 2-2. Mean number of feeding notches per weevil on faba bean and field pea by spring dispersing pre-reproductive (A & D), spring post-dispersal reproductively active (B & E) and fall dispersing reproductively inactive (C & F) weevils in a no-choice (host acceptance) and choice (host preference) study. Weevils (n=4) were introduced to a cage with three seedlings of the same host plant or one seedling of alfalfa, faba bean and a field pea plant (n=10 pots/treatment). Weevils were allowed to feed on host plants for five days and the feeding notches on foliage were counted. Males and females were tested in separate cages. Means with different letters denote significant differences within each panel (Tukey's post hoc, *$P<0.05$*).

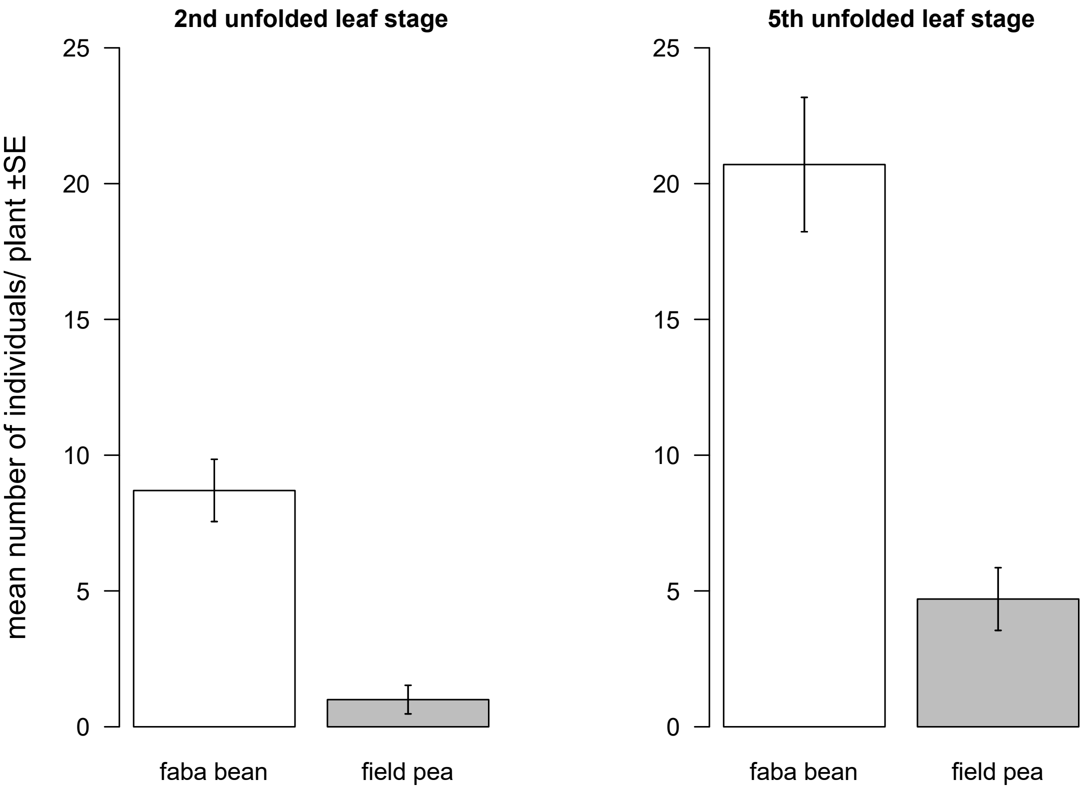

Figure 2-3. Mean number of pea leaf weevil life stages (larvae + adults) collected from faba bean and field pea plants infested with 50-eggs at two different host phenological stages, 2nd and 5th unfolded leaf stages. Pots containing a faba bean or a field pea plant were infested with 50-eggs at two different host phenological stages (n=10/host plant/ phenological stage). The control pots did not receive any eggs. The pots were caged individually and emerging adults or larvae in the root nodules were collected and counted.

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Chapter 3 **Effects of legume-*Rhizobium* symbioses on pea leaf weevil (Coleoptera: Curculionidae) feeding and oviposition**

Abstract

Legume-*Rhizobium* symbioses are host specific in which rhizobia fix atmospheric nitrogen that can be used by the plants and, the bacterial cells are protected by root nodules and receive plant-produced carbon in exchange. These legume-*Rhizobium* interactions affect host quality, hence the above-ground and below-ground insect-plant interactions. In this chapter, I investigate the influence of species-specific legume-*Rhizobium* symbioses on pea leaf weevil, *Sitona lineatus L,* adult and larval interactions with its primary and potential secondary leguminous host plants. First, I tested pea leaf weevil adult host acceptance and larval development on a range of potential legume hosts inoculated with their corresponding *Rhizobium* strains. Second, I investigated how *Rhizobium* inoculation and nitrogen amendment influence adult host acceptance, feeding preference and larval development in a series of laboratory and field studies. I found that the legume-*Rhizobium* association drives pea leaf weevil larval development, but not adult host acceptance. Larvae can survive in nodules associated with *Rhizobium leguminosarum* Frank (Rhizobiales: Rhizobiacea) in field peas and faba bean. Soybean is an occasional host to larvae. Adults, however, feed on most of the potential secondary hosts except on chickpeas and minimal feeding was observed on lentils. *Rhizobium* had inconsistent effects on adult host acceptance and feeding preference in laboratory and field studies, but resulted in increased nodulation and protection of faba bean yield. Nitrogen amendment did not protect faba bean yield from pea leaf weevil damage, even though it decreased larval development and decreased nodulation.

Introduction

Legumes and soil-dwelling *Rhizobium* bacteria (rhizobia), have evolved symbiotic relationships in which rhizobia fix atmospheric nitrogen that can be used by the plant (Lee and Hirsch 2006). In exchange, the bacteria are protected by root nodules and receive plant-produced carbon. Legume species have species-specific relationships with nitrogen-fixing rhizobia and nodulation will not occur in the absence of host-specific strains (Biddle 2017). These legume-rhizobia associations impact soil fertility, plant growth, and above and below ground insect-plant interactions (Brockwell et al. 1995, Laguerre et al. 2007, Dean et al. 2009, Kempel et al. 2009, Katayama et al. 2010, Thamer et al. 2011).

Legume-rhizobia symbioses may influence the underlying host signalling pathways, which in turn influence the semiochemical communication between host plants and insect herbivores. There are two major signalling transduction pathways that activate induced defensive response in plants (Paul et al. 2000). Systemic acquired resistance is induced by salicylic acid (SA) signalling and provides resistance to pathogen infection and sucking herbivores. The wound resistance pathway is induced by jasmonic acid (JA). The phytohormones, SA and JA interact and may inhibit or promote the induction of the other pathway (Stout et al. 1997, 1999, Bostock 1999, Felton et al. 1999, Thaler et al. 1999, Yamada et al. 2012, Liu et al. 2016). The legume-rhizobia symbiosis signalling (Sym) pathway is activated in plant cells upon a perception of the rhizobial nodulation (Nod) factor (Oldroyd and Downie 2008, Oldroyd et al. 2009). Rhizobia are initially recognized as pathogens by the host plant and may induce host immune response (Kouchi et al. 2004, Yahyaoui et al. 2004, Moreau et al. 2011). Host compatible rhizobia, however, have evolved mechanisms to control host SA levels so as to establish successful infections (Martínez-Abarca et al. 1998, Pieterse et al. 2009). These underlying changes in host signalling pathways to promote the rhizobia symbioses may also influence herbivore performance and feeding preference (Pineda et al. 2013, Dean et al. 2014). Plants defend themselves from herbivores and attract natural enemies of the herbivores by emitting herbivore-induced plant volatiles (HIPVs) (Turlings et al. 1995, Jensen et al. 2002, Dicke and Baldwin 2010, McCormick et al. 2012). The synthesis of HIPVs is regulated by herbivore-induced phytohormones; SA, JA and ethylene (ET) (Kessler and Baldwin 2002, Van Poecke and Dicke 2002, Pieterse et al. 2012). Different insect feeding guilds stimulate different combinations of hormonal signalling pathways to produce a specific blend of HIPVs (Zhang et al. 2013, Wei et al. 2014). The hormone, SA regulates the synthesis of volatiles such as methyl salicylate while JA regulates the emission of green leaf volatiles and terpenoids (Dicke and Poecke 2002, Van Poecke and Dicke 2002, Maffei et al. 2011). Beneficial microbes modulate JA and ET signalling pathways to enhance plant defence response to insect herbivores (Van Oosten et al. 2008, Van Wees et al. 2008, Van der Ent et al. 2009, Pangesti et al. 2015).

Nitrogen is a limiting factor for insect herbivores, especially for root-dwelling insects, as roots typically have very low nitrogen content (Mattson 1980). Legume-rhizobia associations provide a nitrogen-rich environment and some below-ground insect herbivores have overcome nitrogen deficiencies in plant roots by feeding on root nodules containing rhizobia (Johnson and Rasmann 2015). Biological nitrogen fixation by *Rhizobium* affects the nutritional quality of the plant because the form of nitrogen is different from that of nitrogen fertilization (Buchanan et al. 2000). The amount and efficacy of biological nitrogen fixation by nodule-associated rhizobia may, however, vary with the *Rhizobium* strain, soil nitrogen content, host species, agronomic factors and abiotic conditions (Nutman 1976, Salunkhe and Deshpande, 1991, James and

Crawford 1998). High soil inorganic nitrogen content suppresses natural nodulation and nitrogen fixation (Slattery et al. 2004).

The weevil genus *Sitona* (Coleoptera: Curculionidae) are among the few taxa of insects that are well adapted to exploit root-nodule associated bacteria to obtain nitrogen. This genus ranges from obligate root nodule feeding larvae to those that feed on nodules as young larvae and then feed on roots during late larval instars (Quinn and Hower 1986). Nodule feeding increases larval fitness and protects larvae from predators and extreme conditions (Byers and Kendall 1982, Quinn and Hower 1986, Wolfson 1987, Goldson et al. 1988, Gerard 2001, Barnett and Johnson 2013). While larvae are known to actively search for nodules in soil, adults prefer to lay eggs on nodule rich plants (Johnson et al., 2004, 2012).

The pea leaf weevil, *Sitona lineatus* Linnaeus (Coleoptera: Curculionidae) is native to Europe and North Africa and has become an invasive pest in North America (Vankosky et al. 2009, Carcamo et al. 2018). The pea leaf weevil is oligophagous on leguminous (Fabales: Fabaceae) plants (Jackson 1920). Adults feed on above-ground foliage on a variety of legumes but are major pests on field pea (*Pisum sativum*) and faba bean (*Vicia faba*) (Jackson 1920, El-Dessouksi 1971, Nielsen 1990). Adults also feed on other leguminous host plants such as white clover (*Trifolium repens* L.), sweet clover (*Melilotus albus* L.), alfalfa (*Medicago sativa* L.), lupin (*Lupinus albus* L.), red clover (*Trifolium pratense* L.), Siberian pea shrub (*Caragana abrorescens* Lam.) and black locust (*Robinia pseudoacacia* L.) (Fisher and O'Keeffe 1979, Fisher and O'Keeffe 1979b, 1979a, Hamon et al. 1987, Hoebeke and Wheeler 1985, Jackson 1920, Murray and Clements 1992, Schotzko and O'Keeffe 1988, 1986, Vankosky et al. 2009, Williams et al. 1991). Adults, however, reject lentils (*Lens culinaris* Medik.) and common beans (*Phaseolus vulgaris* L.) (El-Dessouksi 1971, Fisher and O'Keeffe 1979a, 1979b, Schotzko and

O'Keeffe 1988). Pea leaf weevil larvae feed on the *Rhizobium* bacteria associated with root nodules along with root nodule tissue of the host plant (Johnson and O'Keeffe 1981, Hamon et al. 1987). Larval feeding has only been recorded on field peas, faba bean and chickpeas (Fisher and O'Keeffe 1979, Hoebeke and Wheeler 1985, Williams et al. 1991). Pea leaf weevil damage can reduce soybean yield, although larval damage has not been recorded on soybean (North et al. 2016). Lentils do not support pea leaf weevil larval development even though lentil root nodules are associated with the same *Rhizobium* strain, *Rhizobium leguminosarum biovar viciae* Frank (Rhizobiales: Rhizobiacea), that is associated with field pea and faba bean. It is unclear whether there is an influence of host-specific *Rhizobium* strains on adult host preference and larval development of pea leaf weevil.

Feeding by specialized and generalized herbivores can influence host nutrition and soil fertility while soil nutrient fluctuations can influence the soil fauna through bottom-up trophic cascades. High soil nitrogen conditions can delay peak nodulation (Gibson and Harper 1985) and reduce the root nodule production in field peas thereby decreasing food availability and potentially decreasing the size of the larval population (Vankosky et al. 2011, Cárcamo et al. 2015). Field pea plants subjected to pea leaf weevil adult feeding and oviposition showed a reduction in nitrogen fixation (Jensen et al. 1986, Corre-Hellou and Crozat 2005), but inconsistence effects of herbivory on nitrogen fixation, yield and seed nitrogen content was observed in other studies (El-Dessouksi 1971, Lohaus and Vidal 2010, Vankosky et al. 2011, Vankosky et al. 2011a). These interactions of pea leaf weevil, nitrogen fixing bacteria and soil nitrogen content may influence pea leaf weevil interactions with the host plants.

In this chapter, I investigate whether pea leaf weevil adult host acceptance and larval development are influenced by the *Rhizobium* strain using a range of primary and secondary host plants. I also investigate the effect of *Rhizobium* and/or nitrogen on adult host preference and acceptance, oviposition and larval development.

Materials and Methods

Insects

Pre-reproductive pea leaf weevil adults were collected in spring (April-May) on alfalfa using sweep nets at fields near Lethbridge Research and Development Center (LRDC), Lethbridge, Alberta, Canada (49.7004°N - 112.7632°W). Collected weevils were fed on alfalfa *ad libitum* in mesh cages (47.5cm X 47.5cm X 93cm) before the experiment (23°C, 16:8 L:D). Conical flasks (0.5l) containing cotton swabs were used to provide water. Weevils were starved for at least 24 hours and weighed to the nearest 0.01 mg (Mettler Toledo, XS105, Columbus, OH) before the experiments. Pea leaf weevil eggs were identified using their oblong and oval shapes, colour and size (~0.36 mm). Eggs are yellow-white when first deposited and brown-black after melanisation in 2-3 days of oviposition (Jackson 1920). Eggs were easily visible when floated in clear solutions and on white surfaces.

Plants

Plants were grown under controlled conditions approximately 21°C under a 16L:8D photoperiod supplemented with high pressure sodium light in a greenhouse. Plants were grown in low nitrogen soil (Appendix 3-1) inoculated with the corresponding *Rhizobium* inoculant at seeding (Table 3-1). Plants were watered as typically two-three times a week.

Adult host acceptance and larval development on primary and secondary legume plants inoculated with associated Rhizobium bacteria

A greenhouse study was conducted during spring (May- August) 2018 to test the hypothesis that legume-*Rhizobium* symbiosis will affect pea leaf weevil adult feeding and larval development. Seven different legume species were inoculated with their corresponding *Rhizobium* species (Table 3-1) and treated with fungicide (325ml/ 100kg seeds, APRON MAXX® RTA®, Syngenta Canada Inc., Guelph, ON, Canada) at seeding. Plants were potted singly in pots (diameter =15.24 cm) and two to three-week-old plants (10 plants/ legume species) were caged individually using a mesh sleeve. Two pairs of male and female weevils were introduced onto the soil of each caged pot. The control pots did not receive any weevils. The experiment was conducted as a completely randomized design under controlled conditions (21°C, 16:8 L:D) in a greenhouse under natural light conditions. After a 2.5 month growing period, the plants were harvested and adult feeding notches on the foliage were counted along 5 cm portions of the top, middle and bottom of the plant along the stem. The soil from each pot was soaked in water and any weevil life stages that floated to the surface were collected and counted. Remaining life stages that did not float were collected by sieving soil through metal sieves. Roots were washed and blotted with paper towels. Root nodules were counted and assessed for larval nodule damage by detecting larval entrance or exit holes of each nodule and opening each nodule to observe the presence of larvae. A growth score for each plant was calculated by adding individual scores for plant growth and vigour, nodule colour and number, and nodule position following the 20/20 seed labs protocol (Nodulation assessments 2019) (Appendix 3-2).

Adult host acceptance and larval development on faba bean vs soybean

Since soybean supported larval development in the first lab experiment, a field cage study was conducted to assess pea leaf weevil larval development on soybean under field conditions. The study was conducted at a site near Lethbridge (49.7004°N, -112.7632°W), Alberta, Canada in 2019. Faba bean and soybean seeds were inoculated with their corresponding *Rhizobium* species (Table 3-1) and treated with fungicide (325ml/ 100kg seeds, APRON MAXX® RTA®, Syngenta Canada Inc., Guelph, ON, Canada) and seeded at early May 2019. Crops were planted parallel to each other with a 4 m buffer between the two. In April, eight cages (1m X 1m X 1.5m) (n=4/host plant) were placed along two linear transects in faba bean (Zero tannin, cultivar CDC Snow Drop, 49 plants/ m^2) and soybean (AC Edwards, 53 plants/m^2) plots after seeding. Each cage was thinned to 20 plants, and 10 male and 10 female weevils were added to each cage when seedlings reached the 5th unfolded leaf stage (Staging guide faba bean 2021). The number of foliar feeding notches from adult feeding was counted 24 hours after weevil introduction. One plant per cage was randomly selected and dug up at the early flower stage (~2.5 months after weevil introduction) using a hand trowel to collect the soil and root nodules around the roots. Soil samples collected to include all of the roots of the plant and stored in 4.5l plastic bags and transported to a laboratory root-washing station. The soil was soaked in water and all life stages of weevils that floated to the surface were collected and counted. The roots were washed, and blotted with paper towels and assessed for larval feeding damage (Nodulation assessments 2019) (Appendix 3-2).

Adult host acceptance, feeding preference and oviposition between Rhizobium treated vs untreated faba bean

A greenhouse study was conducted to test the hypothesis that *Rhizobium* inoculation will influence adult host acceptance and oviposition on faba bean in spring 2018. Faba bean plants were treated with the corresponding *Rhizobium* inoculant (Table 3-1) at seeding and a subset of plants served as uninoculated controls. Two pots (diameter=10.6 cm) containing *Rhizobium* treated or untreated plants (no-choice test), or one pot with and one without *Rhizobium* (choice-tests) were placed in a white plastic container (7.6 l) (Fig. 3-1). For pots with two plants, a piece of fluted polypropylene sheet (coroplast) was buried perpendicular to the soil surface to separate them and to ensure that there was no *Rhizobium* spore transmission between the plants. The space between the pots was filled with soil and the top of the soil surface was covered with coroplast and two layers of paper towels. The containers were caged and (n=10/treatment) plants were exposed to four reproductively active female weevils. After 10 days, weevils were recovered and the number of feeding notches on foliage was counted. The number of eggs laid in each half of the container corresponding to the inoculated and uninoculated plants was counted.

Adult host acceptance, feeding preference and oviposition between nitrogen treated vs untreated faba bean

A greenhouse study was conducted to test the hypothesis that nitrogen fertilization influences adult host acceptance and oviposition on faba bean in spring 2018. Faba bean plants were treated with the corresponding *Rhizobium* inoculant (Table 3-1) at seeding. Plants were grown individually in pots (diameter=10.6 cm). Half of the plants were treated with nitrogen (46-0-0, NPK) (0.05 g/pot) and the others remained as unfertilized controls. Plastic containers (7.6l) containing two pots of the same treatment (no-choice) or two different treatments (choice) were caged, and the study was repeated following the same steps as outlined above (Fig. 3-1). Four females (n=10 pots) or two male and two female weevils (n=10 pots) were introduced to each pot under no-choice or choice conditions. The feeding notches and the number of eggs laid were counted 10 days after weevil introduction.

Field study to test effect of Rhizobium and nitrogen on pea leaf weevil adult and larval damage on faba bean

A field choice study was conducted to test the effects of *Rhizobium* and nitrogen on pea leaf weevil adult and larval damage on faba bean. The study was conducted at a site near

Lethbridge (49.7004°N, -112.7632°W), Alberta, Canada in 2019. The study was conducted in a randomized block design with four blocks. Blocks were separated 2 m apart from each other and each block was divided into four plots. The plots were 5 m X 5.6 m and each half of the plot was assigned to one of the four choice treatments: i) control vs nitrogen (163 kg/ha, 46-0-0 NPK) treated plants, ii) control vs *Rhizobium* (1.2 kg/ 1000 kg seeds) (Nodulator® FB Peat, 2008027A, BASF Canada, Ontario, Canada) treated plants, iii) control vs nitrogen and *Rhizobium* treated plants and, iv) nitrogen vs *Rhizobium* treated plants (Fig. 3-2). Faba bean seeds were treated with fungicide (325ml/ 100kg seeds, APRON MAXX® RTA®, Syngenta Canada Inc., Guelph, ON, Canada) at seeding. Before seeding, soil samples from each site were tested for nutrient availability. Five soil cores (17 cm X 30 cm) were obtained from each of two different depths, 0-15 cm and 15-30 cm by walking along a "W" shape across the field and samples were collected at each corner of the "W". Samples from each depth were combined to make two soil samples. Soil samples were tested for nutrients (nitrogen (NO_3^-), phosphorous, potassium and sulphur) at Down to Earth Labs, Lethbridge, Alberta, Canada. All plots were treated with fertilizer (11-52-0; NPK, 79 kg/ha before seeding and 20 kg/ha at seeding) based on the nutrient recommendations. Faba bean was directly seeded with a target seeding rate of 49 plants/m^2 in early May.

For the mark-recapture experiment, pea leaf weevil adults were coloured using fluorescent powder (Fluorescent pigment, DayG10 ColourCorp, 4732 Clair Avenue, Cleaveland, Ohio) and colour coded to correspond with the four host plant choice treatments described above (i. blue, ii. orange, iii. pink, iv. green respectively). The powder was applied topically by allowing weevils to walk on a petri dish with the powder (~0.2 mg) until weevils were covered with the powder (~2 min). One hundred and fifty coloured weevils were introduced in the middle between each choice treatment at the 3rd unfolded leaf stage of seedlings. Six pitfall traps

(Vernon, Intko supply, Vedder Rd. Chilliwack, BC, Canada) were placed within a 1m radius of the release point, so that 3 traps were located on each side of the choice treatment (Fig. 3-2). Dry traps were coated with Insect-a-Slip Insect Barrier (PTFE DISP30, BioQuip, CA, USA) along the upper rim (2 cm) to prevent weevils from crawling out of the trap. Pitfall traps were checked every day for four consecutive days following the release and marked, and unmarked weevils were collected. Plants were assessed for adult feeding notches at the 2nd -3rd unfolded leaf stage. Root nodules were assessed for pea leaf weevil larval damage at the early flower stage. Two plants per treatment plot were dug up using a hand trowel to collect the soil and root nodules around the roots. Soil samples were stored in 4.5l plastic bags and transported to a laboratory root- washing station. The soil was soaked in water and all life stages of weevils that floated to the surface were collected. The roots were washed, and blotted with paper towels and assessed for larval feeding damage (Nodulation assessments 2019). A soil core (4 cm X 3 cm) was used to collect pea leaf weevil eggs from the soil. A metal core was placed around the plant at the 4th to 5th unfolded leaf stage and soil within the collar was collected. Two soil samples were collected from each treatment plot and stored at 5°C and pea leaf weevil eggs were extracted from the soil using a flotation technique (Danthanarayana 1967). The soil was weighed (Mettler Toledo, NewClassic ML), sieved (0.5 mm mesh size) and soaked in 500 ml of water overnight. Floating materials were sieved and placed in a beaker that contained 100 ml of 1.2sg of Magnesium Sulphate (#057800056683, Life Brand) for 5-6 minutes. Floating materials were filtered through a filter paper (Whatman #1) and eggs on the filter paper were collected using a paintbrush. Plants were harvested with a Wintersteiger plot combine (1.5 m header width, Wintersteiger, Austria), air dried, cleaned and the seeds were weighed (Model 6500, ISI Scan software version 4.0, FOSS North America, Eden Prairie, Minnesota).

