Presence and Distribution of Alfalfa Weevil and *Sitona* Species in Alfalfa Fields Grown for Seed in Alberta

by

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Abstract

The alfalfa weevil (*Hypera postica* (Gyllenhal), Curculionidae: Coleoptera) is a major pest of alfalfa (*Medicago sativa* L., Fabaceae) that feeds on alfalfa foliage. High density larval populations cause significant damage through leaf skeletonization. This study examines the location of adult alfalfa weevil in alfalfa fields grown for seed at various times throughout the growing season using different sampling techniques. A secondary objective is to assess the diversity and abundance of broad nosed weevils (*Sitona* spp. (Curculionidae: Coleoptera)) in these same fields. The study was conducted in 18 alfalfa fields grown for seed in southern Alberta over three growing seasons (2021-2023) with two fields from 2021 repeated in 2022. Alfalfa fields were sampled using various capture methods to monitor flying weevils and those present on the ground and plant.

Five fields with established alfalfa stands grown for seed were selected in 2021 and 2022 and eight fields in 2023. Alfalfa weevils captured in emergence cages, pitfall traps, sweep net samples and soil samples were collected in all the study years. Additionally, double-sided sticky cards and malaise traps were used in 2021 to capture flying alfalfa weevils. In 2022, trials using water traps and baited pitfall traps tested the attractiveness of alfalfa weevil to water and putative pheromone components ((*Z*)- and (*E*)-3,3-dimethylcyclohexane- $\Delta^{1,\alpha}$-acetaldehyde (1:1)) and the aggregation pheromone of the pea leaf weevil (4-methyl-3,5 heptanedione). In 2023, solo cup pitfall traps were replaced with directional pitfall traps separated by a barrier to determine the directionality of walking alfalfa weevils. In 2023, the effect of alfalfa stand age on weevil abundance was tested.

Sweep samples were an effective tool for capturing and monitoring adult alfalfa weevils, and also predicted subsequent larval densities. Adult alfalfa weevils were captured in low numbers in pitfall traps, water traps positioned on the ground and in soil samples taken at various times throughout the season. Sticky cards and malaise traps failed to capture any flying alfalfa weevils, indicating less reliance on flight for dispersal by alfalfa weevils. More adult alfalfa weevils were captured in emergence cages positioned outside alfalfa fields than in those positioned within, revealing that some adult alfalfa weevils overwinter outside the field. Pitfall traps (2022) baited with pea leaf weevil aggregation pheromone attracted numerically but not statistically more alfalfa weevil adults than traps baited with putative alfalfa weevil pheromone components or solvent only control traps. More female alfalfa weevils were consistently captured than male alfalfa weevils in the overwintered generation collected in the spring during the three experimental years. More overwintered alfalfa weevil adults were collected from mature than young alfalfa stands, suggesting the preference for or lack of dispersal from established alfalfa fields by adult alfalfa weevils. Weekly average temperature and relative humidity affected the number of alfalfa weevil adults that were captured in sweep samples.

Other weevil species including *Sitona* spp. were collected consistently using various sample methods. Two species of broad-nosed weevils were identified from the by-catch of sweep net samples, the alfalfa curculio (*Sitona lineellus* Bonsdorff) and to a lesser extent the pea leaf weevil (*Sitona lineatus* L.), in addition to non-*Sitona* weevil species.

Overall, the current research has identified effective monitoring tools for alfalfa weevil adults, the abundance of alfalfa weevil throughout the growing season and potential overwintering locations of adult alfalfa weevils. The common occurrence of alfalfa curculio in alfalfa fields grown for seed highlights the need for more research on this species in Alberta.

Preface

This research work was conducted by P. Chennamkulangara under the supervision of Dr. Maya Evenden which is intended for publication. I performed the experiments, collected and analyzed data.

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'It always seems impossible until it is done' – Nelson Mandela

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1. Introduction

1.1 Significance of alfalfa

Alfalfa (*Medicago sativa* L.) (Fabaceae), known as the queen of forages, is a major perennial legume crop produced on ~ 3.06 million hectares (ha) covering nearly 5% of Canada's farmland (Attram et al. 2016, Statistics Canada 2021). Alfalfa originated in Asia and was introduced to different parts of the world as livestock feed (Veronesi et al. 2010). World wide, the majority of alfalfa production occurs in North America; with considerable production in France, Italy and Argentina (Alfalfa seed marketing in Canada 2024). Due to its high protein content, alfalfa is an essential component of poultry and livestock feed, and a good source of protein for humans, mostly as sprouted seeds (Mielmann 2013, Firdaous et al. 2017, Boucher et al. 2023, Hadidi et al. 2023, Francis et al. 2022). Alfalfa is grown for seed in arid and semi-arid regions of Alberta, Saskatchewan, and Manitoba (Sim and Meers 2016). Alberta alone produces nearly 2,964 tonnes of alfalfa seed annually (AFIN 2012, Statistics Canada 2021). Canada also plays a vital role in export of alfalfa seeds (14 million kg), mostly from Alberta, Saskatchewan and Manitoba (Alfalfa seed marketing in Canada 2024). Apart from growing alfalfa for forage and seed, alfalfa is additionally incorporated in crop rotations to enhance the soil quality through nitrogen fixation (Summers 1998). Alfalfa is highly dependent on bees for pollination and provides nectar in return (Pankiw et al. 1956, Bosch and Kemp 2005).

1.2 Pollinators in alfalfa

Insect pollination is an important ecosystem service for global food production in which 70% of crops depend on insects for some sort of pollination (Klein et al. 2007, Potts et al. 2010). Bees (Hymenoptera: Anthophila) are considered the most important pollinators and managed honey bees (*Apis mellifera* L. (Hymenoptera: Apidae)) are often required in cropping systems (Hung et al. 2018). But for some crops (alfalfa, almond, cherry, blueberry), other bee species are important pollinators, including solitary alfalfa leaf cutter bees (*Megachile rotundata* Fab. (Hymenoptera: Megachilidae)), bumble bees (*Bombus* spp. (Hymenoptera: Apidae)), mason bees (*Osmia* spp. (Hymenoptera: Megachilidae)), carpenter bees (*Xylocopa* spp. (Hymenoptera: Apidae)) and other native bees (Delaplane and Mayer 2000, Garibaldi et al. 2013).

Pollination in alfalfa relies heavily on insects as the alfalfa flowers have a tripping mechanism to expose the anther and stigma when the insect lands on the flower (Bohart 1957). Both alfalfa leaf cutter bees and honey bees are used in alfalfa for pollination services (Haedo et al. 2022), but alfalfa leaf cutter bees are more effective (Bohart 1957). The efficiency of honey bee pollination of alfalfa in the Great Plains of North America is only 0-1% (Delaplane and Mayer 2000), and hence alfalfa growers rely on alfalfa leaf cutter bees in those areas. The introduction of leaf cutter bees to Canada coincided with a noticeable increase in alfalfa seed yield from an average of 50 kg to 1,100 kg seed/ha (Richards 1993). Apart from managed bees, bumble bees and some native bees pollinate alfalfa crops to some extent (Haedo et al. 2022, Zhang et al. 2022). Unmanaged alkali bees (*Nomia melanderi* Cockerell (Hymenoptera: Halictidae)), native to western North America, are effective in alfalfa pollination in parts of the United States of America (USA) (Bohart 1957, Cane 2008).

Insecticides increase mortality of adult and larval stages of leaf cutter bees because they use alfalfa leaves with insecticide residue for nesting (Waller 1969). Fewer leaf cutter bee eggs hatch in fields treated with the insect growth regulator, Novaluron compared to those receiving no Novaluron (Pitts-Singer and Barbour 2017). The pyrethroid insecticide, Deltamethrin is more toxic to alfalfa leaf cutter bees in a laboratory setting than to bumble bees and *Osmia lignaria*. Likewise, the neonicotinoid, Clothianidin is toxic to leaf cutter bees, bumble bees, *O. lignaria* and honey bees (Scott-Dupree et al. 2009). Alfalfa seed producers face the challenge of managing pest insects with tools that do not impact the pollinators that are required for a high yielding seed crop.

1.3 Insect pests on alfalfa

Being an economically significant crop, the beneficial and detrimental insects on alfalfa need to be studied in order to take necessary decisions for crop protection. Harper (1988) surveyed insect and mites in alfalfa fields of Alberta and discovered 437 arthropod species. Significant insect pests on alfalfa in Alberta are alfalfa weevil (*Hypera postica* (Gyllenhal) (Curculionidae: Coleoptera) (Hobbs et al. 1959), broad nosed weevils (*Sitona* spp. (Coleoptera, Curculionidae) (*Sitona lineatus* L., *Sitona lineellus* Bonsdorff, *Sitona hispidulus* F., *Sitona cylindricollis* Fåhraeus) (Loan 1961), alfalfa blotch leafminer (*Agromyza frontella* Rondani

(Diptera: Agromyzidae)) (Soroka and Otani 2011), lygus bugs (*Lygus* spp. (Hemiptera: Miridae)) (Butts and Lamb 1991, Carcamo et al. 2003), alfalfa plant bug (*Adelphocoris lineolatus* Goeze (Hemiptera: Miridae)) (May et al. 2003, Soroka and Murrel 1993), pea aphid (*Acyrthosiphon pisum* Harris (Hemiptera: Aphididae)) (Harper and Kaldy 1982, Uddin 2005), and the spotted alfalfa aphid (*Therioaphis maculate* Buckton (Hemiptera: Aphididae)) (Sim and Meers 2016).