Data Analysis

Data were analysed using R version 3.6.3 (R Core Development Team 2018). Initial models contained all explanatory variables and interactions between all explanatory variables. The model simplification was achieved by ANOVA hypothesis testing ($P < 0.05$) for full and reduced models, until the most parsimonious model remained. Model residuals were checked for normality using the Shapiro Wilk test. The models were checked for homogeneity of variance and for over-dispersion using the one sample Kolmogorov–Smirnov test in DARMa package (Hartig 2018). Model fit was evaluated using qq-plots. A Tukey's post hoc test was conducted to test the separation of means of models (Tukey 1977). Mixed effect models were analysed using lme4 library (Bates et al. 2015).

Adult host acceptance and larval development on primary and secondary host plants

The effect of host type on the number of offspring that emerged from each host was analysed using the Kruskal Wallis test (Kruskal and Wallis 1952) and pairwise mean comparisons were conducted using a Wilcoxon-Signed-Rank test (Rey and Neuhäuser 2011) with Bonferroni adjusted alpha levels of 0.00014 per test (0.05/36). A linear model tested the effect of host plant on adult feeding. The mean number of adult notches per individual weevil was square root transformed to meet model assumptions. The effect of the host type and presence or absence of pea leaf weevil eggs on the subsequent plant growth score was analysed with a generalized linear model using Poisson error distribution.

Adult host acceptance and larval development on faba bean vs soybean

A linear model analysed the effect of host type on the mean number of adult feeding notches. The effect of host type on the percentage of nodules fed on by larvae and on the number of offspring produced were tested using separate generalized linear models with Poisson and negative binomial error distributions, respectively.

Adult host acceptance and preference between Rhizobium or nitrogen treated vs untreated faba bean

The effect of *Rhizobium* treatment or nitrogen treatment on the mean number of feeding notches per individual and the mean number of eggs per female were analysed using separate linear models in choice and no-choice assays. The mean number of eggs per female was square root transformed to meet model assumptions in both choice and no-choice nitrogen assays.

Adult host choice between Rhizobium or/and nitrogen-treated faba bean in the field

A nested generalized mixed-effect model with Poisson error distribution compared the total number of adults captured in pitfall traps in treatment plots in the choice test. Nested linear mixed effect models were used to compare the average number of notches, eggs, larvae, nodules, yield and percentage of fed nodules between two treatments in each choice test. The choice nested within the block was the random factor in each model. The average number of larvae in all four treatment choices and the average number of nodules in the choice between control vs nitrogen and *Rhizobium* were square root transformed to meet the model assumptions. The mean number of nodules fed on in the control vs nitrogen and *Rhizobium* and nitrogen vs *Rhizobium* choices were square root transformed.

Results

Adult host acceptance and larval development on primary and secondary host plants

Adult weevil feeding was affected by the host plant (F=14.52, df=6, P= 2.909e-10) (Fig. 3-3A). Adults fed on most test plants, except for chickpeas. The highest number of feeding notches occurred on faba bean and the lowest on lentil. When pea leaf weevils were offered a variety of primary hosts and potentially secondary leguminous plants, the number of offspring that emerged varied with the plant species (χ^2=56.59, df=6, P=2.212e-10). Larval development occurred only on the known primary hosts, peas and faba bean, and to a lesser extent on the secondary soybean host (Wilcoxon test, P<0.05) (Fig. 3-3B). The plant growth score was affected by the plant host species (χ^2=40.50, df=6, P=3.622e-07) but not the presence of pea leaf weevils (χ^2=3.23, df=6, P=0.07).

Adult host acceptance and larval development on faba bean vs soybean in the field study

The number of notches produced from pea leaf weevil adult feeding did not differ between faba bean and soybean (F=5.57, df=1, P=0.05). The percentage of damaged nodules caused by larval feeding, however, differed between the two-host plants, and soybean had no damaged nodules (F=6.93, df=1, P=0.03). Soybean produced a low number of offspring compared to faba bean (χ^2=10.65, df=6, P= 0.001) (Fig. 3-4).

Adult host acceptance and preference between Rhizobium or nitrogen treated vs untreated faba bean in the greenhouse

The mean number of feeding notches did not differ between *Rhizobium*-treated and untreated plants in both no-choice (F=1.73, df=1, P=0.21) (Fig. 3-5A) and choice (F=0.57, df=1, P=0.46) (Fig. 3-5B) assays. The mean number of eggs laid per female was also not affected by *Rhizobium* treatment in no-choice (F=1.25, df=1, P= 0.28) (Fig. 3-5C) and choice (F=0.001, df=1, P=0.97) (Fig. 3-5D) assays. In the no-choice assay, males fed more on nitrogen-treated faba bean plants compared to untreated plants, but females fed equally on both nitrogen-treated and untreated plants (F=5.19, df=5, P=0.0002) (Fig 3-6 A). The mean number of notches per weevil was not different between nitrogen-treated and untreated plants in the choice assay (F= 1.57, df= 5, P=0.18) (Fig 3-6 B). In the no-choice assay, females laid more eggs on the soil around nitrogen-treated plants than untreated plants (χ^2=7.62, df=3, P=0.0001) (Fig 3-6 C). There was no difference in the mean number of eggs laid between nitrogen-treated and untreated plants in the choice test (Fig 3-6 D). Unsurprisingly, the treatment with more females had more eggs compared to the treatment with two females and two males (F=4.45, df= 3, P= 0.009).

Adult host choice between Rhizobium or/and nitrogen-treated faba bean in the field

More adults were collected in pitfall traps from nitrogen-treated plots compared to untreated plots (Table 3-2). The mean number of adults collected in pitfall traps, however, was higher in *Rhizobium*-treated plots compared to nitrogen treated-plots (Table 3-2). The mean number of notches was similar across most choices presented in field plots, except that weevils made more notches on control plants compared to *Rhizobium*-treated plants (Table 3-2). The mean number of eggs laid by females was also similar across all field plot choices (Table 3-2). More larvae developed on control faba bean plants compared to nitrogen-treated faba bean plants in field plots (Fig. 3-7A) (Table 3-2), but otherwise it was similar across all other field plot choices. Nitrogen-treated plants produced fewer root nodules compared to control and *Rhizobium*-treated plants (Fig. 3-7B) (Table 3-2). There were no differences in the percentage of fed nodules across all choices presented in the field plots (Table 3-2). The *Rhizobium*-treated plants produced more yield compared to untreated plants, but the yield did not differ in other field plot choice comparisons.

Discussion

This study provides evidence to support the hypothesis that the legume-*Rhizobium* association drives pea leaf weevil larval development, but not adult host acceptance. Pea leaf weevil adults accepted all legume species with a variety of *Rhizobium* species inoculants, except chickpeas and the feeding was minimal on lentil. This is in contrast to other studies which showed adult pea leaf weevil feeding on the foliage of chickpeas under both field and laboratory no-choice conditions (Taylor et al. 1991). Feeding on chickpea and other less suitable hosts may occur only in areas where farmers do not plant suitable hosts. This disparity in results could also be linked to the host-specific defensive characters, such as glandular hairs on the foliage, of different host species and varieties within species. Inoculation of chickpeas with *Rhizobium* increases the production of plant defensive compounds, phenolics, which may negatively affect the adult feeding (Arfaoui et al. 2007). Pea leaf weevil feeding varies with the variety of field peas (Landon et al. 1995). Although lentil has the same *Rhizobium* strain as faba bean and field peas, adult weevils fed only minimally on lentil in my study and completely rejected lentil foliage in other studies (Fisher and O'Keeffe 1979a, 1979b, Schotzko and O'Keeffe 1988).

A significant number of pea leaf weevil offspring were collected only from the primary host plants, field pea and faba bean. A very low number of offspring developed on soybean, but other tested host plants were not suitable for larval development. As adult insects fed on most of the tested legume plants, there is a distinct difference in the plant-insect interaction with weevil life stage. Field pea, faba bean and lentil root nodules are all associated with the same *Rhizobium* strain, *Rhizobium leguminosarum biovar viciae*, but no offspring developed on lentils. Lentils can produce a higher amount of defensive phenolics as a response to microbial attack and this may have minimized the establishment of pea leaf weevil larvae in the root nodules (Vermerris and Nicholson 2006, Mirali et al. 2016, Bazghaleh et al. 2018). Soybean has a symbiotic relationship with *Bradyrhizobium japonicum* (Biddle 2017) and has rarely been reported as a host for pea leaf weevil larval development (North et al. 2016). But, larval feeding on soybean root nodules has been observed by other insects pests such as two-stripped leaf beetle, *Medythis nigrobilineata* Motschulsky (Coleoptera: Chrysomelidae) and bean leaf beetle, *Cerotoma* *trifurcate* Forster (Coleoptera: Chrysomelidae) (Lundgren and Riedell 2008, Takei et al. 2014). As I observed minimal larval development on soybean in this study, it does not appear that soybean is a primary host for pea leaf weevil larvae, and they may have a species-specific affinity to a specific *Rhizobium* species or strain for development. There is a possibility that rhizobia increase the biosynthesis of jasmonates at the process of wounding. Jasmonic acid induces the production of Nod factor by *B. japonicum* that facilitates the *Bradyrhizobium*-soybean symbiosis (Mabood et al. 2006). The biosynthesis of SA is inhibited during the initial stage of symbiosis and it may allow pea leaf weevil larvae to colonize soybean root nodules (Martínez-Abarca et al. 1998). Other nodule characters such as nodule size, age, texture and host defences may play a role and interact with the *Rhizobium* strain to impact larval success on legume hosts. Pea leaf weevil adults need to feed on field peas and faba bean to reach reproductive maturity (Jackson 1920), and females may have failed to lay eggs on lentils or other tested host plants in this study because they were not reproductively mature.

Adults equally preferred and accepted *Rhizobium*-treated and untreated faba bean plants in laboratory assays, but different preferences occurred in the field plot choice experiments. Adults fed more and preferred un-treated control plants over *Rhizobium*-treated plants in the field choice assay, even though the number of adults caught in pitfall traps was similar between the two treatments. The differences in adult feeding between laboratory and field assays may be caused by variation in *Rhizobium* activity under field and lab conditions. In the field, legume plants encounter a variety of rhizobia in the soil, which are genetically distinct from natural strains (Dean et al. 2009, Wang et al. 2009). Rhizobia-plant associations also depend on the soil characters, local environmental conditions and /or plant genotypes (Taylor et al. 1991, Streeter 1994, Denton et al. 2002, Thrall et al. 2007). Beneficial microbes modulate the plant hormonal system that regulates plant defence against herbivores (Van Oosten et al. 2008, Van Wees et al. 2008, Van der Ent et al. 2009, Pineda et al. 2012, Pangesti et al. 2015). I only observed an effect of *Rhizobium* inoculation on adult feeding in the field and lower feeding on *Rhizobium*-treated plants may be due to an increase in concentration of defence compounds. Similar to what I found in faba bean, *Rhizobium* inoculation suppresses adult pea leaf weevil feeding on field peas (Vankosky et al. 2011a). Females laid an equal number of eggs on faba bean regardless of *Rhizobium* inoculation in both lab and field studies. *Rhizobium* inoculation increased root nodulation and protected field pea yield (Vankosky et al. 2011). In contrast, I did not observe an effect of *Rhizobium* inoculation on root nodulation, but inoculation protected faba bean yield in the current study. Therefore, *Rhizobium* inoculation is the most effective way to protect faba bean yield from pea leaf weevil feeding damage. This increase in yield may result from increased nitrogen availability and a combined effect of increased nodulation to withstand the effect of larval damage and decreased adult feeding on inoculated plants.

Nitrogen fertilization can influence plant host quality by limiting the biological nitrogen fixation of legume-associated bacteria. Only pea leaf weevil males preferred the foliage of nitrogen-treated plants in choice experiments but fed on both fertilized and control plants equally in no choice experiments. Males produce the aggregation pheromone, 4-methyl-3,5-heptanedione, which attracts both males and females (Blight et al. 1991, Evenden 2018), but it is not known whether host quality has any effect on pheromone production. Females, however, do not respond to nitrogen-treated plants preferentially over control plants in either choice or no-choice laboratory assays. Females only lay more eggs on nitrogen-treated plants when there is no-choice, which further highlights the non-responsiveness of female to differences in foliage quality. More adults were caught in plots containing nitrogen-treated plants compared to control plants in the field, but the adult feeding did not differ between the two treatments. Differences in male feeding in field plots may have been masked by the extensive feeding of females (Wijerathna et al. unpublished data). I show that nitrogen amendments inhibit root nodulation in faba bean, as observed in field peas (George 1962, Allen and Allen 1981, Cárcamo and Vankosky 2011), which subsequently leads to reduced pea leaf weevil larval development on nitrogen-treated faba bean plants. Adults prefer control plants without *Rhizobium* when they have a choice between control and *Rhizobium* inoculated plants. When there is a choice between nitrogen and *Rhizobium*-treated plants, however, more adults are attracted to the *Rhizobium*-treated plants, which produce more nodules compared to the nitrogen-treated faba bean. Larval development did not differ, however, on plants treated with *Rhizobium* or nitrogen. These differences highlight variation in host quality between biological nitrogen fixation by *Rhizobium* and nitrogen fertilization (Buchanan et al. 2000).

In conclusion, the pea leaf weevil-legume-rhizobia relationship is complex and both host plant and *Rhizobium* interaction contribute to the pea leaf weevil host preference and offspring development. It is likely that pea leaf weevil reproductive development is not completed when adults feed on secondary host plants, and hence no oviposition occurs on the soil around secondary hosts. Another possibility is that eggs laid on soil surrounding secondary hosts are not viable or larvae cannot access root nodules. Future studies should focus on pea leaf weevil oviposition after feeding on potential hosts and on egg viability and larval success on these hosts. The secondary hosts may support adult survival when primary hosts are not available, but offspring success is driven by the legume-rhizobia interaction. The underlying mechanism of how the legume -rhizobia association affects host quality, and the chemical signature of the host should be further studied, as pea leaf weevil adults respond to host plant volatiles (Blight and

Wadhams 1987, Blight et al. 1991, Landon 1997, Evenden et al. 2016, St Onge et al. 2018). Identification of volatiles released by host plants with and without *Rhizobium* inoculation coupled with electrophysiological and behavioural assays to test adult weevil response to variation in host volatile profiles may provide a better understanding of pea leaf weevil-legume-rhizobia relationship. Inoculation of faba bean with *Rhizobium* has the potential to protect faba bean yield from pea leaf weevil damage.

Table 3-1. The inoculation rates and product details of *Rhizobium* inoculum corresponding to each legume species.

legume species		***Rhizobium* species and variety**	**application rate (mg/pot)**	**product details**
common name (variety)	**scientific name**			
Alfalfa (AC, Blue Jay)	*Medicago sativa* Linnaeus	*Rhizobium leguminosarum biovar trifoli*	1	Dormal inoculant, 850057A, Becker Underwood, MO, USA
Chickpeas (Leader)	*Cicer arientinum* Linnaeus	*Mesorhizobium circeri*	20	Tag team granular, Novozymes BioAg 3935 Thatcher Ave Saskatoon, SK Canada
faba bean (Zero tannin, CDC Snow Drop)	*Vicia faba* Linnaeus	*Rhizobium leguminosarum biovar viceae*	2	Nodulator® FB Peat, 2008027A, BASF Canada, Ontario, Canada
Lentil (Impulse)	*Lens culinaris* Medikus	*Rhizobium leguminosarum biovar viceae*	20	Nodulator Duo SCG, 2008027A, BASF Canada, Ontario, Canada
lupin	*Lupinus polyphyllus Lindl.*	*Bradyrhizobium sp.*	1	Garden inoculant, Mckenzie seeds, 1000 Parker Blvd, Brandon, Manitoba, R7A 6E1
Pea (Smiroff AAC, Lacombe)	*Pisum sativum* Linnaeus	*Rhizobium leguminosarum biovar viceae*	20	Nodulator Duo SCG, 2008027A, BASF Canada, Ontario, Canada
Soybean (AC Edwards)	*Glycine max* Williams	*Bradyrhizobium japonicum*	30	Cell-Tech® west Granular, Novozymes BioAg 3935 Thatcher Ave Saskatoon, SK Canada

Table 3-2. Mixed effect model results for field choice test conducted in 2019 to test pea leaf weevil adult host preference, oviposition and larval development on faba bean. Separate mixed effect models were used to analyse each response variable between each choice treatment. Plots were arranged to present weevils with four choices, control vs nitrogen, control vs *Rhizobium*, control vs nitrogen & *Rhizobium* and nitrogen vs *Rhizobium* in a field plot study.

response variable	**choice test**	χ^2	**df**	**p**
total number of adults	control vs nitrogen	3.98	1	**0.04**
	control vs *Rhizobium*	0.005	1	0.94
	control vs nitrogen & *Rhizobium*	1.66	1	0.19
	nitrogen vs *Rhizobium*	23.01	1	**1.607e-06**
average number of notches/10 plant	control vs nitrogen	0.15	1	0.70
	control vs *Rhizobium*	11.20	1	**0.0008**
	control vs nitrogen & *Rhizobium*	1.52	1	0.22
	nitrogen vs *Rhizobium*	0.82	1	0.36
average number of eggs/plant	control vs nitrogen	0.26	1	0.61
	control vs *Rhizobium*	0.78	1	0.37
	control vs nitrogen & *Rhizobium*	1.95	1	0.16
	nitrogen vs *Rhizobium*	1.36	1	0.24
average number of larvae/plant	control vs nitrogen	37.15	1	**1.096e-09**
	control vs *Rhizobium*	1.99	1	0.15
	control vs nitrogen & *Rhizobium*	0.08	1	0.76
	nitrogen vs *Rhizobium*	3.03	1	0.08
average number of nodules/plant	control vs nitrogen	17.63	1	**2.678e-05**
	control vs *Rhizobium*	0.77	1	0.37
	control vs nitrogen & *Rhizobium*	0.53	1	0.46
	nitrogen vs *Rhizobium*	7.60	1	**0.005**
average number of damaged nodules	control vs nitrogen	1.36	1	0.24
	control vs *Rhizobium*	1.67	1	0.19
	control vs nitrogen & *Rhizobium*	1.40	1	0.23
	nitrogen vs *Rhizobium*	0.01	1	0.91
yield	control vs nitrogen	1.42	1	0.23
	control vs *Rhizobium*	8.18	1	**0.004**
	control vs nitrogen & *Rhizobium*	0.23	1	0.62
	nitrogen vs *Rhizobium*	1.77	1	0.18

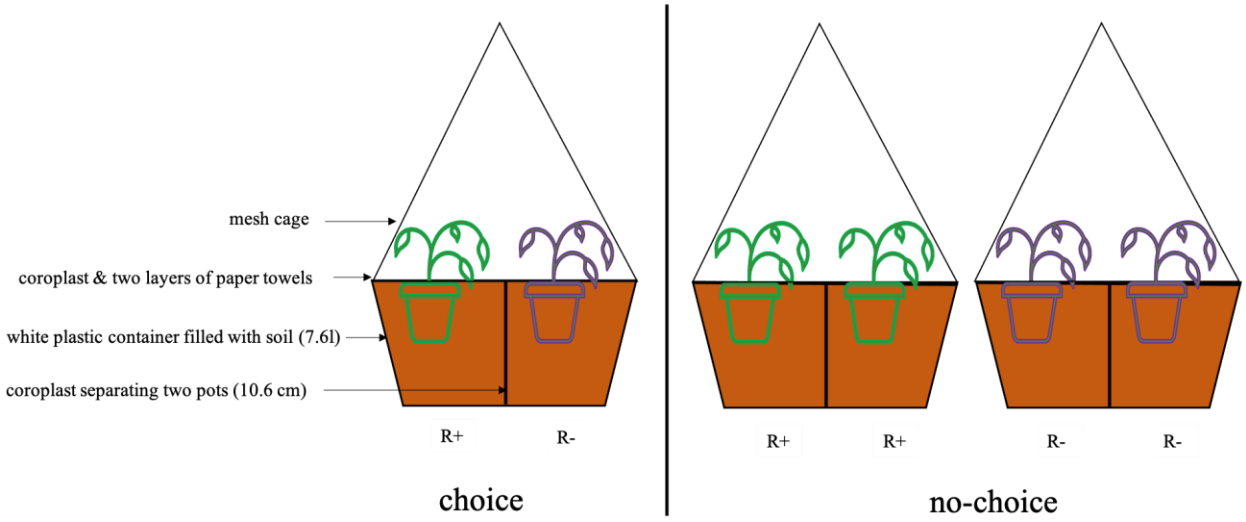

Figure 3-1. Schematic diagram of experimental set up to test the effect of *Rhizobium* on pea leaf weevil host acceptance and female oviposition in the greenhouse. Faba bean seeds were treated with *Rhizobium* at the planting. Treated (R+) and untreated (R-) plants were exposed to two females and two males in a mesh cage under choice or no-choice conditions. The number of adult feeding notches and the number of eggs laid per female were counted after 10 days of experimental period.

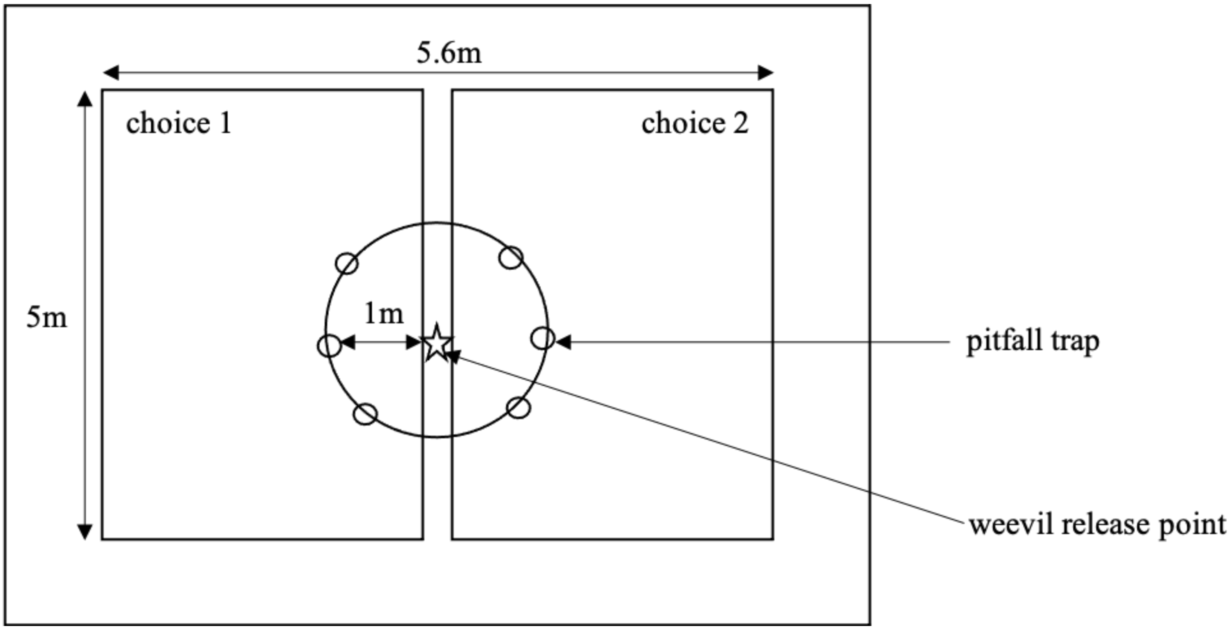

Figure 3-2. Schematic diagram of marked pea leaf weevil release point and locations of pitfall traps in between two choices of each choice treatment. A field choice study was conducted to test the effect of *Rhizobium* inoculation and nitrogen fertilization on adult host preference, oviposition, and larval development. Weevils were marked using fluorescent powder according to the treatment: i) control vs nitrogen (blue), ii) control vs *Rhizobium* (orange), iii) control vs nitrogen and *Rhizobium* (pink) and, iv) nitrogen vs *Rhizobium* (green). One hundred and fifty coloured weevils were released at the release point between two choices of each treatment (n=4/treatment). Traps were checked for consecutive days after the released day and the number of marked and unmarked weevils were collected and counted.

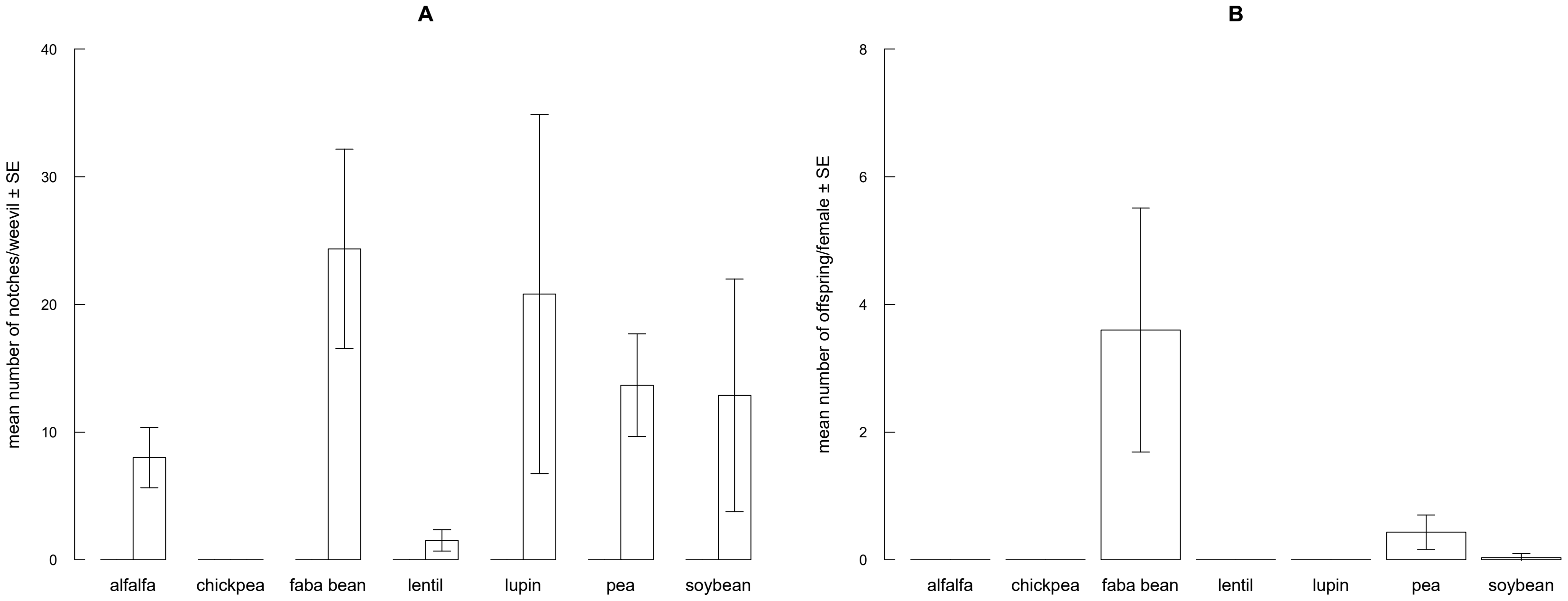

Figure 3-3. Mean number (± SE) of adult feeding notches (A) and the mean number of offspring (B) that emerged from seven different legume species in a no-choice greenhouse assay. Two female and male pea leaf weevils were introduced to each cage containing one of the legume plants (n=10/plant host) and weevils were allowed to feed, mate and oviposit. Notches were counted on 5cm portions along the stem from the bottom, middle and top of each plant and the average notches were calculated. The offspring were collected from the soil of the cage after 2.5 months of development.

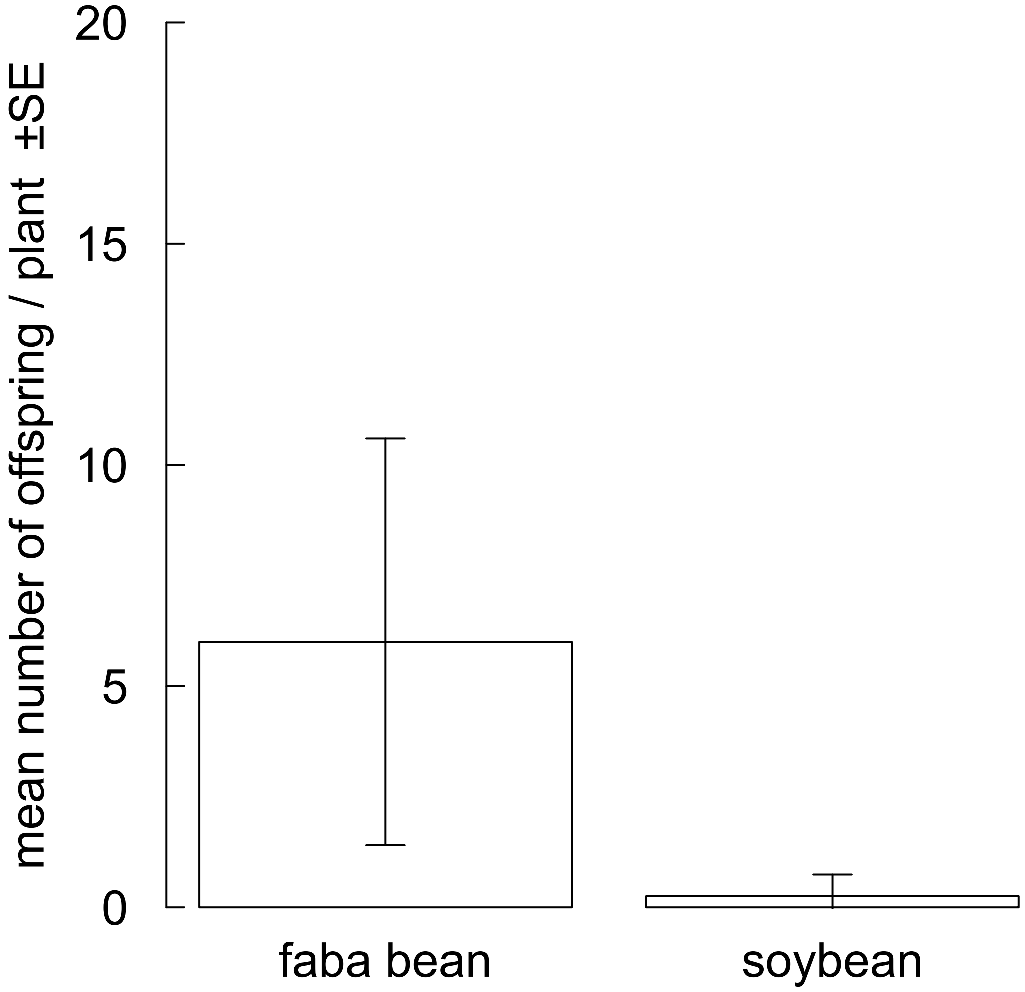

Figure 3-4. Mean number (±SE) of pea leaf weevil offspring emerged from caged faba bean and soybean plants in the field. A cage study (n=4/host) was conducted to test pea leaf weevil larval development on soyabean under field conditions. Ten males and females were introduced to each cage containing 20 plants. Two plants were harvested with the soil at the early flower stage, and the offspring were collected from the soil.

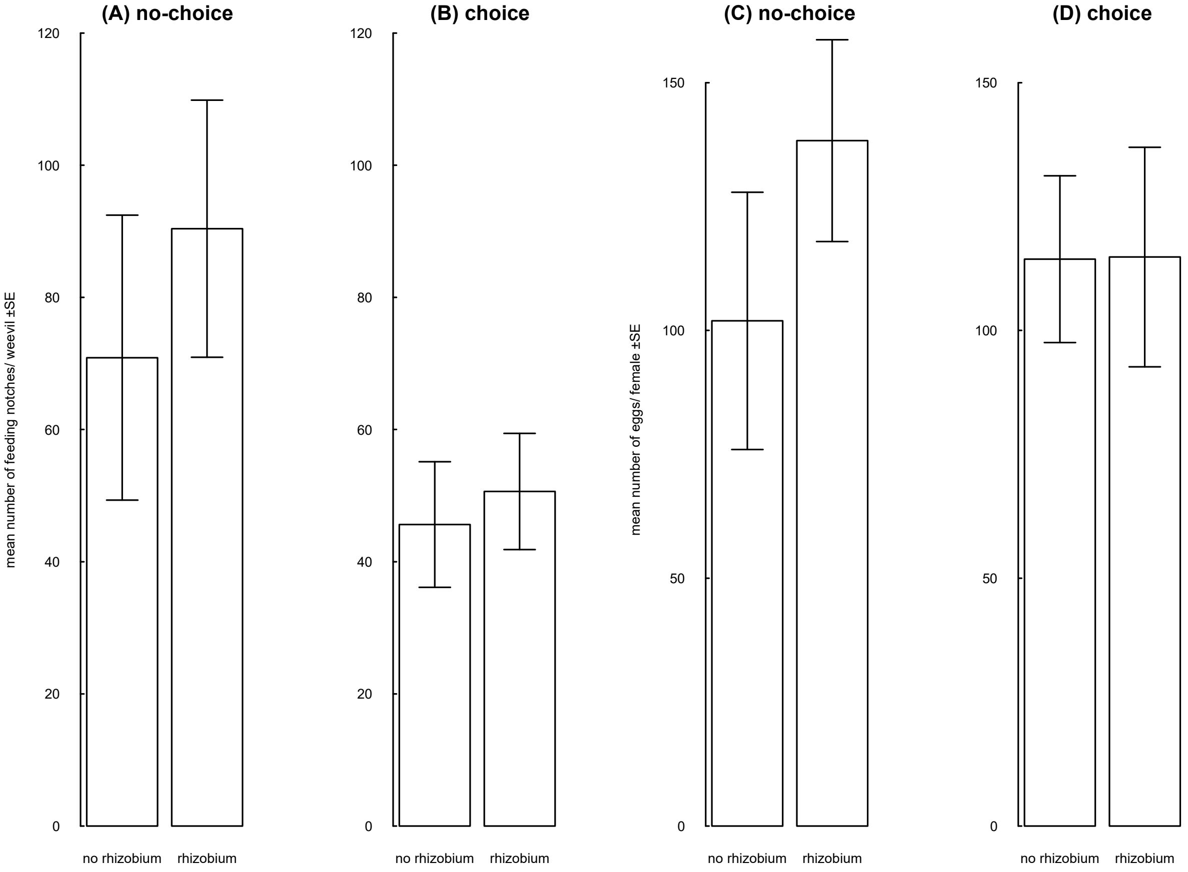

Figure 3-5. Mean number (±SE) of notches and mean number of eggs laid per female pea leaf weevil on *Rhizobium*-treated and untreated faba bean plants in a no-choice (A, B) and a choice (C, D) greenhouse assay. Four female pea leaf weevils were introduced to a caged pot containing a faba bean plant at the 2nd unfolded leaf stage, inoculated with *Rhizobium* and a plant without *Rhizobium* inoculation (n=10) or to caged pot containing two *Rhizobium* inoculated plants or two control plants (n=10/treatment). The number of feeding notches per weevil and the number of eggs laid per plant per female were collected and counted after 10 days of feeding and oviposition period.

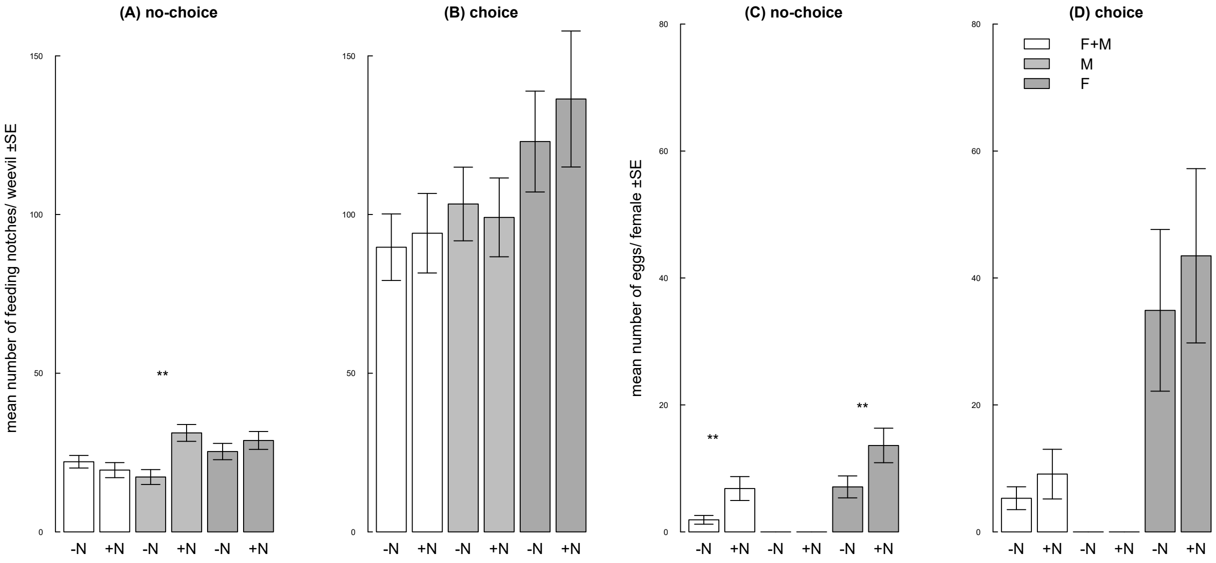

Figure 3-6. Mean number (±SE) of notches and mean number of eggs laid per female pea leaf weevil on nitrogen-treated and untreated faba bean plants in a no-choice (A, B) and a choice (C, D) greenhouse assay. Four female, four male or two female and two male pea leaf weevils were introduced to a caged pot containing a faba bean plant at the 2nd unfolded leaf stage, treated with nitrogen and a plant without nitrogen (n=10) or to a caged pot containing two nitrogen-treated plants or two control plants (n=10/treatment). The number of feeding notches per weevil and the number of eggs laid per plant per female were collected and counted after 10 days of feeding and oviposition period

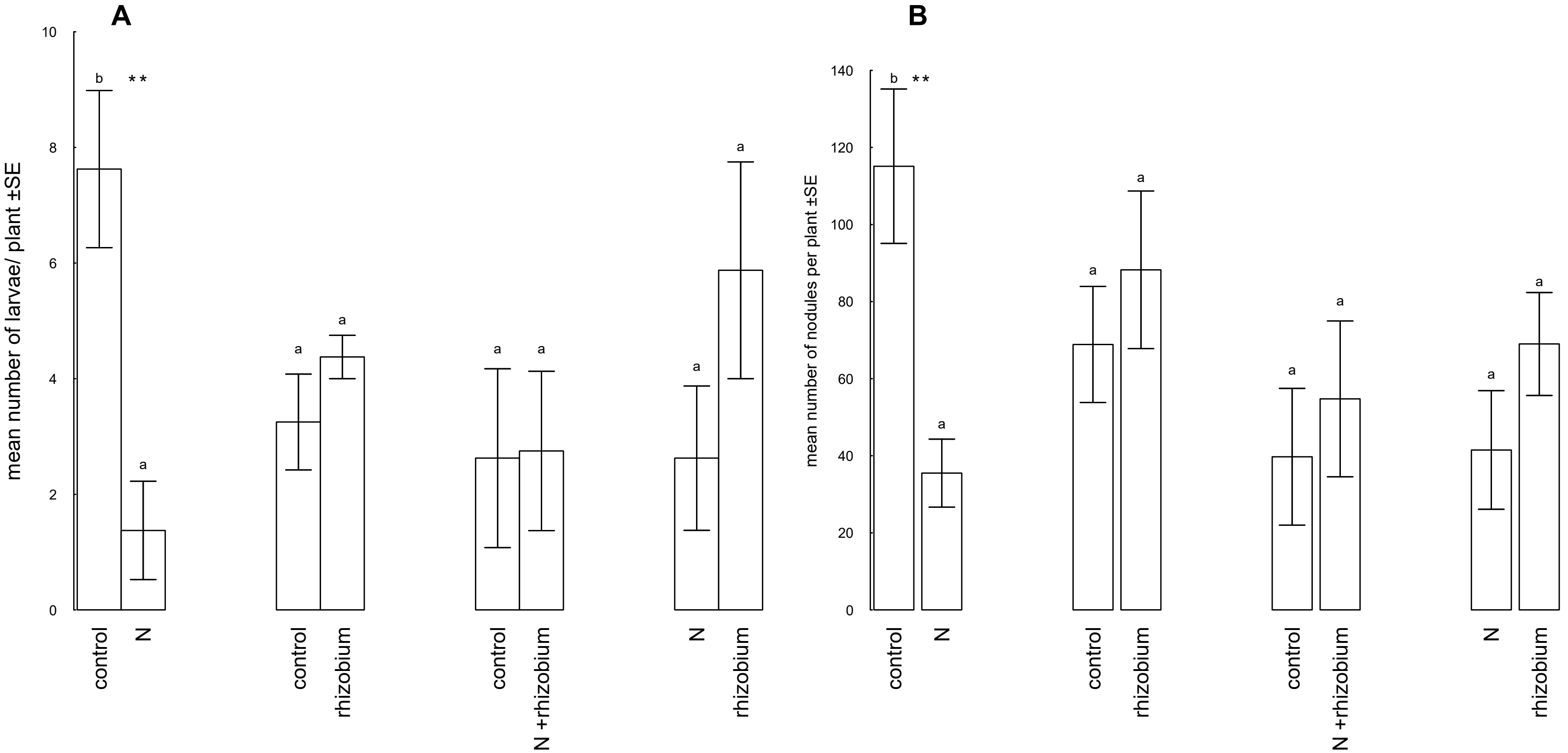

Figure 3-7. Mean number (±SE) of pea leaf weevil larvae per faba bean plant (A) and the mean number of nodules produced by faba bean plants (B) in a field study that tested the pea leaf weevil host preference and larval development. Plots (n=4/choice) were arranged to provide weevils with four choices, control *vs* nitrogen, control *vs Rhizobium*, control vs nitrogen and *Rhizobium* and nitrogen *vs Rhizobium.* The control plots were only treated with fungicide. Two plants were harvested with the soil at the early flower stage, and the offspring from each plant was collected from the soil.

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Appendix 3-1. Low nitrogen soil recipe used in the greenhouse studies.

1 bale peat moss

1bag Turface

2 bags vermiculite

Nutrient mix:

1000g Calcium Carbonate

Ground Limestone, Graymont Western Canada Inc., Prod # 09FG-GRA-009922

1200g 0-21-0, Phosphate, ADM, Prod#070623

20g Fritted trace elements, Everris, Micromax, Micronutient Granular, Prod# E90505C

15g chelated Iron 14%, Lidochem, Lidoquest Zn14, Prod# ZNEDTA14HEB

7g chelated Zinc 13%, Lidochem, Lidoquest Fe13, Prod# FeEDTA13HEB

Appendix 3-2. The protocol for legume plant nodulation assessment used at early-late flower stages. The protocol adapted from Nodulation assessment 2019 (https://2020seedlabs.ca/nodulation-assessments/).

Assessment Criteria

1. Plant and Growth Vigour

- Plants green and vigorous (healthy) 5
- Plants green and relatively small 3
- Plants slightly chlorotic (less green) 2
- Plants very chlorotic 1

2. Nodule Colour and Number

- Greater than five clusters of pink pigmented nodules 5
- Three to five clusters of predominantly pink nodules 3
- Less than three clusters of nodules, or whitish/greenish nodules 1
- No nodules, or white/green nodules 0

3. Nodule Position

- Crown and lateral root nodulation 3
- Generally crown nodulation 2
- Generally lateral root nodulation 1

Total score = score for plant and growth vigour + score for nodule colour and number + score for nodule position

Total Score = 11 to 13

Nodulation Assessment: Effective Nodulation

Nitrogen Fixing Potential: Good. No further Steps required

Appendix 3-2. continious….

Total Score = 7 to 10

Nodulation Assessment: Less effective nodulation

Nitrogen Fixing Potential: Reduced. Check inoculation method for errors; could also be a result of less-than-optimal growing conditions.

Total Score = 1 to 6

Nodulation Assessment: Unsatisfactory

Nitrogen Fixing Potential: Poor. Re-evaluate inoculants used, inoculation method and growing conditions.

Chapter 4 **Cold hardiness and overwintering survival of the pea leaf weevil (Coleoptera: Curculionidae) in its expanding range**

Abstract

Predicting population dynamics and the development of strategies to manage invasive agricultural pests requires a better understanding of their life history including cold hardiness. Pea leaf weevil (*Sitona lineatus* L.) is an invasive agricultural pest of field peas (*Pisum sativum* L.) and faba bean (*Vicia faba* L.). Pea leaf weevil has expanded its range in North America to include the Prairie Provinces of Canada. Here, I investigate the overwintering survival and cold hardiness of pea leaf weevil in its expanded range. I also investigate the influence of overwintering temperature and the duration on pea leaf weevil post-overwintering interaction with its host plants and the effect of juvenile hormone in modulating lipid metabolism of overwintering weevils. The overwintering survival of pea leaf weevil adults increased with soil temperature and varied with region and microhabitat. More weevils survived winters at tree shelterbelts compared to open alfalfa fields. Weevil supercooling point varied throughout its expanding range but not between the two microhabitats. The average threshold lethal temperature of pea leaf weevil at all three sites was -9.38°C. Weevils that overwintered for a longer duration and at a higher temperature fed more on faba bean foliage and laid more eggs compared to those which overwintered for a shorter duration at a lower temperature. My study highlights that warm winters would increase overwintering survival and post-overwintering fitness, facilitating further northern pea leaf weevil invasion northward in the Prairie Provinces of Canada.

Introduction

Overwintering biology and cold hardiness of invasive insect herbivores are good indicators of survival in their expanded range (Bale and Hayward 2010). Insects can survive in severe winter climates using various behavioural and physiological mechanisms (Danks 1996). Insects select microhabitats to minimize energy drain during the overwintering period (Marshall and Sinclair 2012, Sinclair 2015). Small changes in microhabitat selection can greatly influence overwintering survival, but selection of an optimal microhabitat is not always possible (Sinclair 2015, Sunday et al. 2014). Insects can also physiologically cope with sub-zero temperatures either through freeze tolerance or by avoiding or delaying freezing by supercooling (Baust and Rojas 1985, Salt 1961). The supercooling point, the temperature at which spontaneous freezing occurs, is a commonly used measure of cold hardiness (Zachariassen 1985).

Overwintering temperatures and duration can impact not only overwintering survival but also sublethal effects and the subsequent performance of individuals following overwintering (Marshall and Sinclair 2012, Williams et al. 2015). Insects store specific energy sources, lipids in the form of triglycerides and carbohydrates in the form of glycogen and trehalose, in preparation for winter (Hahn and Denlinger 2007). Longer winters could lead to increased energy consumption during the overwintering period leading to starvation through resource depletion, trade-offs with cryoprotectants, or increased costs associated with repairing cold injuries (Han and Bauce 1998, Koštál et al. 2006, Sgolastra et al. 2011, Sinclair 2015). Overwintering temperatures can also influence individual survival through alteration of the metabolic-rate-temperature relationship that dictates high energy use at high overwintering temperatures (Williams et al. 2012). Selection of microhabitats can modify overwintering temperatures to which insects are exposed, as snow cover can increase mean soil temperatures and result in minimal temperature fluctuation (Pauli et al. 2013). Overwintering conditions are not always directly linked to individual fitness through loss of energy reserves, as some insects feed post overwintering, but indirect effects such as cold injury to the digestive system can affect fitness through energy expenditure to repair damaged tissues (Sinclair and Chown 2005, Teets et al. 2011).

Hormones, such as juvenile hormone, ecdysteroids, or diapause hormone, alone or interactively regulate adult diapause in insects. Diapausing adults cease their reproduction; females stop oocyte development while males fail to mate with receptive females. Diapausing insects have smaller accessory glands and remain inactive during diapause (Denlinger et al. 2005). Juvenile hormone plays a major role in regulating reproductive diapause in many insects (de Kort 1989, 1990, Larrere et al. 1993, Matsuo et al. 1999, Tanigawa et al. 1999, Watanabe and Tanaka 2000, Kopper et al. 2001, Evenden et al. 2007, Sim and Denlinger 2013, Liu et al. 2016, Zhu et al. 2017, Ma et al. 2021). Diapausing insects have low levels of juvenile hormone titre and the termination of diapause is associated with a rise in juvenile hormone titre (de Kort 1990). Application of juvenile hormone to diapausing adult female insects promotes oviposition (de Kort 1990, Evenden et al. 2007, Liu et al. 2016), while removal of the *corpora allata*, the site of juvenile hormone synthesis, induces the diapause state (de Kort 1990). Juvenile hormone can influence physiological status by reducing lipid accumulation in diapausing insects (Liu et al. 2016, Sim and Denlinger 2013) and this reduction of energy resources may affect cold tolerance (Denlinger et al. 2005, 1988). Hormonal disruption of adult diapause has potential as a pest management tactic for insect pests that overwinter as adults, including some important invasive insect pests (Krysan 1990, Žďárek et al. 2000). The condition of insects in the post-overwintering physiological stage will affect subsequent feeding and dispersal which can influence oviposition and population dynamics of invasive insect pests.

Pea leaf weevil, *Sitona lineatus* Linnaeus (Coleoptera: Curculionidae), is an oligophagous pest of leguminaceous (Fabales: Fabaceae) plants (Jackson 1920). It is native to Europe and North Africa and has become an invasive pest in North America (Vankosky et al. 2009) damaging its primary hosts field pea (*Pisum sativum*) and faba bean (*Vicia faba*) (El-Dessouksi 1971, Jackson 1920, Nielsen 1990). Pea leaf weevil has expanded its range in North America to include the Prairie Provinces of Canada (Bloem et al. 2002, Cárcamo et al. 2018, Gavloski 2019, Hoebeke and Wheeler 1985, Hubert and Reehe 1961, “Pea Leaf Weevil Detected in Western N.D.” 2017). The life cycle, life history and pest management strategies of the pea leaf weevil were reviewed by Cárcamo et al. (2018), Evenden (2018) and Vankosky et al. (2009). Less is known, however, about the overwintering biology of the weevil, which when combined with estimates of population density taken in the fall (St. Onge et al. 2018), is crucial for pest management decisions prior to spring planting. Pea leaf weevils are univoltine (Jackson 1920) and overwinter as non-reproductive adults in field shelterbelts or semi-perennial forage pulse crops where they can resume feeding if the temperature permits (Schotzko and O’Keeffe 1986). Pea leaf weevils do not undergo a true diapause and the reproductive tract slowly develops through the overwintering period (Schotzko and O’Keeffe 1986).

Although the pea leaf weevil is well established in North America and continues to spread toward northern and eastern areas of the Prairie Provinces of Canada, it is not understood how well this invasive pest copes with the harsh winter conditions in the Canadian Prairies. Cold hardiness is an essential component for winter survival of pea leaf weevil in its expanded northern range in North America. An understanding of the overwintering strategy of the pea leaf weevil, together with measurements of overwintering survival may provide insight into its future invasion success and improve local forecasting to plan pest management strategies. In this study, my objectives were to investigate: 1. cold hardiness of the weevil by determining its supercooling point, 2. the overwintering survival of the pea leaf weevil in its expanding range under two microhabitats, 3. the effect of juvenile hormone on control of overwintering survival, and 4. the ideal overwintering temperature and duration to optimize pea leaf weevil performance to facilitate laboratory colony establishment.

Materials and Methods

Weevils

Pea leaf weevils were collected in August of all study years from alfalfa using sweep nets at fields near Lethbridge Research and Development Center (LRDC), Lethbridge, Alberta, Canada (49.7004°N - 112.7632°W). Collected weevils were held in mesh cages (47.5 cm X 47.5 cm X 93 cm) at 23°C, 16:8 L:D and fed alfalfa *ad libitum* before each experiment (2 alfalfa pots/cage) pots. Conical flasks (0.5 l) containing cotton swabs were used to provide water.

Field overwintering survival

A field study was conducted to investigate the overwintering survival and cold hardiness of pea leaf weevils in its expanding range in the Canadian Prairie Provinces. The study was conducted at three sites, one in each of southern, central and north-central Alberta, Canada. These sites, located in Lethbridge (49.7004°N, -112.7632°W), Lacombe (52.4540°N, -113.7517°W), and Edmonton (50.5030°N, -113.5290°W), span some of the broad climatic conditions found throughout the invasive range of the pea leaf weevil in Alberta. The experiment was repeated at all three study sites in 2016 and 2017 and only at the Lethbridge site in 2018. Cylindrical microcosm cages (10 cm X 20 cm) constructed from 70 mesh (7250C, Dacron

Chiffon, BioQuip Products Inc, CA, USA), contained a 6 cm layer of alfalfa or leaf-litter on top of a 6 cm layer of soil to mimic the overwintering habitat. Each microcosm contained 10 male and 10 female weevils. All weevils used in this study were collected from the Lethbridge site. In mid-October of each study year, microcosms were positioned in one of two microhabitats at each site, a tree shelterbelt or 10 m into an adjacent alfalfa field. Ten microcosms were buried 10 m apart in each microhabitat at each site along a transect (100 m). The soil temperature and humidity were measured at each location with HOBOs (U23-001A, U23 Pro v2 temperature/relative humidity data logger) which were positioned 6 cm above and below the soil surface. Microcosms were collected in the spring when the air temperature was above 12°C for at least one week. The microcosms were transported to the laboratory and searched for living and dead weevils. Live weevils were stored at 10°C and used for supercooling measurements.

Supercooling point determination

Supercooling points of the living weevils that successfully overwintered in the field study were determined as a measure of cold hardiness. Live adults were attached dorsally using petroleum jelly to a fine thermocouple (0.26 mm in diameter, T type copper-constantan, Omega Engineering, Laval, QC, Canada) threaded through the lids of 2 ml screw-cap cryogenic vials. One to six vials containing weevils (n=1 weevil/vial) were placed in a cooling thermostat (LAUDA-Brinkmann, LP ©. Delran, New Jersey, United States), and the temperature was dropped to -30°C at 1°C/min intervals. A micro logger (21X, Campbell Scientific Inc. ©. Logan, Utah, United States) connected to the thermostat recorded the temperature data at one-second intervals. The temperature at which heat crystallization occurred was recorded as the supercooling point.

Effect of juvenile hormone on pea leaf weevil quiescence

A laboratory study was conducted to test whether pea leaf weevil quiescence is influenced by juvenile hormone. Weevils were separated according to sex and were treated topically with 0.5µl of a 20 µg/µl solution of juvenile hormone III (65%, J2000, Sigma Aldrich, Oakville, ON, Canada) in acetone (HPLC grade, A949-4, Fisher Scientifics) or acetone alone as a control (n=100/treatment/sex). Treated and untreated males and females were overwintered at 5°C in cylindrical microcosm cages (10 cm X 20 cm) that contained a 6 cm layer of leaf-litter on top of a 6 cm layer of soil to mimic the overwintering habitat. Each microcosm contained 20 male or 20 female weevils. Microcosms were misted with distilled water once a week. Weevils were recovered after four months of overwintering and assessed as living or dead. Weevils were stored at -20°C before lipid extraction. Weevil lipid content was extracted in petroleum ether (Fisher Chemical, Fair Lawn, NJ). Weevils (n=10/treatment/sex) were dried at 60°C for 24 h and weighed to the nearest 0.01mg (Mettler Toledo, XS105, Columbus, OH). Weevils were placed individually in perforated vials (0.2ml) and flushed with petroleum ether in a Soxhlet apparatus (45/50 Pyrex, Fisher, Canada) for 6 h. After lipid extraction, weevils were dried at 60°C for 24 h and re-weighed. The weight difference before and after extraction accounted for the body lipid content.

Effect of laboratory overwintering temperature and duration on feeding and oviposition

The effects of overwintering duration and temperature on pea leaf weevil survival, feeding and oviposition were investigated in the laboratory with the aim to optimize their survivorship and performance to establish robust colonies for research. Weevils were overwintered at one of two temperatures, 5°C or 10°C. Five male and seven female pea leaf weevils were introduced into individual mesh (described above) microcosms (10 cm X 20 cm) or to plastic containers (1

L) containing a surface layer of leaf litter (6 cm) on top of a soil layer (6 cm). Five microcosms and five containers were held at each of the two test temperatures for either 10 or 20 weeks under dark conditions. At the end of the overwintering period, weevil survival was recorded and live weevils from each treatment were introduced into a mesh cage (2.54 cm X 2.54 cm X 2.54 cm) with alfalfa plants (1 plant pot/cage, pre-flowering stage, AC Blue Jay) for at least 14 days. Following the foraging period on alfalfa, two pairs of males and females, observed in copula, were introduced to a caged faba bean plant (n= 8-10 cages/ temperature-duration combination). The number of feeding notches on plants and the number of eggs laid were counted at the end of 14 days on the caged faba bean plants.

Data analysis

Data were analysed using R version 3.6.3 (R Core Development Team 2020). Mixed effect models were constructed in lme4 library (Bates et al. 2015). Initial models contained all explanatory variables and interactions between all explanatory variables. The model simplification was achieved by ANOVA hypothesis testing ($P < 0.05$) for full and reduced models until the most parsimonious model remained. Model residuals were checked for normality using the Shapiro Wilk test. Model fit was tested visually using qq-plots. The models were checked for homogeneity of variance and over-dispersion using the one-sample Kolmogorov–Smirnov test in DARMa package (Hartig 2018). A Tukey's post hoc test was conducted to test the separation of means of models (Tukey 1977).

The effect of overwintering site and microhabitat on proportion of overwintering survival was analysed using generalized mixed-effect models with a beta-binomial error distribution. The effect of overwintering site and microhabitat on supercooling point was analysed using a general mixed effect model. The microhabitat was nested within the study site and within the study year and was treated as the random effect in these models. A linear model was used to analyse the correlation between the proportion of live weevils and the mean overwintering soil temperature over the overwintering period. The proportion of live weevils was transformed to square root. A linear model analysed the effect of juvenile hormone treatment and sex on body lipid content by body weight comparisons after overwintering. The treatment, sex and interaction between treatment and sex were the fixed factors in the model.

The effect of temperature and duration of the overwintering period on weevil survival was analysed using generalized mixed effect models with a binomial error distribution. The proportion of live weevils (total, female, and male) was the response variable in each model with overwintering temperature and duration of the overwintering period as fixed factors. The weevil survival was not different between overwintering habitats (mesh microcosm or plastic containers) and therefore, habitat was treated as a random factor in each model. The proportion of total live weevils was transformed to square root to meet the model assumptions. The effect of overwintering temperature and duration of the overwintering period on the subsequent mean number of eggs laid and the mean number of feeding notches per individual on caged faba bean plants was analysed using a linear model. A linear regression tested the relationship between the mean number of eggs per female and the mean number of feeding notches. The mean number of eggs was square root transformed to meet model assumptions. Fisher’s test was used to conduct the pairwise comparisons of Pearson correlation coefficients of linear regression models for temperature-duration combinations.

Results

Field overwintering survival

There is an interaction effect of overwintering site and microhabitat on the proportion of live weevils recovered from microcosms positioned at the various field sites (F=3.47, df=6, P=0.0038) (Fig. 4-1). Significantly more weevils survived the winter in tree shelter microhabitat in Lethbridge than those in alfalfa microhabitat and in both microhabitats at the more northern sites of Edmonton and Lacombe (Scheffe post hoc, P<0.05). Weevil overwintering survival showed a positive correlation with the soil temperature (F=6.33, df=1, P=0.03) (r^2=0.3) (Fig. 4-2). The supercooling point was affected by the overwintering site (χ^2=4.90, df=1, P=0.02). The mean supercooling point for weevils at the most northern site in Edmonton was -4.49±1.6 °C (n=3). In central Alberta at the Lacombe site, the mean supercooling point was -7.5±3.4 °C (n=6) and at the most southern site in Lethbridge was -9.7± 4.4 °C (n=83). The supercooling point of weevils did not differ by overwintering microhabitat (χ^2=0.10, df=1, P=0.74) (tree shelter= -9.7± 4.5 °C (n=71), alfalfa=-10.16± 3.6°C (n=12). Male and female weevils have similar supercooling points (χ^2=0.04, df=1, P=0.84).

Effect of Juvenile hormone on pea leaf weevil quiescence

None of the juvenile hormone- treated, acetone-treated or untreated control weevils survived the four-month overwintering period in the laboratory at -5ºC. Weevils were unable to adapt to the constant exposure at -5°C during the overwintering period. After removal from the overwintering conditions, lipids were extracted from the weevils from all treatments. An interaction effect between treatment and sex significantly affected body lipid content (F=3.98, df=2, P=0.02) (Fig. 4-3). Juvenile hormone-treated female weevils had lower body lipid content compared to control and acetone-treated weevils, but males in all treatments had a similar amount of body lipid (Tukey's post hoc, $P<0.05$).

Effect of overwintering temperature and duration on feeding and oviposition

Weevil overwintering survival did not vary between the milder overwintering temperatures of 5 and 10 °C (total: $\chi^2=0$, df=1, P=1, male: $\chi^2=0.73$, df=1, P=0.39, female: $\chi^2=0.65$, df=1, P=0.41) and duration (total: $\chi^2=0$, df=1, P=1, male: $\chi^2=0.03$, df=1, P=0.86, female: $\chi^2=2.33$, df=1, P=0.13). The overwintering duration (F=1.17, df=1, P=0.0001) and temperature ($\chi^2=6.13$, df=1, P=0.018), however, affected subsequent oviposition. Oviposition was higher when weevils were overwintered at 10°C compared to 5°C and for 20 weeks compared to 10 weeks (Tukey's post hoc, $P<0.05$). Weevils fed more after being held at 10°C compared to 5°C (F=8.03 df=1, P=0.007), and after a longer overwintering period of 20 weeks compared to the 10-week overwintering treatment (F=9.45 df=1, P=0.004). Weevil oviposition increased as feeding increased (F=84.85, df=1, P=6.952e-11, $r^2=0.7$) (Fig. 4-4). This positive correlation between feeding and oviposition, however, was affected by the temperature-duration treatment combination (Fig. 4-4) (F=3.99, df=3, P=0.01). The correlation coefficient was higher when weevils overwintered at 5°C for 20 weeks compared to 10°C for 10 weeks (z=2.65, df=1, P=0.01).

Discussion

Predicting pea leaf weevil population dynamics and invasion and the development of management strategies to target spring weevils requires a better understanding of its cold hardiness and factors influencing cold hardiness. The supercooling point provides useful information on the lower limit of the lethal temperature of freeze-intolerant species and is a good starting point to understand insect overwintering survival (Moore and Lee 1991, Renault et al.

2002). The average threshold lethal temperature of pea leaf weevil for all three sites is -9.38°C (± 0.46, range: -18.5 to -0.90). The value, however, varied depending on the site where they overwintered. Weevils that overwintered in Edmonton had a higher supercooling point (-4.49±1.6 °C) compared to those that overwintered at Lethbridge (-9.7± 4.4 °C). These differences in freeze tolerance between sites may not solely relate to the below-ground average temperature differences between sites. The mean below-ground temperature was lower in Edmonton (-3.79±0.15°C) and Lacombe (-4.97±1.04°C), than at Lethbridge (-2.47±0.67°C). Although high overwintering temperatures can reduce overwintering capacity in some insects through an increase in metabolic rate and reduction in the energy available (Sinclair 2015, Williams et al. 2012), the weevils in this study that overwintered at the lowest soil temperature had the lowest freeze tolerance. It is therefore unlikely that temperature alone affected energy use in overwintering weevils. Energy resources at the end of the overwintering period are not only used for activity in the post-winter period, but also are used for tissue repair related to the winter damage (Sinclair 2015). The colder the overwintering temperatures, the more severe is the tissue damage (Košťál et al. 2006). The few pea leaf weevils that survived the overwintering period at the Edmonton site may have spent more energy on tissue repair than the weevils that overwintered at the Lethbridge site, causing weevils to be less freeze tolerant after overwintering in Edmonton. These results should be viewed with caution, however, as the number of weevils used in testing for the super cooling point was relatively low at the Edmonton (n=3) and Lacombe (n=6) sites, due to low overwintering survival at these locations.

Weevils successfully overwintered in both microhabitats within sites, and weevils had similar freeze tolerance when located in tree shelterbelts or alfalfa fields over the winter. Although some insects choose overwintering sites based on the insulating properties of the microhabitat to tolerate cold temperatures (Moore and Lee 1991), microhabitat did not affect the supercooling point of pea leaf weevils in our study. It is not clear which mechanisms contribute to the differences in freeze tolerance of pea leaf weevil between sites. The expanded range of the pea leaf weevil now extends north of Edmonton and into the Peace River region of Alberta. The low overwinter survival of weevils in microcosms in Edmonton suggests that these microhabitats do not adequately reflect the overwintering habitat of pea leaf weevil. Variation in microhabitats, cold tolerance strategy, snow cover and pre-overwintering conditions may collectively influence weevil ability to tolerate freezing (Marshall and Sinclair 2012, Pauli et al. 2013, Sgolastra et al. 2011). Future work should focus on field studies to understand the natural overwintering microhabitats and mechanisms of cold tolerance of the pea leaf weevil.

Despite the negative relationship between soil temperature and the supercooling point, pea leaf weevil overwintering survival was lowest at the low soil temperatures. This suggests that a warming climate might enhance the overwintering success of frontier populations of pea leaf weevil, allowing invasion to new habitats and range expansion (Olfert et al. 2012). Climate models predict pea leaf weevil range expansion beyond the current range in North America, and higher soil temperatures with climate change may facilitate pea leaf weevil overwintering survival in the expanded range (Olfert et al. 2012, 2017). Pea leaf weevil overwintering survival is highest at the southern site in Lethbridge and lowest at the northern site in Edmonton. High survival in Lethbridge may relate to the increased ability of weevils to tolerate cold temperatures with a lower supercooling point than weevils at other sites. Although there was no statistical difference in supercooling point between microhabitats, more weevils survived when overwintered in tree shelter belts compared to open alfalfa fields. Insects choose overwintering habitats based on the size, shelter, position, and moisture content of the microhabitat, and freeze intolerant insects avoid sites with high moisture (Salt 1961). Tree shelterbelts may provide extra insulation to overwintering weevils.

The differences in pea leaf weevil cold hardiness, as measured by the supercooling points of weevils at the different sites, and overwintering survival may relate to the physiological mechanisms that regulate overwintering and the production of cryoprotectants in this species. Juvenile hormone terminates reproductive diapause, increases the synthesis of vitellogenins and prompts egg-laying by adult female insects post overwintering (de Kort 1990, Denlinger et al. 2005, Matsuo et al. 1999). Female weevils that were treated with juvenile hormone III before being subjected to cold (-5°C) overwintering temperatures had lower levels of body lipid content compared to control females at the end of the overwintering period. This may relate to increased metabolic activity as a result of induction of reproductive activity by juvenile hormone stimulation. Juvenile hormone depletes the haemolymph lipids through reduction of lipogenic enzyme activity and total and triglyceride lipid biosynthesis (Mulye and Gordon 1993; Zera and Zhao 2004, Zibaee et al. 2011). Further studies on the trade-off between increased reproductive development and lipid reduction as a result of juvenile hormone activity in overwintering females may provide a better understanding of the timing of reproductive activity in the pea leaf weevil and lead to better management strategies against this invasive weevil.

The performance of insect herbivores following overwintering can be directly or indirectly related to the post-overwintering energetic condition (Sinclair 2015). Long, warm winter conditions can cause increased energy use and decrease the subsequent fitness of insect herbivores. Pea leaf weevils were well adapted to the mild overwintering temperatures of 5°C and 10°C tested in this study and there was no difference in weevil survival between the two temperature regimes in either overwintering duration scheme. I chose these mild temperatures to allow weevil survival in order to develop a method to overwinter pea leaf weevil colonies in the laboratory. Weevil energy state, however, may be affected by the overwintering temperature-duration combinations which could impact subsequent foraging behaviour and fitness. Energy balance and cryoprotectant management are regulated by energy signalling pathways in insects (Teets et al. 2011). The climatic cues experienced by the insects in the winter period can be transduced through the insulin pathway to dictate the subsequent behaviour (Wigby et al. 2011). In the current study, weevil feeding and oviposition post overwintering were higher when weevils experienced a higher overwintering temperature for a longer duration. Similarly, Schotzko and O'Keeffe (1988) showed that pea leaf weevil laid more eggs after a relatively long winter period. Pea leaf weevils do not undergo a true diapause and the reproductive system can mature over the period of quiescence, if conditions permit (Landon et al. 1995, Schotzko and O'Keeffe 1986). High temperatures for long periods during quiescence may allow for the initiation of reproductive tract development, and hence an increase in metabolic rate of pea leaf weevils. Pea leaf weevils must feed on reproductive host plants following overwintering to initiate oogenesis (Jackson 1920, Landon et al. 1995). Pea leaf weevils that experienced the high overwinter temperature for the long duration fed more following overwintering than weevils held at the other winter temperature/duration regimes. Weevils that initiate reproductive development in the overwintering period may have less energy resources at the end and must respond rapidly to host plants. The duration of the winter period at the high temperature seemed to dictate weevil fitness, as egg laying is directly related to adult feeding after winter in this species (Schotzko and O'Keeffe 1988). In some insects, reproductive maturation enhances insect response to host plant volatiles (Anton et al. 2007), which could be important for pea leaf weevil host colonization in the spring. The initiation of reproductive development in spring may be faster when overwintering temperatures are high over an extended period. Early resumption of reproductive development results in increased fitness of female weevils that lay more eggs under these conditions. Warm winters will benefit pea leaf weevil invasion and range expansion through an increase in both overwintering survival and post-overwintering population growth.

In conclusion, pea leaf weevil overwintering survival increases with soil temperature. The supercooling point of the pea leaf weevil varies with the region where it overwinters in its expanding range. The microhabitat plays an important role in pea leaf weevil overwintering survival, but more than one factor may contribute to cold hardiness. Juvenile hormone influences lipid metabolism in the pea leaf weevil and has the potential for development as a management tool against this species when the physiological mechanisms that regulate pea leaf weevil cold tolerance are determined. Overwintering conditions experienced by pea leaf weevils dictate overwintering survival, weevil fitness and potentially post-overwintering population growth and feeding damage. Warm winters under a changing climate would be expected to increase pea leaf weevil fitness and facilitate range expansion.

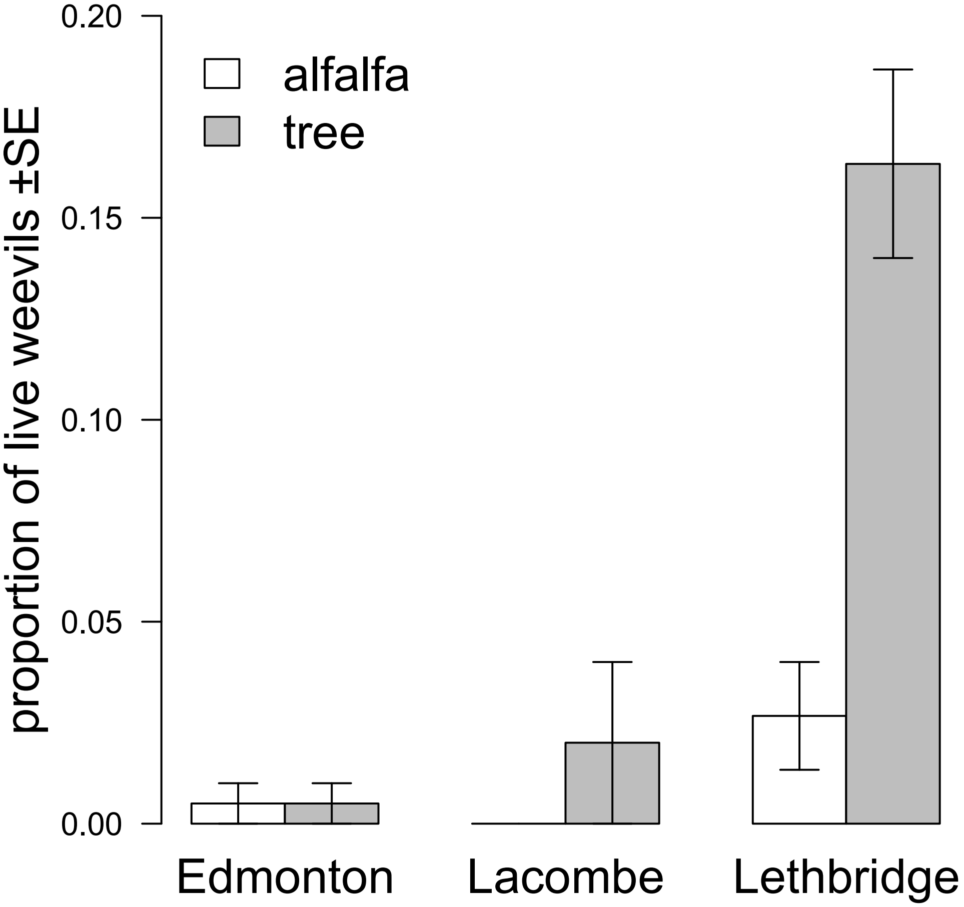

Figure 4-1. Proportion of live weevils overwintering in the field. A field study was conducted to investigate pea leaf weevil overwintering survival at three sites, Edmonton, Lacombe and Lethbridge, Alberta from 2016-2018. Twenty weevils (n=10/sex) were introduced to a mesh microcosm containing soil and alfalfa leaf litter (n=20/site) and buried along a tree or in alfalfa field transects in October of each year. Microcosms were recovered in April and the number of live weevils were recovered and counted.

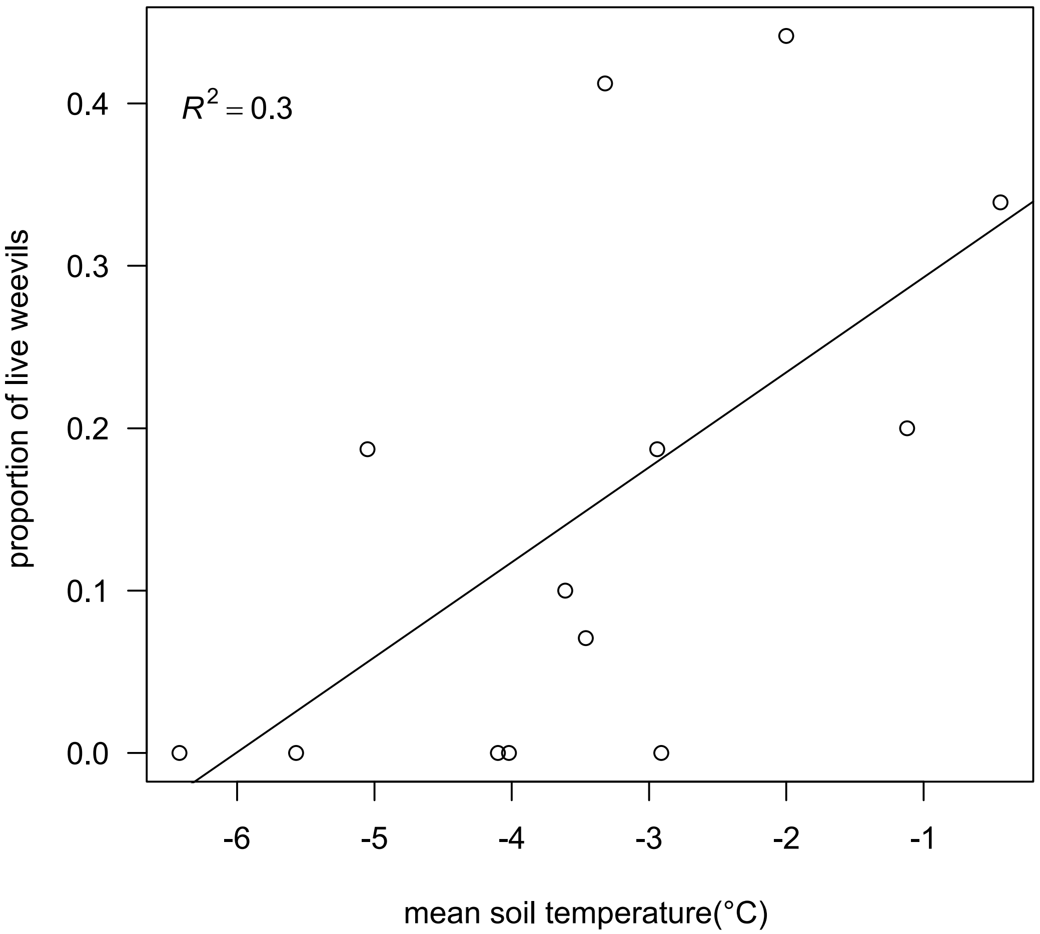

Figure 4-2. Correlation between mean overwintering soil temperature and the proportion of weevils that survived (r^2=0.3) after overwintering in the field. A field study was conducted to investigate the pea leaf weevil overwintering survival at three sites, Edmonton, Lacombe and Lethbridge, Alberta from 2016-2018. Twenty weevils (n=10/sex) were introduced to mesh microcosms containing soil and alfalfa leaf litter (n=20/site) and buried in two microhabitats along tree or in alfalfa field transects in October of each year. Microcosms were recovered in April and the proportion of live weevils in each microcosm was recovered and counted. Below ground soil temperature was recorded using HOBOs buried 6 cm below the soil surface.

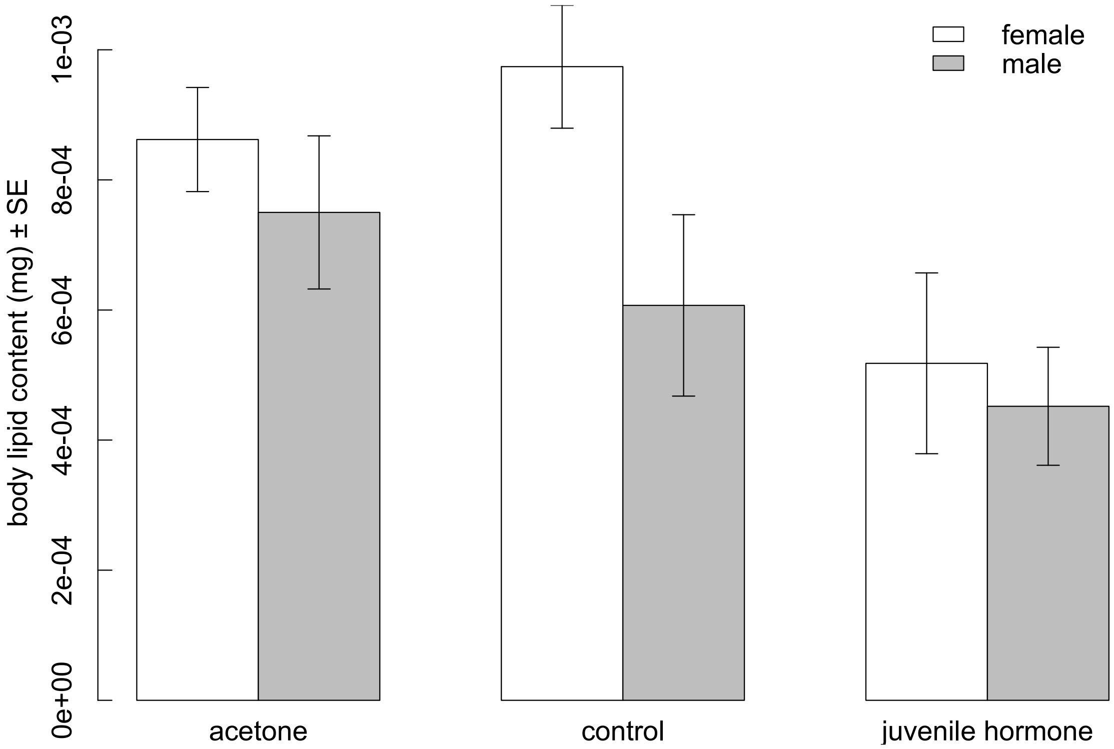

Figure 4-3. Post overwintering body lipid content (mg) of pea leaf weevils treated with juvenile hormone III diluted in acetone or acetone alone before overwintering at -5 °C in the lab, compared to that of similarly handled but untreated control weevils. Weevils were treated topically with juvenile hormone III or acetone. Treated and untreated control weevils were placed in separate mesh microcosms containing soil and leaf litter and microcosms were placed at a constant temperature of -5°C for overwintering. Dead, overwintered weevils (n=10/treatment/sex) from each treatment group were subjected to lipid extraction in petroleum ether using a Soxhlet apparatus.

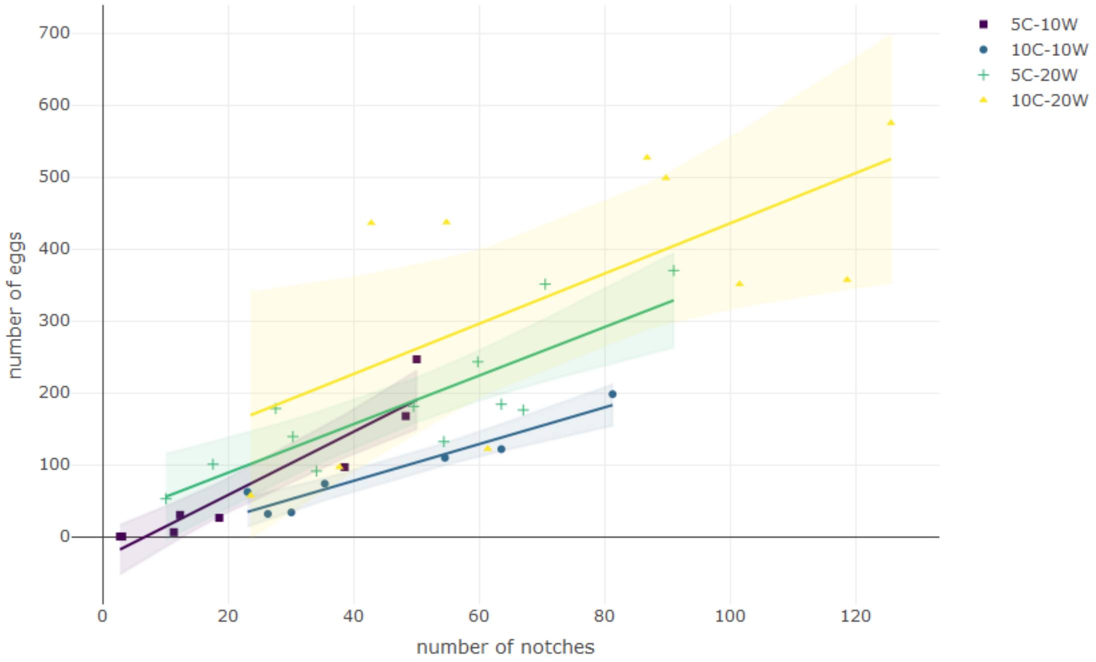

Figure 4-4. Correlation between pea leaf weevil adult feeding after overwintering and subsequent oviposition of weevils held under four different overwintering temperature-duration regimes. A laboratory overwintering study was conducted to investigate the effect of overwintering duration and temperature on pea leaf weevil feeding and oviposition. Weevils overwintered for 10 or 20 weeks (W) at 5□C or10 □C. Following overwintering, two female and two male weevils from each treatment were introduced to a cage with a faba bean plant (n= 8-10 cages/ temperature-duration combination). Weevils were allowed to feed and oviposit for 10 days. The number of feeding notches on foliage and number of eggs laid were counted after 14 days

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Chapter 5 **Management of pea leaf weevil (Coleoptera: Curculionidae) and development of a nominal threshold in faba beans**

Abstract

Pea leaf weevil, *Sitona lineatus* (Linnaeus) (Coleoptera: Curculionidae), can reduce yield of field pea (*Pisum sativum* Linnaeus) and faba bean (*Vicia faba* Linnaeus). Adults feed on the foliage and larvae feed on root nodules and nodule-associated *Rhizobium* bacteria. In this chapter, I developed a data-based nominal threshold for pea leaf weevil in faba bean. I further tested the efficacy of insecticidal seed treatment and foliar insecticide (thiamethoxam and lambda-cyhalothrin, respectively), and nitrogen amendment for pea leaf weevil control using a multi-year field plot study at two sites in Alberta, Canada. Pea leaf weevil feeding damage significantly reduced faba bean yield. Thiamethoxam reduced adult and larval damage, and protected faba bean yield, while neither lambda-cyhalothrin nor a nitrogen amendment was effective in protecting yield. The percentage of seedlings with feeding on the terminal leaf had a negative relationship with yield and was used to estimate a nominal threshold near 15% of seedlings with terminal leaf damage. Since lambda-cyhalothrin is not effective in managing pea leaf weevil on faba bean, there is a need to research additional integrated pest management strategies to reduce prophylactic insecticidal seed treatments.

Introduction

Insect pests can have large ecological and socioeconomic impacts (Pimentel et al. 2002, Pimental 2004, Schlaepfer et al. 2011, Early et al. 2016) and often require control with chemical insecticides. Judicious chemical control that is incorporated into Integrated Pest Management (IPM) programs requires knowledge of pest population density in relation to plant tolerance, natural enemies, and abiotic conditions in the habitat (Richards et al. 2015). Overuse of insecticide to manage agricultural insect pests can cause insecticide resistance, affect non-target species, increase production costs and harm wildlife and human health (Okoth et al. 2014, Alyokhin and Miller 2015). A major goal of IPM is to minimize insecticide use through application of economic thresholds (ET) (Stern et al. 1959, Pedigo et al. 1986, Sharma et al. 2011, Batistela et al. 2012, Ramsden et al. 2017). Economic thresholds for insecticide application can be incorporated with more environmentally friendly approaches such as ecological pest management (Tshernyshev 1995) and biovigilance (Delos et al. 2006). The precision of ETs, however, may be influenced by the method by which an EIL is calculated (Riley 2008). In cases in which some information is lacking, a Nominal Threshold can be developed in lieu of an ET, and still accomplish the goal to reduce insecticide use (Poston et al. 1983, Tangtrakulwanich et al. 2014). The nominal threshold can be a subjective value, based on the experience of the manager rather than on a calculated EIL (Poston and Pedigo 1983). The threshold calculated in the current study is a data-based estimate that compares adult feeding damage to yield but lacks a comparison with insect population density, and thus is referred to as a nominal threshold. For cost calculations, a common foliar insecticide registered for pea leaf weevil was used although its efficacy was poor in the current study.

Pea leaf weevil (*Sitona lineatus* (L.) (Coleoptera: Curculionidae)) is native to Europe and North Africa and has become an invasive pest in North America (Vankosky et al. 2009). Adults are oligophagous on leguminaceous (Fabales: Fabaceae) plants (Jackson 1920) and feed on a variety of legumes, but the species is only a major pest of its reproductive hosts, field pea (*Pisum sativum*) and faba bean (*Vicia faba*) (Jackson 1920, El-Dessouksi 1971, Nielsen 1990). Since the 1930s, pea leaf weevil has become established in the northwestern regions of United States (USA) including Washington, Oregon, northern California and northwestern Idaho (Hubert and Reehe 1961, Hoebeke and Wheeler 1985). Pea leaf weevils were first recorded in northeastern USA in 1984 (Hoebeke and Wheeler 1985) and in Florida in 2002 (Bloem et al. 2002). Recently, pea leaf weevil has been recorded in North Dakota, USA (Pea leaf weevil detected in western N.D, 2017). The pea leaf weevil was first recorded in southern Alberta, Canada in 1997 in field peas (Vankosky et al. 2009). Since then, it has expanded its range to the east and north in the Prairie Provinces of Canada and now infests field peas and faba beans grown in central Alberta, Saskatchewan, Manitoba and the Peace River Region of Alberta (Cárcamo et al. 2018, Gavloski 2019). Unlike pea leaf weevil management in peas (Cárcamo et al. 2018), there are no established ET or IPM strategies available for pea leaf weevil management in faba bean.

Pea leaf weevil adults feed on host plant foliage making "U" shaped notches along leaf margins (Jackson 1920). Field pea and faba bean are the preferred hosts of reproductively active weevils (Jackson 1920, Landon et al. 1995). Larvae feed on *Rhizobium leguminosarum* Frank (Rhizobiales: Rhizobiacea) bacteria associated with host plant root nodules along with root nodule tissue (Johnson and O'Keeffe 1981, Hamon et al. 1987). Feeding by pea leaf weevil larvae and adults can cause yield losses in both field peas and faba bean (El-Dessouksi 1971, Nielsen 1990). In field peas, larval damage is highest at the early flowering stage (Jackson 1920), where it reduces yield (Hunter 2001, Corre-Hellou and Crozat 2005), seed nitrogen content and soil nitrogen input (Doré and Meynard 1995, Corre-Hellou and Crozat 2005). Larval damage reduces pod production in faba bean (El-Dessouksi 1971). Pea leaf weevil adult feeding can reduce pod production and subsequent yield in white clover (*Trifolium repens*) (Murray and Clements 1992). The severity of damage caused by adult feeding, however, depends on the host plant stage (Williams et al. 1995). At very high densities, adult feeding can destroy developing seedlings (Jackson 1920).

Current pea leaf weevil management practices in faba beans in Alberta are based on monitoring adult damage in field peas, although most of the economic yield losses occur as a result of larval feeding (Vankosky et al. 2009). The nominal threshold to trigger foliar insecticide application against the pea leaf weevil in field peas is 30% of seedlings with adult damage on terminal leaves (Cárcamo and Vankosky 2011). Determining larval damage is difficult and time-consuming compared to adult damage assessment. The correlation between the level of adult feeding and future larval damage in field peas is equivocal, as some studies have found a correlation (Cantot 1989) and others have not (Cárcamo and Vankosky 2011). Current chemical management of pea leaf weevil relies mainly on insecticidal seed treatments to minimize larval feeding (Vankosky et al. 2009). Nominal thresholds do not provide immediate guidance for insecticidal seed treatments in this system, but it can be useful to evaluate seed treatment efficacy and plan for future years. Foliar insecticides are inconsistent in yield protection against the pea leaf weevil in both field peas and faba bean, as they have no direct effect on larvae (Bardner et al. 1983, Cárcamo and Vankosky 2011). The efficacy of foliar insecticides that target adults, to affect subsequent larval damage depends on the timing and frequency of applications, weevil density and abiotic factors (Bardner et al. 1983, Vankosky et al. 2009, Cárcamo et al. 2018).

Insecticidal seed treatment, however, protects yield by directly affecting larvae and reducing adult feeding through systemic action on foliage (Cárcamo et al. 2012). Although insecticides are commonly used in pea leaf weevil management in faba bean, no economic or nominal thresholds have been determined for use in the crop in Alberta or elsewhere. In addition to insecticides, nitrogen fertilization has been tested on field peas as a potential management tactic against the pea leaf weevil (Vankosky et al. 2011a). Nitrogen fertilization replaces biological nitrogen fixation in fulfilling the plants' nitrogen needs. Fertilization reduces root nodule production in field peas, thereby decreasing food availability and potentially decreasing the size of the larval population.

The objectives of this chapter were to develop a nominal threshold for pea leaf weevil in faba beans in Alberta, to assess the potential to manage it with insecticides registered in Canada on other field crops, and to explore the potential to use nitrogen fertilization in lieu of insecticides.

Materials and Methods

Study sites and treatments

A field plot study was conducted at sites near Lethbridge (49.7004°N, -112.7632°W) and Lacombe (52.4540°N, -113.7517°W), Alberta, Canada from 2016-2018. The experiment was a randomized block design with four blocks separated by 15 m at each site. Each block was divided into five plots. The plots were 9 m X 10 m at the Lethbridge site and 7.6 m X 10.6 m at the Lacombe site with a 4 m buffer between plots. Each plot was randomly assigned one of five treatments: 1) control with only fungicide (Trilex EverGol, 1.2 l/ha) seed treatment, 2) fungicide and insecticidal seed treatment (thiamethoxam (30 g/100 kg weight of seed), 3) fungicide seed treatment and foliar insecticide (lambda- cyhalothrin (84 ml/ha)) at the 2nd unfolded leaf stage, 4) fungicide seed treatment and lambda-cyhalothrin at 2nd and 4th unfolded leaf stages, 5) fungicide and thiamethoxam, and lambda-cyhalothrin at 2nd unfolded leaf stage. In 2017 and 2018, a sixth treatment was added in the form of urea (46-0-0) amendment at an amount equivalent to the cost of thiamethoxam treatment (50 kg nitrogen/ha) in addition to the start-up fertilizer amount added to all plots as described below.

Before seeding, soil samples from each site were tested for the availability of nutrients (nitrogen, phosphorous, potassium and sulphur). Five soil cores were obtained from each of two different depths, 0-15 cm and 15-30 cm and samples from each depth were combined to make two soil samples. Soil samples from Lacombe were tested at Exova, Edmonton, Alberta, Canada. Soil samples from Lethbridge were tested at Western Ag Innovations, Saskatoon, Saskatchewan, Canada in 2016 and at Down to Earth Labs, Lethbridge, Alberta, Canada in 2017 and 2018. Based on these nutrient recommendations, the fertilizer (11-52-0; NPK) was applied at seeding (Appendix 5-1). Faba bean seeds (Zero tannin, cultivar CDC Snow Drop) were treated with *Rhizobium* inoculant (1.222 kg/ 1000 kg seeds) (Nodulator® FB Peat, 2008027A, BASF Canada, Ontario, Canada) and directly seeded at 0.2 m row spacing in Lethbridge and 0.3 m row spacing in Lacombe with a target seeding rate of 48.44 plants/m^2. Plots were seeded in early May of all study years at both locations and plants were irrigated as necessary at the Lethbridge site while the Lacombe site was rain-fed. Annual average precipitation 1995-2020 was 0.88 m at Lethbridge and 1.2 m at Lacombe site (Historical climate data, no date). Weeds were controlled with herbicides as necessary following local standard agronomic recommendations to prevent effects of weeds and insects attracted to weeds (Appendix 5-2).

Above ground damage assessment

Pea leaf weevil adult damage was assessed soon after seedling emergence around the 2nd -3rd unfolded leaf stages and ~two weeks later at the 4th -5th unfolded leaf stages (Staging guide faba bean,no date). A 0.25m^2 quadrat was placed ~ 2 m from two edges of each plot and the number of plants in each quadrat was recorded. Ten plants per quadrat were randomly selected and assessed for damage by counting the number of "U"-shaped notches made by adult pea leaf weevils per leaf pair per node, including the terminal leaf (Cárcamo and Vankosky 2011, Vankosky et al. 2011a). The growth stage of each sampled plant was recorded following the BBCH scale (Weber and Bleiholder 1990).

Below ground damage assessment

The root nodules of two plants per plot at least 2 m apart were assessed for pea leaf weevil larval damage at early and late flowering stages (BBCH 50-59 and BBCH 65-67, respectively) at Lethbridge and only at late flower at Lacombe. Growth stages were determined following the scale developed by Weber and Bleiholder (1990). Plants were dug up using a hand trowel to collect the soil around the roots, so as to capture as many nodules as possible. Soil samples were stored in 4.5 l plastic bags and transported to a laboratory root- washing station. The soil was soaked in water and all life stages of weevils that floated to the surface were collected. The roots were gently washed, and blotted with paper towels before assessment of larval feeding damage on individual root nodules. The total nodule number, and nodule health contributed to a growth score that included plant growth and vigor, nodule color and number, and nodule position (Nodulation assesments, 2019). In addition, the number of nodules with leghaemoglobin and the number of tumescent nodules (> 1 fused nodules) were recorded (Cárcamo and Vankosky 2011, Vankosky et al. 2011a). Legheamoglobin is found in nodules containing *Rhizobium* bacteria and larval feeding preference may be associated with the presence of legheamoglobin (Danthanarayana 1967). The number of larvae recovered from within the nodules was added to those counted in the floatation process. All stages of the weevils were preserved in 70% (v/v) ethanol.

Yield assessment

Seeds were harvested with a Wintersteiger plot combine (1.5 m header width, Wintersteiger, Austria). From each plot, an area of 1.5 m X 10 m was harvested and the samples were air dried and cleaned. Harvested seeds were weighed, and thousand kernel weights were calculated. The sampled seeds were analysed for protein, calcium and phosphorous content using near-infrared spectroscopy analysis (Model 6500, ISI Scan software version 4.0, FOSS North America, Eden Prairie, Minnesota).

Data analysis

Data were analysed using R version 3.6.3 (R Core Development Team 2018). Initial models contained all explanatory variables and interactions between all explanatory variables. In all analyses, model simplification was achieved by ANOVA hypothesis testing ($p < 0.05$) for full and reduced models, until the most parsimonious model remained. Models were compared using AIC values and models with the lowest AIC values were selected. Model residuals were checked for normality using the Shapiro Wilk test. The models were checked for homogeneity of variance using Levene's test and for over-dispersion using the one sample Kolmogorov–Smirnov test in DARMa package (Hartig 2018). Model fit was tested using qq-plots. A Tukey's post hoc test was conducted to test the separation of means of each model (Tukey 1977). Mixed effect models were analysed using lme4 library (Bates et al. 2015). The model assumptions (Shapiro-Wilk test and Levene's test) were met in all linear models unless specified. Generalized mixed effect models were analysed using Poisson error distribution with a log link function. General mixed effect models were analysed with a gaussian error distribution with identity link function.

Data for the two study sites from all three study years were analysed to assess the above ground damage. Separate generalized mixed effect models were used to analyse the effect of insecticide treatment on the mean number of plants per 0.25 m^2 quadrat, the mean number of feeding notches per plant and the mean number of plants with terminal leaf damage at the 3rd and 5th unfolded leaf stages of plants. The nested random factors were study year, study site, block and quadrat. Study site was specified as a random factor because variation between sites was minimal. The number of notches per plant at the 3rd unfolded leaf stage was transformed to fourth root to meet model assumptions.

Separate generalized mixed effect models were used to analyse the effect of insecticide treatment on the mean number of nodules, mean tumescent nodules, mean number of nodules with leghaemoglobin and the plant growth score at each nodule assessment stage (late flower stage for both sites and early flower stage for Lethbridge). Study year, block and subsample were treated as nested random effects in each model. The percentage of root nodules damaged by larvae per plant was analysed using separate, nested general mixed effect models. Insecticide treatment, study site and the interaction between insecticide treatment and study site were fixed factors in the late flower assessment models at both study sites. Since the early flower assessment was only done at the Lethbridge site, the insecticide treatment was the fixed factor in this model. Nodule feeding data at the early and late flower stages were square root transformed to meet model assumptions. The effects of the number of nodules with leghaemoglobin and percentage of damaged nodules on plant growth were analysed using a generalized linear model. The response variable was the growth score for each plant; calculated following the 20/20 seed lab protocol (Nodulation assesments, 2019). The relationships between the percentage of plants with terminal leaf damage and the percentage of damaged nodules were analysed using separate linear models for the two different notch assessment stages.

A general mixed effect model with insecticide treatment, site and year as fixed effects was used to find the effect of year on faba bean yield. Block and plot were treated as random effects in the model. There was an effect of year on yield (χ^2=59.75, df=2, P< 0.0001) and it was driven by the low yield in 2018 (Tukey's post hoc, P<0.05). Faba bean yield analysis was conducted on data from 2016 and 2017, for both sites (Lacombe, Lethbridge). Data from 2018 were excluded from the analysis because the low yield, likely caused by dry weather conditions, diluted treatment effects. The effects of insecticide treatment on faba bean yield and seed protein content were analysed with separate linear mixed effect models and the effect of insecticide treatment on thousand seed weight was analysed with a generalized linear model. The fixed factors were insecticide treatment and study site. The nested random factors included study year, block and the plot. The correlation between yield and the percentage of plants with terminal leaf damage, and yield and percentage of nodule damage were analysed using separate linear regression models. Yield data that fell under 2500 (n=24/120 excluded) and over 4400 (n=5/120 excluded) kg/ha were removed as outliers and used to calculate the correlation between yield and terminal leaf damage.

Similar to a past study in field peas (Cárcamo and Vankosky 2011), the foliar insecticide did not protect faba bean yield, but development of a nominal threshold was still deemed valuable for pea leaf weevil management in faba bean (Tangtrakulwanich et al. 2014). Therefore, we proceeded to develop a nominal threshold using the standard framework to calculate an Economic injury level (EIL) based on the current prices for foliar insecticides. The economic injury level for faba bean was calculated using the equation EIL= g $kgha^{-1}$/b $kgha^{-1}$ / % of plants with terminal leaf damage (Pedigo et al. 1986) where (g) is the gain threshold and (b) is the slope coefficient of the regression between yield and the percentage of plants with terminal feeding damage. This quantifies the yield per 1% terminal leaf damage or the slope of the regression equation. The gain threshold was calculated using an average price of faba bean of February, May and July of 2019 in Alberta ($332.33 CAD/metric tonne) (Faba bean bench mark July 19/19, 2019) and using a typical cost of spraying insecticide for southern Alberta ($22.24/ha) (Aerial application for crop spraying, 2012) (22.24 /332.33=0.06692 metric tonne/ha~ 67 kg/ha). The regression model was simulated 1000 times using "sim" function in R package arm to obtain point estimates for the slope of the model (Gelman and Hill 2007). These point estimates of the slope were used to calculate a posterior distribution for EIL using the EIL=g/b equation. A Markov chain Monte Carlo was used to obtain 95% confidence intervals with the R package MCMCglmm (Hadfield 2010).

Data from 2017-2018 for both study sites were analysed to investigate nitrogen effects on adult feeding on foliage and larval feeding on root nodules. Generalized mixed effect models were used to compare the mean number of plants with terminal leaf damage at 3rd and 5th unfolded leaf stages of plants among the variously treated plants. Data were analysed in separate models with insecticide and nitrogen treatment as the fixed factor. The nested random factors were study year, study site, block and quadrat. The number of notches per plant at the 3rd unfolded leaf stage was square root transformed to meet the model assumptions. The percentage of root nodules damaged by larvae per plant was analysed using separate nested general mixed effect models at each nodule assessment stage (early flower stage for both sites and late flower stages for Lethbridge). A general mixed effect model was used to analyse the effect of insecticide and nitrogen treatment on yield. Data from 2017 were selected for yield assessment because yield was very low in 2018. Insecticide treatment and study site were fixed factors for the late flower assessment models. Since the early flower assessment was only done at the Lethbridge site, the insecticide treatment was the fixed factor in this model. Study year, block, and subsample were treated as nested random effects in each model.

Results

Above ground damage assessment

Pest pressure was high and consistent at the two study sites (Lethbridge and Lacombe) in 2016 and 2017, but low in 2018. Seedlings at the 3rd unfolded leaf stage had an average of 149.79±7.57 and 145±8.44 adult feeding notches per plant and 5.12±0.33 and 4.11±3.4 plants with terminal damage out of 10 plants at Lethbridge and Lacombe, respectively. There was an average of 14 plants per 0.25m^2 quadrat at both unfolded leaf stages and establishment was not affected by treatment at 3rd or 5th unfolded leaf stages (Table 5-1) of assessment. The average number of adult feeding notches per plant at the 3rd and 5th unfolded leaf stages were affected by the insecticide treatment (Table 5-1). Thiamethoxam reduced the overall adult feeding notches compared to lambda-cyhalothrin at the 3rd unfolded leaf stage. Neither thiamethoxam nor lambda-cyhalothrin effectively protected seedlings from adult damage at the 5th unfolded leaf stage. The number of plants with terminal leaf damage (out of 10 plants) was lower for those treated with thiamethoxam compared to the lambda-cyhalothrin treated plants and plants from control plots at the 3rd unfolded leaf stage (Table 5-1). Plots that received insecticide (thiamethoxam or lambda-cyhalothrin) had fewer plants with terminal leaf damage compared to those from the control plots when seedlings reached the 5th unfolded leaf stage (Table 5-1) (Fig. 5-1).

Below ground damage assessment

There was an interaction effect of the insecticide treatment and the study site on the mean number of nodules produced by faba bean plants at the late flower stage (Table 5-2). Plants produced more nodules at the Lacombe site compared to Lethbridge at the late flower assessment stage (Tukey's post hoc, $P<0.05$). Plants in the thiamethoxam treatment had fewer nodules compared to plants in control plots at both study sites (Tukey's post hoc, $P<0.05$). There was no consistent effect of lambda-cyhalothrin on nodule numbers at the late flower assessment stage.

There were more tumescent nodules on thiamethoxam-treated faba bean plants compared to untreated faba bean plants at late and at early flowering stages (Table 5-2). Insecticide-treated plants had a higher number of nodules with leghaemoglobin compared to control plants at both early and late flowering stages at the Lethbridge site (Table 5-2). At Lacombe, however, all treatments reduced the number of nodules with legaemoglobin compared to controls except the plants that received lambda-cyhalothrin twice.

Treatments affected faba bean nodule damage (%, Fig. 5-2) in a similar pattern to the percentage of terminal leaf damage. There was an interaction effect of insecticide treatment and study site that affected the percentage of nodules damaged by pea leaf weevil larvae at the late flower stage (Table 5-2) (Fig. 5-2). At both sites, thiamethoxam reduced the percentage of damaged nodules compared to untreated faba bean plants (Tukey's post hoc, $P<0.05$) (Table 5-2). The plants treated with lambda-cyhalothrin + thiamethoxam had a higher percentage of damaged nodules compared to the faba bean plants treated only with thiamethoxam at both study sites. Nodule assessment at early flower at the Lethbridge site showed that the thiamethoxam treatment substantially reduced the percentage of damaged nodules compared to the untreated controls and lambda-cyhalothrin treatment (Table 5-2) (Fig. 5-2). Plants that received both lambda-cyhalothrin and thiamethoxam had a higher percentage of damaged nodules compared to the plants that only received thiamethoxam (Tukey's post hoc, $P<0.05$) (Table 5-2).

There was a positive correlation between plant growth and the number of nodules with leghaemoglobin ($r^2=0.15$, $\chi^2=49.18$, df=1, $P<0.0001$). The overall plant growth, however, was not affected by the percentage of damaged nodules ($r^2=0.004$, $\chi^2=1.39$, df=1, $P=0.24$). The percentage of damaged nodules increased with the percentage of plants with terminal leaf damage ($r^2=0.2$, $F=40.96$, df=1, $P<0.0001$) (data not shown).

Nitrogen treatment

The percentage of plants with terminal leaf damage at the 3rd unfolded leaf stage was not different between nitrogen-treated (70.28±7.37) and control (78.09±5.23) plants (Tukey's post hoc, $P>0.05$). At the 5th unfolded leaf stage, nitrogen treatment reduced the percentage of plants (77.50±8.6) with terminal leaf damage compared to the control plants (90.94±4.14). The percentage of damaged nodules was not affected by the nitrogen treatment at either early or late flower nodule assessment stages (Tukey's post hoc, $P>0.05$). Nitrogen-treated plants had a similar number of damaged nodules (46.64±4.01, 34.83±5.30) compared to control (39.81±2.38, 33.64±5.65) plants at early and late flower nodule assessment stages, respectively. There was no difference in yield between nitrogen-treated (2952.50 kg/ha±172.22) and control (2972.08 kg/ha±133.12) faba bean plants (Tukey's post hoc, $P>0.05$).

Yield and seed quality assessment

Yield was affected by the insecticide treatment (Table 5-3), and there was no difference in yield between sites ($\chi^2=1.95$, df=1, $P=0.16$). Yield was higher in thiamethoxam-treated plots compared to the non-treated control and lambda-cyhalothrin treatments (Tukey's post hoc, $P<0.05$). Around 700-800 kg/ha of seed yield was protected by the two treatments that contained the thiamethoxam, at both study sites, compared to the untreated control. The thousand seed weights were affected by the thiamethoxam treatment (Table 5-3), but not by the study site (χ^2=2.75, df=1, P=0.09). Thiamethoxam-treated faba bean plants had higher thousand seed weights compared to untreated controls and plants treated with lambda-cyhalothrin (Tukey's post hoc, P<0.05). Lambda-cyhalothrin application had no effect on the seed protein content at both sites (Table 5-3).

Faba bean yield was related to the percentage of seedlings with damage on the terminal leaf at the 3rd unfolded leaf stage (r^2=0.2, F=22.65, df=1, P<0.0001) (Fig. 5-3) but not at the 5th unfolded leaf stage (r^2=0.01, F=2.50, df=1, P=0.12). The slope coefficient (b) (-4.91) of the regression equation was significant (Y = 3,692.49 – 4.91X; r^2 = 0.2 (n=90)). The gain threshold (g) was 67 kg/ha. Therefore, 67 kg/ha of yield needs to be saved from pea leaf weevil damage to cover the cost of management based on the current cost of management. Because currently there is no effective post-seeding control measure, and this value depends on the value of the crop and the cost of control, we can only suggest a nominal threshold from the calculated economic injury level. The EIL (EIL=g/b) was 13.7% (95% CI, 8%-19%) of seedlings with terminal leaf damage at the 3rd unfolded leaf stage and a nominal threshold of 15% was selected to simplify it as half of the current nominal threshold used in field peas. Also, there is evidence from laboratory studies that ground predators attack some life stages of pea leaf weevils (Vankosky et al. 2011b) and increasing the threshold from 13-15%, may protect some beneficial natural enemies.

There was no correlation between faba bean yield and the percentage of damaged nodules at early (r^2= 0.01, F=1.47, df=1, P=0.24) or full flower (r^2= 0.005, F=1.46, df=1, P=0.23) stages of nodule assessment (data not shown).

Discussion

Thiamethoxam protected faba bean seedlings from pea leaf weevil foliar damage, but the efficacy of lambda-cyhalothrin was inconsistent. My results agree with a study of pea leaf weevil and root rot management in Alberta, Canada, in which thiamethoxam reduced the adult foliage damage at the 3rd unfolded leaf stage of faba bean plants (Willsey et al. 2021). My results also agree with other research in which insecticidal seed treatments reduce adult damage in field peas better than foliar insecticides (Vankosky et al. 2009, Seidenglanz et al. 2010, Cárcamo et al. 2012). Seed treatments with systemic insecticides provide superior control to foliar applications because of consistent protection of the vulnerable seedling stage from adult feeding during host colonization (Wnuk and Wiech 1996). In the current study, lambda-cyhalothrin reduced the percentage of seedlings with terminal leaf damage at the 5th unfolded leaf stage but not at the younger 3rd unfolded leaf stage. This inconsistency of lambda-cyhalothrin efficacy may relate to the differences in the timing of adult flights into fields (Fisher and O'Keeffe 1979, Hamon et al. 1987, Nielsen and Jensen 1993) in relation to variable spring weather conditions in Alberta (Cárcamo and Vankosky 2011). This may lead to a mismatch between the timing of foliar application and weevil colonization of fields. The effectiveness of foliar insecticides is also rate dependent and may vary with the weevil density.

Thiamethoxam was effective in protecting faba bean from pea leaf weevil larval damage in the current study and in a recent study (Willsey et al. 2021). In field peas, thiamethoxam directly kills larvae and reduces female oviposition leading to fewer larvae (Cárcamo et al. 2012). These effects may have contributed to the lower percentage of damaged nodules in thiamethoxam-treated faba bean plants compared to untreated plants. The plants treated with thiamethoxam had fewer nodules at the late flower stage but a higher growth score compared to untreated plants.

Faba bean may have enough active nodules to compensate for the nodule reduction in thiamethoxam-treated plants. Faba bean produces more nodules per plant than field peas (Wijerathna, Cárcamo and Evenden, unpublished data).

The percentage of damaged nodules and nodule production varied with study site. Nodule production was higher at the more northerly site of Lacombe than at the site near Lethbridge. The differences in soil types and other abiotic factors such as annual precipitation between sites may have contributed to the variable nodule numbers. Because faba bean plants produce more nodules at the Lacombe compared to the Lethbridge site, plants may withstand more larval damage in Lacombe before yield is affected. Thiamethoxam-treated faba bean plants had a higher percentage of damaged nodules in Lacombe compared to Lethbridge. This suggests that thiamethoxam efficacy may differ with geographic location or may become ineffective at high pest pressure. These results illustrate the need for local research to develop local pest management recommendations. Even though the percentage of damage is higher at Lacombe compared to Lethbridge, faba bean yield did not differ between sites. Management practices that increase nodulation at the Lethbridge site may reduce yield loss through an increase in plant tolerance.

Foliar insecticides can reduce the larval population through an effect on female pea leaf weevils (Bardner et al. 1983), but do not directly impact eggs and larvae (Steene et al. 1999). Asynchrony between foliar applications and adult weevil dispersal to the crop may allow female oviposition before or after the application (Seidenglanz et al. 2010). A combined application of lambda-cyhalothrin and thiamethoxam increased larval damage in faba bean compared to plants that received only thiamethoxam at the Lethbridge site. This interesting finding could suggest that there is an interaction between the two insecticides that renders thiamethoxam less effective.

Foliar applications may also reduce control efficacy because of negative impacts on natural enemies which feed on pea leaf weevil eggs (Vankosky et al. 2011b, Cárcamo and Vankosky 2013), and adults (Wijerathna, field observation). The effect of insecticides used in pulse crops on natural enemies of pea leaf weevil requires further study (Cárcamo et al. 2018).

As reported by Willsey et al. (2021), supplemental nitrogen treatment did not affect larval damage or yield. The levels of nitrogen applied in this study may have been insufficient to inhibit the nodule production in faba bean, in contrast to previous studies in field peas (George 1962, Vankosky et al. 2011a). Nitrogen increases field pea yield under certain levels of pea leaf weevil pressure. Although high nitrogen rates can increase field pea (Vankosky et al. 2011a) and faba bean yield (Babiker et al. 1995, Elsheikh and Elzidany 1997), this would not be economical in managing pea leaf weevil (Karkanis et al. 2018). The nitrogen amendment reduced the number of plants with terminal leaf damage at the 5th unfolded leaf stage in this study. In contrast, nitrogen-treated field peas were attractive to pea leaf weevil adults (Vankosky et al. 2011a).

Lambda-cyhalothrin was not effective in protecting yield despite multiple applications. My results were consistent with similar investigations on management of pea leaf weevil with foliar insecticides in both faba bean and field pea (Willsey et al. 2021). Other studies of pea leaf weevil management in faba bean found that yield is higher in plants with insecticidal seed treatment compared to foliar insecticide treatment (Ester and Jeuring 1992). Even though the addition of lambda-cyhalothrin to plants treated with thiamethoxam increased the larval damage, yield was still enhanced by thiamethoxam treatment alone in the current study. Lambda-cyhalothrin may have had negative effects on natural enemies, but the long-lasting residual effects of thiamethoxam protected yield in these plants.

Thiamethoxam protected over 0.5 metric ton/ha of faba bean yield and increased thousand kernel weight at both study locations. Similar to the current study, systemic insecticides protected yield, increased the number of pods and seeds per pod in other legume plants (Bardner et al. 1983, Brown et al. 1987, Koch et al. 2005, Nottingham et al. 2017). In contrast, thiamethoxam did not consistently protect field pea (Vankosky et al. 2011c, Willsey et al. 2021) or faba bean (Willsey et al. 2021) yield from pea leaf weevil damage in other field studies in southern Alberta. Plants with thiamethoxam also had larger tumescent nodules than those without it. This may have contributed to increasing atmospheric nitrogen fixation by providing a larger surface area for *Rhizobium* activity. Effects of thiamethoxam on insect-plant interactions are complex and vary among sites and years, but thiamethoxam has greater potential to protect faba bean yield from pea leaf weevil damage than lambda-cyhalothrin.

In this study, I developed a nominal threshold for pea leaf weevil in faba beans using the standard methods to calculate an economic injury level. Based on adult feeding on the foliage, the economic injury level was 13.7%. Because the actual cost of an efficacious foliar insecticide is unknown, I provide a nominal threshold that is rounded to 15% of seedlings with terminal leaf damage at the third unfolded leaf stage. A nominal threshold will help growers estimate pest levels and evaluate their management strategy and plan for the future. For example, some growers prefer to avoid seed treatments, and want to understand yield loss in order to adapt their management strategy in the future. My results suggest that lambda-cyhalothrin is not a viable control option given the low residual life and the complex life cycle of pea leaf weevil. On the other hand, thiamethoxam protected yields but application decisions must be made before the nominal threshold can be estimated. My nominal threshold could be converted into an ET based on the future cost of the crop and future cost of an efficacious foliar insecticide. Timely sampling around the 2nd and 3rd unfolding leaf stage in faba bean will be crucial for application of such insecticide for yield protection.

The nominal threshold developed here for pea leaf weevil damage on faba bean is half of that in field peas, where the ET is 30% of seedlings with damage on the terminal leaves during the second to fifth unfolded leaf stages of the seedlings (El Lafi 1977, Vankosky et al. 2011a). This difference may be due to differences in tolerance to pea leaf weevil damage between the two hosts, or variation in host species characters. The control measures, when an efficacious foliar insecticide becomes available, should be applied at lower damage levels than those suggested for field peas, to minimize economic losses in faba bean. The number of plants with terminal leaf damage was negatively related to the faba bean yield at both 3rd and 5th unfolded leaf stages, but the strength of the relationship was lower at the later stage. This indicates that earlier monitoring for adult damage is more reliable to estimate yield loss and important to detect weevil damage in time for intervention with foliar insecticides.

My results indicate that the systemic insecticide, thiamethoxam, is more effective in reducing pea leaf weevil damage in faba bean compared to the foliar insecticide, lambda-cyhalothrin, in Alberta. The nominal threshold for pea leaf weevil management in faba bean was calculated to 15% of plants with terminal leaf damage based on the cost of foliar applications. This threshold can be applied in long-term decision making on insecticidal seed treatment to manage pea leaf weevil on faba bean. In the future, other IPM strategies remain to be researched to identify viable alternatives to insecticides to manage pea leaf weevil in faba bean.

Table 5-1. Mixed effect model results and treatment (±SE) for plants per quadrat and pea leaf weevil adult feeding at 3rd and 5th unfolded leaf stages of damage assessments of faba bean. Data presented are from a field plot study at Lethbridge and Lacombe from 2016-2018. P values in bold are significant (α=0.05). Means with the same letters within the same row are not significantly different.

			treatment mean ± SE					
parameter	control	thiamethoxam	thiamethoxam + lambda-cyhalothrin	lambda-cyhalothrin at 2nd unfolded leaf	lambda-cyhalothrin at 2nd + 4th unfolded leaf	χ^2 value	df	P value
plants/quadrat-3rd unfolded leaf	13.35±0.45**a**	15.12±0.39**a**	13.58±0.58**a**	12.48±0.36**a**	13.96±0.42**a**	6.29	4	0.18
plants/quadrat-5th unfolded leaf	12.38±0.35**a**	13.46±0.40**a**	14.54±0.53**a**	13.75±0.35**a**	13.83±0.47**a**	4.37	4	0.36
adult feeding notches-3rd unfolded leaf	189.71±8.92**b**	92.73±12.63**a**	88.15±12.89**a**	176.31±10.30**b**	191.04±8.19**b**	330.10	**4**	**<0.0001**
adult feeding notches-5th unfolded leaf	100.54±11.92**a**	157±14.72**ab**	115.79±14.25**ab**	218.12±12.44**b**	198±13.75**b**	1319.5	**4**	**<0.0001**
plants/10 with terminal leaf damage-3rd unfolded leaf	7.17±0.041**b**	2.36±0.03**a**	2.91±0.04**a**	6.0.5±0.06**b**	7.54±2.73**b**	216.16	**4**	**<0.0001**
plants/10 with terminal leaf damage-5th unfolded leaf	9.50±0.01**c**	3.08±3.43**b**	1.09±0.02**a**	2.86±0.03**b**	3.78±0.04**b**	175.76	**4**	**<0.0001**

Table 5-2. Mixed effect model results and treatment (±SE) for nodule characters, plant growth and nodule damage by pea leaf weevil larvae at early and late flowering stages of damage assessments on faba bean. Data presented are from a field plot study at Lethbridge and Lacombe from 2016-2018. P values in bold are significant (α=0.05). Means with the same letters within the same row are not significantly different.

	treatment mean ± SE							
parameter	control	thiamethoxam	thiamethoxam + lambda-cyhalothrin	lambda-cyhalothrin at 2nd unfolded leaf	lambda-cyhalothrin at 2nd + 4th unfolded leaf	χ^2 value	df	P value
	early flower-Lethbridge							
total nodules	45.27±4.07**ab**	48.94±4.20**b**	50.16±4.47**a**	67.78±6.29**c**	49.27±3.94**ab**	127.53	**4**	**<0.0001**
tumescent nodules	6.58±0.71**a**	14.56±1.33**c**	11.94±0.68**b**	12.28±1.29**b**	10.13±0.92**b**	144.02	**4**	**<0.0001**
nodules with leghaemoglobin	19.10±2.09**a**	34.12±2.75**d**	28.75±1.82**c**	37.69±4.24**d**	23.93±2.41**b**	285.34	**4**	**<0.0001**
percentage of damaged nodules	51.57±2.78**c**	9.74±1.13**a**	27.72±3.23**b**	30.31±2.71**b**	37.42±2.55**b**	79.59	**4**	**<0.0001**
growth score	8.75±0.22**a**	10.04±0.18**a**	10.31±0.18**a**	9.91±0.22**a**	9.33±0.67**a**	6.7725		0.15
	late flower-Lethbridge							
total nodules	66.44±6.79**d**	52.58±4.06 **a**	57.11±4.81**b**	72.13±6.69**c**	67.87±6.51**d**	112.74	**4**	**<0.0001**
tumescent nodules	6.25±0.68**a**	14.16±1.24**d**	11.59±0.82**c**	10±2.1.19**b**	10.12±1.04**bc**	39.90	**4**	**<0.0001**
nodules with leghaemoglobin	22.05±2.76**a**	34.97±2.96**cd**	34.56±2.71**cd**	35.19±3.89**bc**	28.28±2.65**b**	468.42	**4**	**<0.0001**

percentage of damaged nodules	44.62±2.66**bc**	12.58±1.43**a**	29.85±2.85**b**	35.28±3.00**bc**	40.71±2.61**c**	20.54	**4**	**0.0003**
growth score	8.50±0.19**a**	9.98±0.19**ab**	10.20±0.21**b**	9.52±0.23**ab**	9.10±0.20**ab**	33.1	**4**	**<0.0001**
	late flower-Lacombe							
total nodules	145.00±15.45**c**	107.65±8.14 **b**	99.42±7.79**a**	111.10±10.56**b**	153.92±14.89**d**	112.74	**4**	**<0.0001**
tumescent nodules	20.29±1.93**a**	36.46±23.34**d**	28.62±1.71**b**	18.04±1.41**a**	13.23±4.04**c**	39.90	**4**	**<0.0001**
nodules with leghaemoglobin	103.40±4.17**d**	86.96±49.03**c**	79.21±4.31**b**	66.62±5.63**a**	111.06±6.96**e**	468.42	**4**	**<0.0001**
percentage of damaged nodules	48.91±2.9**bc**	40.52±26.73**a**	46.02±2.32**b**	57.37±2.44**bc**	50.55±2.18**c**	20.54	**4**	**0.0003**
growth score	9.21±0.22**a**	11.60±1.73**b**	11.60±0.13**b**	9.35±0.21**a**	10.60±1.19**ab**	33.1	**4**	**<0.0001**

Table 5-3. Mixed effect model results and treatment (±SE) for faba bean seed weight, thousand kernel weight and seed protein content. Data presented are from a field plot study at Lethbridge and Lacombe from 2016-2018. P values in bold are significant ($\alpha=0.05$). Means with the same letters in the same row are not significantly different.

	treatment mean ± SE							
parameter	control	thiamethoxam	thiamethoxam + lambda-cyhalothrin	lambda-cyhalothrin at 2nd unfolded leaf	lambda-cyhalothrin at 2nd + 4th unfolded leaf	χ^2 value	df	P value
	Lethbridge							
seed weight (kg/ha)	3297.7±103.8**a**	4030.63±214.5**b**	3989.1±154.7**b**	3328.4±137.3**a**	3191.1±170.8**a**	32.71	**4**	**<0.0001**
thousand kernel weight (g)	289.3±9.8**a**	317.7±12.8**b**	317.8±11.1**b**	286.1±9.3**a**	293.5±10.3**a**	9.46	**4**	**0.05**
seed protein content %	28.6±0.3**a**	28.9±0.3**a**	28.9±0.1**a**	28.6±0.2**a**	27.7±0.2**a**	8.53	4	0.07
	Lacombe							
seed weight (kg/ha)	2954.4±171.8**a**	3741.5±98.8**b**	3751.5±101.4**b**	3300.1±91.8**a**	3298.0±100.4**a**	32.71	**4**	**<0.0001**
thousand kernel weight (g)	305.3±5.1**a**	3212.1±4.4**b**	321.9±3.9**b**	308.7±3.5**a**	310.1±4.4**a**	9.46	**4**	**0.05**
seed protein content %	27.7±0.2**a**	29.2±0.3**a**	28.3±0.2**a**	27.1±0.2**a**	26.9±0.3**a**	8.53	4	0.07

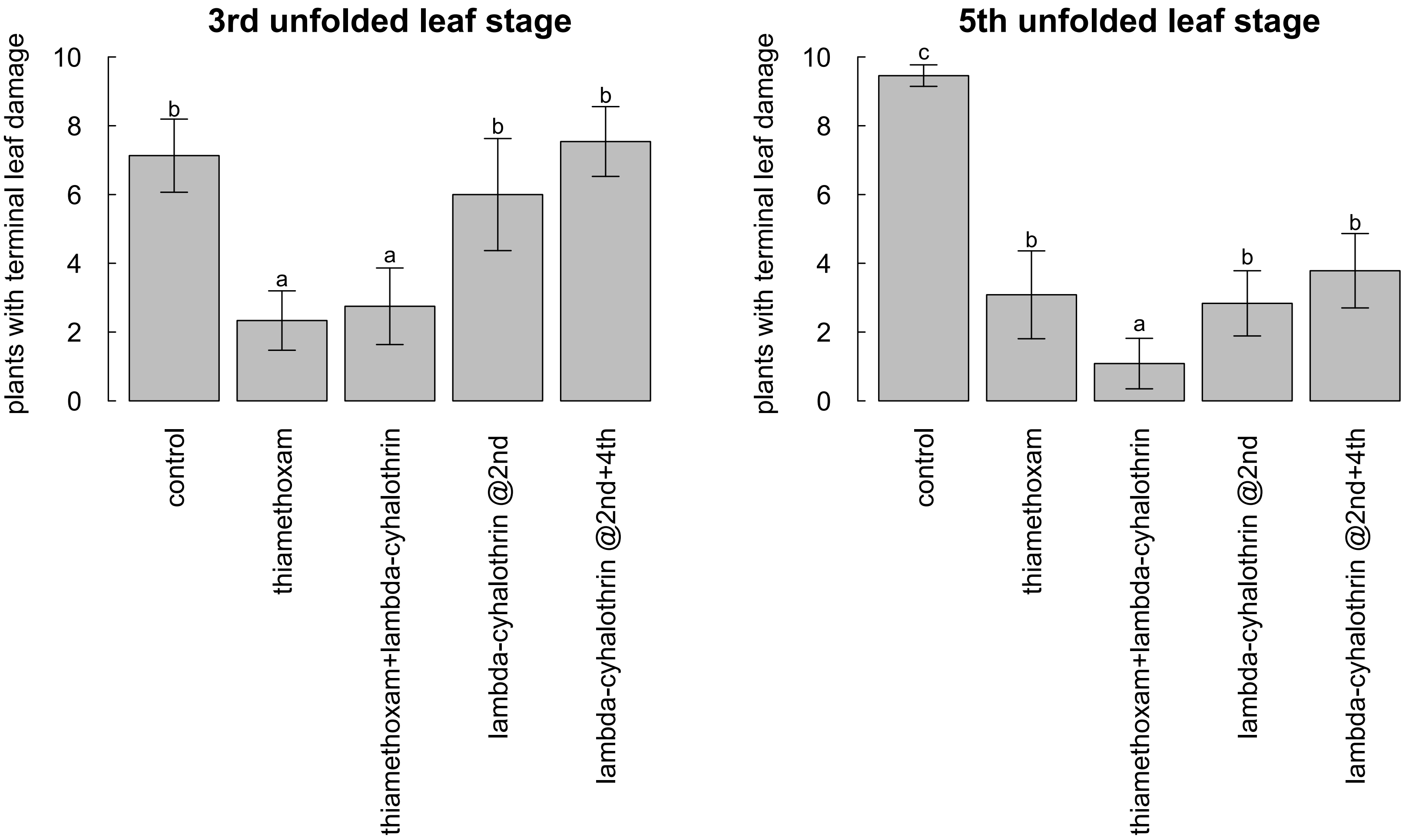

Figure 5-1. Mean number of faba bean plants with terminal leaf damage (out of 10 plants) at the 3rd and 5th unfolded leaf stages under different insecticide treatments. Error bars represent the standard errors.

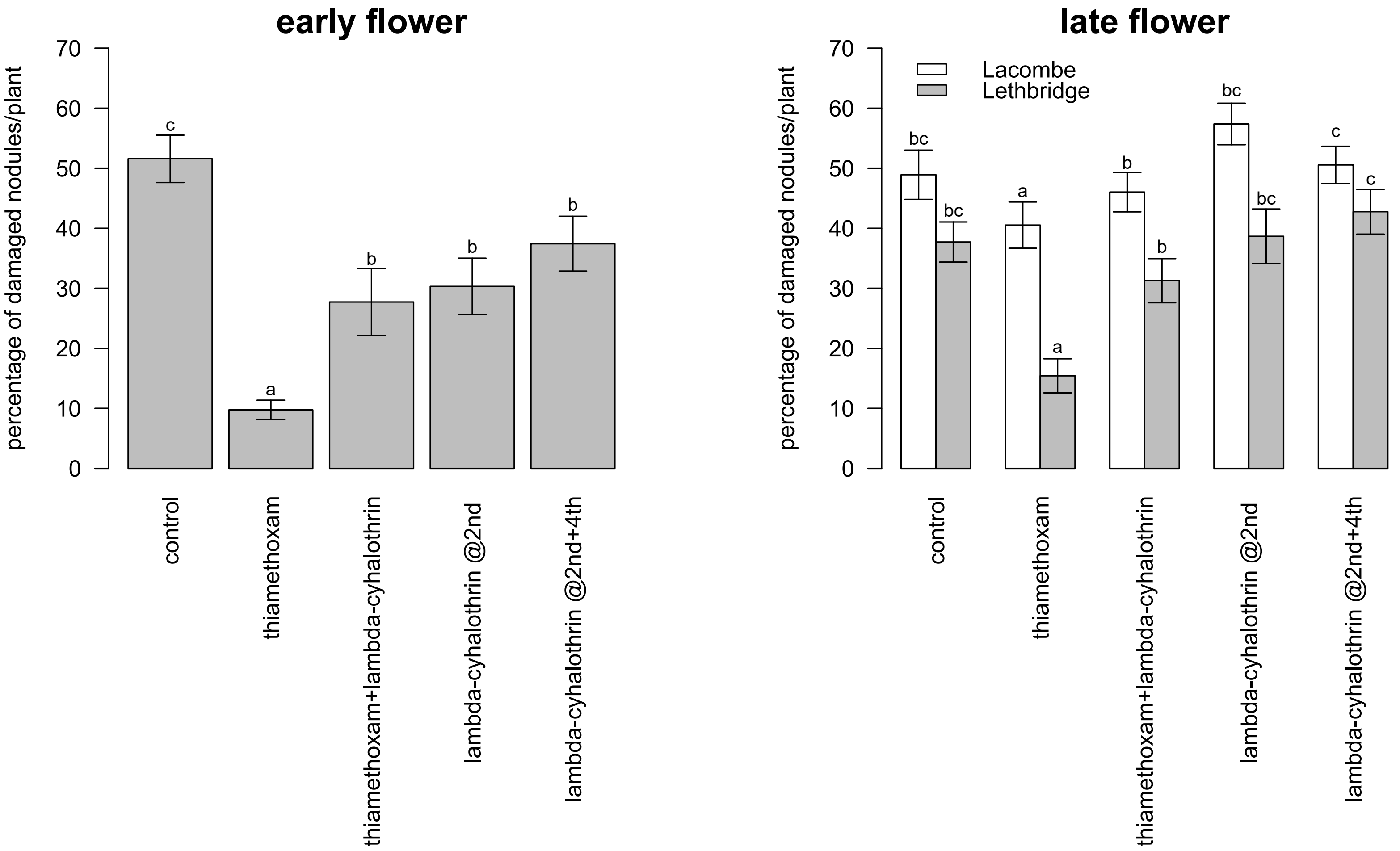

Figure 5-2. Percentage of nodules damaged by pea leaf weevil larvae at late and early flower assessment stages of faba bean plants under different insecticide treatments. Letters on the figure represent the significant difference between treatments within each site. Error bars represent the standard errors.

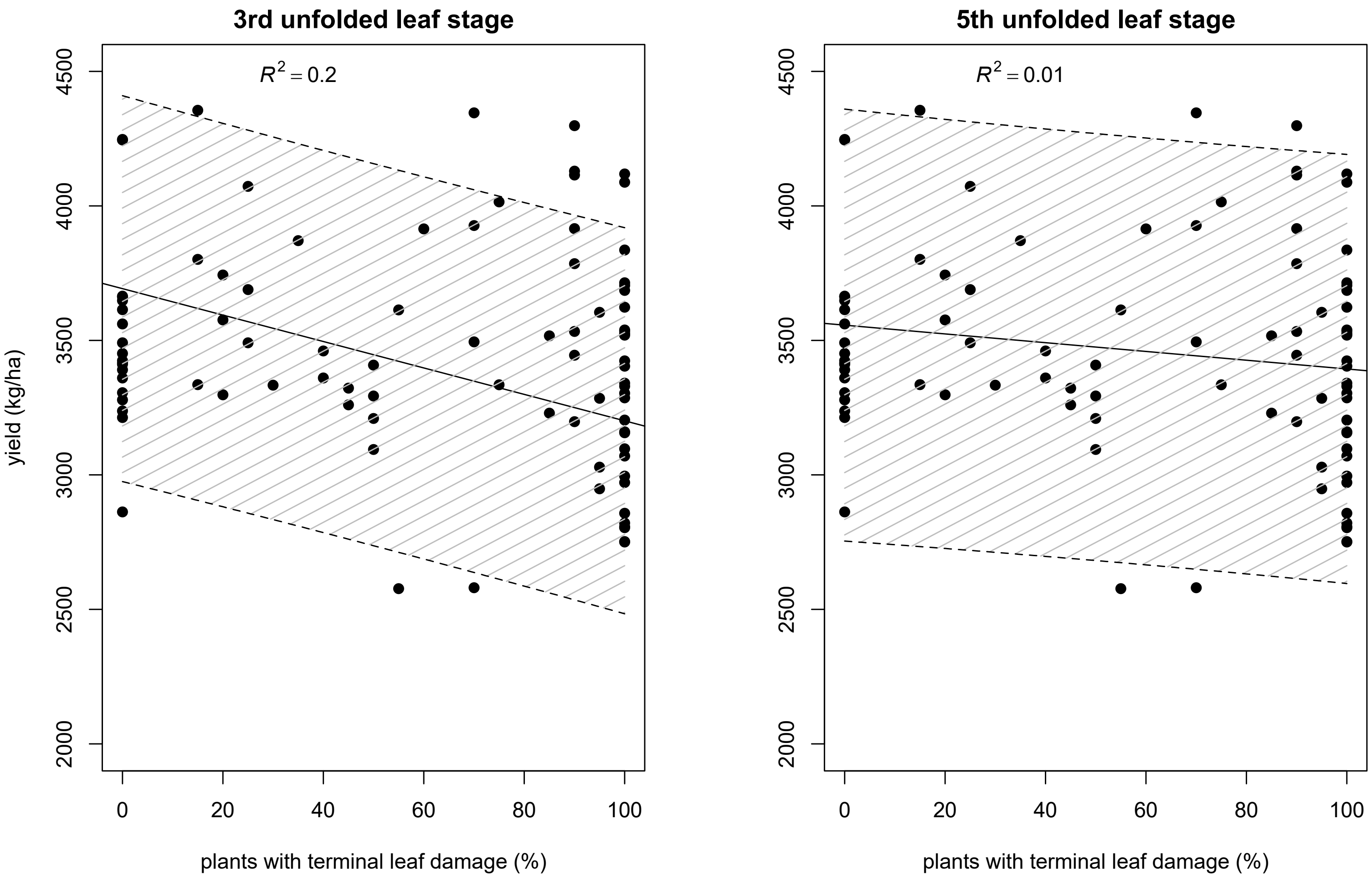

Figure 5-3. Relationship between yield and the percentage of plants with terminal leaf damage at 3rd and 5th unfolded leaf stages. The shaded area represents the 95% confidence interval. 3rd unfolded leaf stage: y= -4.911x + 3692.5 (p<0.0001), 5th unfolded leaf stage.

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Appendix 5-1. Details of pre-seeding soil nutrient recommendations for the faba bean field plot study for 2016-2018 at two study sites

year	site	N (kg/ha)	P_2O_5 (kg/ha)	K_2O_2 (kg/ha)
2016	Lethbridge	195	76.2	22.4
	Lacombe	0	116.7	151.3
2017	Lethbridge	0	56	0
	Lacombe	0	118.8	174.8
2018	Lethbridge	16.8	66.1	0
	Lacombe	0	67.2	130

Appendix 5-2. Details of herbicide application during the faba bean field plot study from 2016-2018 at two study sites.

year	site	herbicide	application rate	plant phenological stage
2016	Lethbridge	Edge	58.06kg/ha	Pre-seeding stage
		Odyssey + Merge	0.04kg/ha + 0.5% v/v	Early Flower
		Reglone	0.28l/ha	Pre-harvest
	Lacombe	Roundup Weathermax	900g ai/ha	Pre-seeding stage
		Odyssey	30g ai/ha	1-6 unfolded leaf stage
2017	Lethbridge	Edge	9.53kg/ha	Pre-seeding stage
		RoundUp + Heat + Merge	0.4l/ha + 0.025kg/ha + 0.5% v/v	Pre-seeding stage
		Reglone	0.44l/ha	Pre-harvest
	Lacombe	Roundup Weathermax	900g ai/ha	Pre-seeding stage
		Viper	448g ai/ha	3rd unfolded leaf stage
		Roundup Weathermax + Heat	900g ai/ha + 52g ai/ha	Late pod stage
2018	Lethbridge	RoundUp	0.4l/ha	Pre-seeding stage
		Odyssey + Merge	0.04kg/ha + 0.5% v/v	Early Flower
		RoundUp + Heat + Merge	0.4l/ha + 0.02l/ha + 0.08l/ha	Pre-harvest
	Lacombe	Roundup Weathermax	900g ai/ha	Pre-seeding stage

Chapter 6 **General Conclusion**

The pea leaf weevil (*Sitona lineatus* Linnaeus; Coleoptera: Curculionidae) is an invasive pest of field pea (*Pisum sativum*) and faba bean (*Vicia faba*) in North America (Jackson 1920). Larval damage alone or damage by both larvae and adults can cause yield losses in field pea and faba bean (El-Dessouksi 1971, Nielsen 1990). Since the first record of the pea leaf weevil in southern Alberta (Vankosky et al. 2009), its range has expanded to the east and north in the Prairie Provinces. It now causes persistent damage to field peas and faba beans grown in central Alberta and has invaded the Peace River Region (Olfert et al. 2012, Cárcamo et al. 2018). In this thesis, I identify and address knowledge gaps in pea leaf weevil-host plant interactions to understand pea leaf weevil invasion and to enhance Integrated Pest Management (IPM) of this invasive insect pest in the Prairie Provinces.

The second chapter of my thesis focuses on behavioural plasticity in pea leaf weevil-host plant interactions. First, I assess the effect of pea leaf weevil reproductive state in modulating host acceptance and feeding preference between primary host plants, field pea and faba bean. Second, I investigate whether host acceptance and preference influence offspring development of pea leaf weevil. Third, I evaluate how the presence of a secondary host, alfalfa (*Medicago sativa*), influences state-dependent plasticity in host finding of pea leaf weevil adults. The third chapter of my thesis focuses on the effects of host quality on pea leaf weevil-host plant interactions. First, I assess the effect of the species-specific legume-*Rhizobium* symbiosis on pea leaf weevil adult host acceptance and larval development across a range of primary and secondary host plants. Second, I investigate the effect of faba bean host quality in terms of the symbiotic interaction with *Rhizobium* and nitrogen content on adult host acceptance, feeding preference and larval development.

The fourth chapter of my thesis focuses on the overwintering biology of pea leaf weevil to understand the influence of weevil cold hardiness and overwintering survival on subsequent pea leaf weevil-host interactions and pea leaf weevil invasion to novel habitats. In a field study, I investigate the overwintering survival of pea leaf weevil at several locations in its expanded range under two different microhabitats, tree shelterbelts and open alfalfa fields. Second, I investigate pea leaf weevil cold hardiness by determining its supercooling point. Third, I test the effects of juvenile hormone on pea leaf weevil on lipid metabolism. Lastly, I investigate the effect of overwintering temperature and duration on subsequent pea leaf weevil performance on the primary host, faba bean. In the final chapter of my thesis, I develop a nominal threshold for pea leaf weevil management on the primary host, faba bean. I further evaluate the efficacy of chemical control measures currently used against the pea leaf weevil in the Prairie Provinces.

This study will contribute to a better understanding of pea leaf weevil interactions with it primary and potential secondary host plant in its expanding range in Alberta. This study reveals how pea leaf weevil host-plant interactions are shaped by pest-related fitness (plasticity), ecosystem characters (host quality and host plant species) and by changing climatic characters (overwintering survival and cold hardiness). The findings of this work can help to forecast the establishment and spread of invasive species. Ultimately, my thesis will add to a better understanding of pea leaf weevil-host plant interactions and implications of these findings for management of pea leaf weevil in its expanding range.

Behavioural plasticity in pea leaf weevil host location

Host plant choice is crucial for specialist insect herbivores. Host plants influence the fitness of insect herbivores by influencing feeding, mating and oviposition. Insect herbivores must select the most suitable host at the right stage of their life cycle. The preference-performance hypothesis states that females should oviposit on or near the host plants that maximize subsequent larval fitness (Gripenberg et al. 2010). Host selection, however, is challenging because of complexity or lack of reliable host plant cues to assess the host plant quality. Insects overcome these challenges by exhibiting phenotypic plasticity in host selection to maximize their fitness under dynamic environments (Anderson et al. 2013, Anderson and Anton 2014, Lhomme et al. 2018, 2020, Maák et al. 2020). Phenotypic plasticity is the capacity of individuals to alter phenotypic traits as a function of the environmental change (Whitman and Agrawal 2009, Dingemanse and Wolf 2013). Plasticity allows herbivores to adjust their response to host cues based on the current environmental conditions. Insects, however, show variation in their behavioural responses (Wolf et al. 2008) that often relate to physiological state and past host experience of individual insects (Anton et al. 2007, Gadenne et al. 2016, Lemmen-Lechelt et al. 2018).

Pea leaf weevil is oligophagous and uses a variety of legume species as primary and secondary host plants (Jackson 1920, El-Dessouksi 1971, Nielsen 1990). In its one-year life cycle, adult pea leaf weevils experience two different reproductive stages. Adults emerge from overwintering sites in early spring are reproductively immature, and initially feed on secondary host plants such as alfalfa, before dispersing to the primary host plants where they feed and become reproductively active (Hamon et al. 1987). New generation adults overwinter in field margins and tree shelterbelts in fall in a reproductively inactive state (Jackson 1920, Schotzko and O’Keeffe 1988, Murray and Clements 1992, Landon et al. 1995). I found that pea leaf weevil exhibits a state-dependent host preference between primary hosts; pre-reproductive weevils prefer faba bean over field peas, with no such preference evident in reproductively inactive weevils (Chapter 2). This state-dependent plasticity in host preference is important for pea leaf weevil establishment and range expansion because they have access to both primary host plants in pulse growing regions of the Prairie Provinces. Faba bean also supports more offspring development compared to field peas (Chapter 2), probably as a result of the presence of more root nodules on faba bean compared to field pea plants that would support the development of more larvae (Chapters 2 & 3). The behavioural plasticity based on reproductive state in this oligophagous insect herbivore likely results in increased fitness. The lack of feeding preference in reproductively inactive weevils for the two primary hosts could be due to several reasons. There is a cost associated with exhibiting plastic responses (Bernstein and Jervis 2008, Dukas 2008, Mery and Burns 2010), and reproductively inactive weevils may avoid these behaviours to minimize costs in the overwintering period (Chapter 4). Insects store energy resources in preparation for winter (Hahn and Denlinger 2007), and energy use for behavioural plasticity may deplete these resources. Further, pea leaf weevils do not undergo true diapause and can resume feeding under optimal weather conditions during the overwintering period (Landon et al. 1995). As primary hosts are not present on the landscape during winter, there would be no advantage to exhibiting a preference for a specific primary host in reproductively inactive weevils. Reproductively inactive weevils are more likely to feed on any legume rather than expending energy in host searching for primary hosts.

In the presence of the secondary host, alfalfa, the state-dependent host preference disappeared. Interestingly, weevils preferred alfalfa over either of the primary hosts regardless of their reproductive state (Chapter 2). Alfalfa, however, does not support larval development (Chapter 3) and the preference for alfalfa by pre-reproductive and reproductive weevils may relate to host quality, quantity, maternal effects or previous host plant experience (Liu et al. 2008, Bruce and Pickett 2011, Anderson and Anton 2014, Jones and Agrawal 2019). The experience of a poor quality host may put herbivores at a fitness disadvantage (Liu et al. 2008, Jones and Agrawal 2019). In the spring, weevils that emerge from overwintering feed on perennial legumes, such as alfalfa, before orienting to primary hosts for reproduction (Jackson 1920, Schotzko and O'Keeffe 1988, Murray and Clements 1992, Landon et al. 1995). Black vine weevil (*Otiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae)) females preferred to oviposit on the plants on which they previously fed despite the nutritional condition of the host plant (Coyle et al. 2011). In the cabbage looper moth (*Trichoplusis ni* Hubner (Lepidoptera: Noctuidae,) females are attracted to the host plants that they had previously experienced (Landolt and Molina 1996). In the beetle *Sitophilus granarius* L. (Coleoptera: Curculionidae), adult attraction to food odours was influenced by both larval and early adult experience (Rietdorf and Steidle 2002).

From my findings, I infer that the response of the pea leaf weevil to host plants is state-dependent in order to maximize their fitness. This study provides evidence for behavioural plasticity in an oligophagous insect and highlights the importance of considering the physiological state in understanding herbivore-host plant interactions, and in predicting pest population dynamics. Pea leaf weevil physiological state along with host plant information will be important to predict invasion and subsequent management. Since faba bean is a preferred primary host for pea leaf weevil in spring, there is potential to use it as a trap crop in the spring. Trap cropping is a cultural method that involves growing another host species or crop stage in a selected area to attract pests away from a target crop (Cárcamo et al. 2007, Hokkanen 1991, Shelton and Badenes-Perez 2006). Early seeded faba bean could attract dispersing pea leaf weevils from overwintering sites before the emergence of target crops (field peas or faba beans). This trap cropping system could be used along with a push-pull strategy to manage pea leaf weevil damage (Smart et al. 1994). The push-pull strategy of IPM involves the behavioural manipulation of the target pest by behaviour modifying stimuli (Xu et al. 2018). This will deter the pest from the main crop ("push") and attract them into the trap crop ("pull"). But push-pull strategies using faba bean need to be done with caution to prevent larval development before destruction of the trap crop and the cost and effectiveness of trap cropping requires further research.

More research is required to understand the mechanisms underlying host discrimination in pea leaf weevil adults. The response of pea leaf weevil adults to host plant volatiles is plastic and most acute in the spring and fall when weevils are active in the environment. Responsiveness to host plant volatiles declines in adult pea leaf weevil during the overwintering period (Landon 1997). Reproductive state influences response to host volatiles in other insects, and in general herbivores are more responsive to and discriminative of these cues when they are seeking oviposition hosts in a reproductively active state (Anton et al. 2007, Saveer et al. 2012, Kromann et al. 2015, Gadenne et al. 2016, Lemmen-Lechelt et al. 2018, Crava et al. 2019). Further, weevils may use host-specific individual volatiles or specific blends to discern between host plants (Bruce and Pickett 2011). This state-dependent plasticity may occur as a result of neural plasticity at both the central and/or peripheral nervous system (Anton et al. 2007, Lemmen and Evenden 2009). Pea leaf weevil response to host volatiles varies at the peripheral nervous system with physiological state (Landon 1997), but central nervous system processing of volatile cues has not been investigated in pea leaf weevils. The state-dependent plasticity to host plants may result in fitness consequences that affect the invasion success of the pea leaf weevil. Other factors such as previous host experience and maternal effects could be driving the state-dependent plasticity of host choice in the pea leaf weevil. Further exploration of the mechanisms driving the plasticity that I observed is warranted to better understand pea leaf weevil-host plant interactions.

Host quality: legume- *Rhizobium* symbiosis

Pea leaf weevil adults are oligophagous on a variety of legume plants (Jackson 1920, El-Dessouksi 1971, Nielsen 1990, Vankosky et al. 2009). Pea leaf weevil larvae feed on root nodules that house associated *Rhizobium* bacteria along with root nodule tissue of the host plant (Johnson and O'Keeffe 1981, Hamon et al. 1987). While adults feed on a variety of legumes, larval development has been recorded only on primary hosts, field pea and faba bean and to a lesser extent on the secondary host, chickpeas (Fisher and O'Keeffe 1979, Hoebeke and Wheeler 1985, Williams et al. 1991, Vankosky et al. 2009). Legume species have species-specific symbiotic relationships with rhizobia (Biddle 2017). Rhizobia influences host quality through fixing atmospheric nitrogen (Lee and Hirsch 2006). *Rhizobium* bacteria in root nodules may facilitate pea leaf weevil larval development by increasing root nodulation and providing more food resources (Vankosky et al. 2011a). *Rhizobium* activity, however, may be lower in high nitrogen soil because nitrogen reduces the nodulation in legumes that resulted in less larval damage by the pea leaf weevil. The host-specific legume-rhizobia symbioses may influence host signaling pathways to modulate semiochemical communication between pea leaf weevil adults and legume species (Pineda et al. 2013, Dean et al. 2014) which may provide information on host identity and quality to adult weevils. Understanding the relationship between these species-specific legume-*Rhizobium* symbioses and pea leaf weevil is a key component to understanding the host-pea leaf weevil interactions.

I found that the species-specific legume-*Rhizobium* association partially drives success of pea leaf weevil larval development, but not adult host acceptance. Pea leaf weevil adults feed on all the tested leguminous plants inoculated with host-specific *Rhizobium* strain except chickpeas. This is in contrast to previous work that showed pea leaf weevil reproduction on chickpeas resulting from a host-switch between field peas and chickpeas (Williams et al. 1991). This suggests that host-specific legume-*Rhizobium* associations alone may not alter the semiochemical communication between pea leaf weevil adults and host plants. Adults fed on all host plants except lentils and chickpeas. Lentils did not support larval development despite having the same *Rhizobium* strain, *Rhizobium leguminosarum biovar viciae*, as in field peas and faba beans. Future studies should explore whether these symbiotic relationships influence host-pea leaf weevil adult semiochemical communications through identification of the volatiles released by leguminous plants with and without *Rhizobium* inoculation.

I found that the host-*Rhizobium* symbioses contribute to the larval development of pea leaf weevil. Both *Rhizobium leguminosarum biovar viciae* associated hosts, faba bean and field peas support larval development. But lentil with the same *Rhizobium* strain does not support larvae. This could be because adults rejected feeding on lentils and hence either did not fully develop eggs or did not oviposit near lentil plants. Adults may assess host quality during the host acceptance process before oviposition. When tested on faba bean, *Rhizobium* had differential effects on adult feeding in field and laboratory studies. These differences may relate to variability in defence induction by faba bean in response to *Rhizobium* inoculation under field and laboratory conditions. Biological nitrogen fixation by *Rhizobium* varies depending on nitrogen amendments or residual nitrogen levels in the soil from past cropping cycles to the crop which alter the pea leaf weevil-faba bean interaction. In my study, faba bean treated with nitrogen supports fewer larvae due to the reduction of nodule production. Adult host preference also varies with host quality. My findings highlight the legume-*Rhizobium* symbiosis influences host quality and pea leaf weevil establishment. Some larval development occurred on soybean despite the symbiotic relationship with a different *Rhizobium* strain that occurs with field pea and faba bean. Legume-*Rhizobium*-pea leaf weevil interactions are complex and warrant further research. I recommend monitoring pea leaf weevil on soybean as its range expands eastward into Manitoba, because my study suggests that larval development could occur on soybean.

Overwintering biology and post-overwintering host interactions

Pea leaf weevil overwinters as non-reproductive adults in tree shelterbelts or semi-perennial forage crops where they resume feeding under optimal environmental conditions (Schotzko and O'Keeffe 1986). Adults do not undergo true diapause and their reproductive tract slowly develops during the overwintering period (Schotzko and O'Keeffe 1986). Overwintering biology and cold hardiness are major indicators of the population dynamics of an invasive species (Bale and Hayward 2010), and overwintering conditions can influence post-overwintering weevil-host plant interactions (Marshall and Sinclair 2012, Williams et al. 2015). Although the pea leaf weevil is well established in North America and continues to spread toward northern and eastern areas of the Prairie Provinces of Canada, it is not understood how well this invasive pest copes with the harsh winter conditions in the Canadian Prairies.

Insects select microhabitats to minimize energy use during the overwintering period, because small changes in microhabitat can greatly influence overwintering survival (Marshall and Sinclair 2012, Williams et al. 2015). I found that more pea leaf weevil adults survive the winter in tree shelterbelts compared to open crop fields. The supercooling point is not different between weevils that overwintered in the two microhabitats. According to my findings, field margins with leaf and shrub cover facilitate greater overwintering survival and likely provide insulation to overwintering adults. The field margins, however, also provide overwintering sites for natural enemies such as ground beetles, spiders (Corbett and Rosenheim 1996, Ramsden et al. 2014, Gallé et al. 2018). Some species of ground beetles are known to feed on pea leaf weevil eggs (Vankosky et al. 2009, Cárcamo and Vankosky 2013), and carabids prey on adult weevils (Wijerathna, field observation). Future research should investigate how manipulation of overwintering microhabitats could affect pest-natural enemies balance in the pea leaf weevil-legume cropping system.

Pea leaf weevil has expanded to the east and north in the Prairie Provinces and now infests field peas and faba beans grown in central Alberta, Saskatchewan and the Peace River Region (Cárcamo et al. 2018). More recently, pea leaf weevil has been recovered in sampling and trapping efforts in western Manitoba (Gavloski 2019). Local differences in overwintering success will affect pea leaf weevil establishment as it spreads to new areas of the Canadian Prairies. In this study, I found that pea leaf weevil survival is different between regions within Alberta. Adult survival is highest at the southern site in Lethbridge and lowest at the northern site in Edmonton. These differences might be due to the variable climatic conditions between sites. Climate models predict pea leaf weevil range expansion beyond its current range in North America, and higher soil temperatures with climate change may facilitate pea leaf weevil overwintering survival in the expanded range (Olfert et al., 2012, 2017). These findings on differences in overwintering survival between regions should be interpreted cautiously because overall overwintering survival was low at all study regions.

In my study, pea leaf weevil survival increased with soil temperature, and this may have contributed to the poor overwintering survival at the northern site in Edmonton. Although, I expected that the weevils overwintering at the northern site in Edmonton would be the most cold hardy, my results reveal that adults overwintered at the southern site in Lethbridge have higher cold tolerance. The colder overwintering temperatures cause severe winter damage (Koštál et al. 2006) and weevils overwintered in Edmonton may have used more energy in repairing winter damage compared to those that overwintered at Lethbridge (Sinclair 2015). Future experiments investigating energy use under variable overwintering temperatures would reveal mechanisms behind this difference in survival. Weevil origin was held constant in this study, as weevils were collected in Lethbridge and transported to the various overwintering locations. Therefore, it is not clear whether pre-overwintering preparation of weevils, in terms of cryoprotectant production, would vary if weevils were collected locally. A study using weevils that originate at the same site as they overwinter would answer this research question.

Physiological mechanisms regulate overwintering and the production of cryoprotectants. High juvenile hormone titers in adult insects are linked to the metabolism of lipid stores, termination of reproductive diapause, increased vitellogenesis and egg-production to support reproduction in post overwintering female insects (de Kort 1990, Matsuo et al. 1999, Zera and Zhao 2004, Denlinger et al. 2005, Zibaee et al. 2011). I found that topical application of juvenile hormone III reduces the body lipid content of female pea leaf weevils collected in the fall and subjected to overwintering conditions. Further studies are required to determine if there is a trade-off between lipid metabolism and subsequent reproduction in overwintering females. There may be potential to use juvenile hormone III to manage pea leaf weevil and application of juvenile hormone mimics to overwintering weevils should be further investigated.

Overwintering conditions in the laboratory influenced the subsequent performance of pea leaf weevil adults (Schotzko and O'Keeffe 1988, Sinclair 2015). Weevil feeding and oviposition were higher when weevils experienced a higher overwintering temperature for a longer duration (Schotzko and O'Keeffe 1988). The strength of the positive relationship between feeding and subsequent oviposition of pea leaf weevil varied according to the temperature and duration of the overwintering period. Therefore, climatic conditions that pea leaf weevil adults experience through the overwintering period influence post-overwintering interactions with host plants and can affect population density in the subsequent year (Wigby et al. 2011). I recommend that future population prediction models incorporate winter temperatures and duration to predict pea leaf weevil success before prophylactic seed treatments are used for pea leaf weevil control. My findings indicate that warm winters will benefit pea leaf weevil range expansion through an increase in both overwintering survival and post-overwintering population growth. More research is needed to develop models that can predict overwinter survival to help growers determine if prophylactic seed treatment is warranted for pea leaf weevil control.

Integrated pest management

Pea leaf weevil larval and adult damage can cause yield losses in both field peas and faba bean (El-Dessouksi 1971, Nielsen 1990). Adult and larval feeding damage on field peas has been researched in Alberta and a nominal threshold to trigger foliar insecticide application has been developed based on the percentage of terminal leaf damage (Cárcamo and Vankosky 2011, Vankosky et al. 2011a, Vankosky et al. 2011c). Although insecticides are commonly used in pea leaf weevil management in faba bean, no economic or nominal thresholds have previously been determined for use in the crop in Alberta or elsewhere. Seed treatment with thiamethoxam and foliar application with lambda-cyhalothrin are common practices to manage pea leaf weevil on faba bean. Foliar insecticides that target adults, however, are effective only when applied on time and after frequent applications (Bardner et al. 1983, Cárcamo and Vankosky 2011). A nominal threshold provides a guide to foliar applications and is also useful to evaluate seed treatment efficacy of insecticidal treatment.

My work shows seed treatment with thiamethoxam reduces pea leaf weevil adult damage of faba bean seedlings at the most vulnerable stages (2^{nd}- 5^{th} unfolded leaf stages), reduces larval damage (Willsey et al. 2021), and protects yield. Foliar applications with lambda-cyhalothrin, however, are inconsistent in reducing adult damage, and do not reduce larval damage or protect yield. Foliar insecticides are also inconsistent in protecting field pea from adult pea leaf weevil damage, compared to insecticidal seed treatments (Seidenglanz et al. 2010, Cárcamo et al. 2012). Efficacy of foliar applications varies with the timing of primary host colonization by weevils in the spring, and with weevil density (Fisher and O'Keeffe 1979, Hamon et al. 1987, Nielsen 1993, Cárcamo et al. 2018). Variable spring conditions in pulse growing regions of Alberta will influence insecticidal control of pea leaf weevil by foliar insecticides. Future research should investigate pea leaf weevil flight patterns and overwintering survival under variable weather conditions before prophylactic foliar applications are recommended. In this study, I recommend a nominal threshold of 15% plants with terminal leaf damage at the 2^{nd} unfolded leaf stage. But this nominal threshold is proposed in the absence of an effective foliar insecticide. This nominal threshold, however, could assist in assessing the efficacy of seed treatments and to make decisions on future management strategies. There is a need for future research into control tactics that can be used post primary host colonization against pea leaf weevil.

An unusual finding from my study showed that the combined application of foliar insecticide and seed treatment insecticide increased the larval damage compared to faba bean plants that received only the seed treatment. This suggests that foliar application renders the seed treatment less effective. Foliar application may have negative indirect effects on natural enemies of pea leaf weevil (Vankosky et al. 2011b, Cárcamo and Vankosky 2013) and this potential needs to be further studied in the pea leaf weevil-faba bean system. My study also highlights the need for local pest management recommendations to manage pea leaf weevil, given that the percentage of root nodules that support larval development varies between study sites. This may affect the pea leaf weevil adult and larval density and hence the weevil-host plant interactions and agronomic yield.

Final thoughts

Pea leaf weevil has complex interactions with its host plants. Pea leaf weevil interactions with the host plants are modified by individual fitness, ecosystem characters and local climate dynamics. State-dependent behavioural plasticity modifies host preference and allows pea leaf weevil to maximize fitness through reproduction and survival. The presence of secondary host plants alters the state-dependent host preference and can cause a fitness disadvantage through preference of secondary hosts over primary host plants that support larval development. Although larval development is mainly observed on field peas and faba beans, pea leaf weevil adults forage on a wide variety of secondary host plants, and these secondary hosts may facilitate weevil survival in the absence of primary hosts. Legume-rhizobia symbioses influence pea leaf weevil larval development but, not adult host acceptance. To make these interactions more complex, overwintering conditions influence pea leaf weevil post-overwintering feeding and oviposition. The pea leaf weevil damage may intensify as a warmer climate persists by increasing the overwintering survival. Monitoring pea leaf weevil at regional and microhabitat levels are good indicators of overwintering survival to predict subsequent interactions with host plants. Recommendations on overwintering survival, availability and diversity of host plants and host quality at the local level are important factors to consider when applying the proposed nominal threshold in IPM practices.

An increase in temperature as a result of global warming will facilitate population growth and range expansion of pea leaf weevil. The increased social pressure on diets based on plant-based proteins (Neacsu et al. 2017) have provided a market for increased production of pulses such as soybean in the western Prairie provinces (Alberta, Saskatchewan, and Manitoba) (https://soycanada.ca). These expansions of pulse growing areas and increasing temperatures will allow pea leaf weevil to become established in previously non-suitable habitats. I recommend ecologically based pest management strategies such as trap crops, legume-species based crop rotations, and *Rhizobium* inoculations to minimize the pesticide use in pest management.

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