1.3.1 Plant bugs

Plant bugs (Hemiptera: Miridae), namely lygus bugs (*Lygus* spp.) and alfalfa plant bugs (*Adelphocoris lineolatus* (Goeze)), cause damage to alfalfa that decreases the seed yield. In Alberta, four species of lygus bugs, *Lygus lineolaris* (Palisot de Beauvois), *L. keltoni* (Schwartz and Foottit), *L. borealis* (Kelton), and *L. elisus* (Van Duzee) were recorded from sweep net samples of alfalfa (Sim and Meers 2016, Cárcamo et al. 2002). In the Prairie Provinces, lygus bugs feed on alfalfa, canola (*Brassica napus* (Brassicaceae)), soybean (*Glycine max* (Fabaceae)), and sunflower (*Helianthus annuus* (Asteraceae)), whereas alfalfa plant bugs feed on alfalfa, canola, and red clover (*Trifolium pratense* (Fabaceae)) (Craig 1963, May et al. 2003). Both adult and nymphal plant bugs feed on sap from bud, flowers and young pods (canola) using piercing and sucking mouth parts (Craig 1963). Lygus bugs have several generations in a year while the alfalfa plant bug has only one generation per year in the Prairies (Craig 1971).

Alfalfa plant bugs overwinter as eggs and lygus bugs overwinter as adults (Aasen and Bjorge 2009). The number of plant bugs count in alfalfa is mainly reduced by native parasitic wasps, including *Peristenus braunae* (Goulet) and *Peristenus digoneutis* (Loan) affecting alfalfa plant bugs and lygus bugs, respectively in southern Alberta (Braun et al. 2001, Goulet and Mason 2006, De Clerck-Floate and Cárcamo 2011).

1.3.2 Aphids

Pea aphid (*Acyrthosiphon pisum* Harris) and spotted alfalfa aphid (*Therioaphis maculate* Buckton) are the major aphid pests of alfalfa in Alberta (Sim and Meers 2016). Aphids produce honey dew as a result of feeding which promotes growth of sooty mould that covers plants and reduces photosynthetic activity (Flessa et al. 2022). Sooty mould infection reduces alfalfa plant height (by ~45 %), fresh weight (by ~38%), dry weight (by ~44%) and fibre content (by ~13%)

under greenhouse conditions (Harper and Kaldy 1982). Spotted alfalfa aphid is a more important pest of alfalfa than the pea aphid, because it produces a toxin that results in necrosis of plant tissue around feeding sites (Sim and Meers 2016).

1.3.3 Broad nosed weevils

Several weevil pests (Curculionidae: Coleoptera) infest alfalfa and cause damage by defoliation or root feeding. The root weevils, commonly called broad-nosed weevils belonging to the genus *Sitona*, are considered pests of plants in the family Fabaceae (Gözüaçik 2023). The *Sitona* weevils have a short rostrum with the distinguishing feature of an oval or round scar on the mandibles (Bright 1994). There are ~100 species of *Sitona* around the world, most of which are important agricultural pests (Bright 1994, Bright and Bouchard 2008, Danthanarayana 1969). In Canada, there are six native and five introduced species of *Sitona* (Cárcamo and Vankosky 2013). The life cycle of the *Sitona* species is similar, except that host preference varies by species (Carcamo and Vankosky 2013).

Sitona spp. larvae largely feed on the roots of the host plant (Wildermuth 1910, Prescott and Reeher 1961, Gerard et al. 2010) including the root nodules that contain the *Rhizobium* nitrogen-fixing bacteria (Johnson and O'Keeffe 1981, Carcamo and Vankosky 2013, Hoebeke and Wheeler 1985). Adults feed on the foliage and cause less direct damage than larvae (Johnson and O'Keeffe 1981, Corre-Hellou and Crozat 2005). The primary hosts required for reproduction of the pea leaf weevil, *Sitona lineatus* L. are field peas (*Pisum sativum* L.) and faba beans (*Vicia faba* L.) (Vankosky et al. 2009, Carcamo and Vankosky 2011, Nielsen 1990). *Sitona lineellus* Bonsdorf (*Sitona scissifrons* Say) feeds on alfalfa, vetches (*Vicia* spp.) and field peas (*Pisum sativum*) (Loan 1963). *Sitona flavescens* (Marshall) and *Sitona hispidulus* (Fabricius) prefer clover (*Trifolium* spp.) and alfalfa (Soroka and Otani 2011) whereas *Sitona cylindricollis* (Fahraeus) prefers sweet clover (*Melilotus officinalis* L.) (Craig 1978).

Sitona weevils overwinter mostly as adults and emerge from overwintering sites in spring. However, *Sitona hispidulus* is found to overwinter in egg and adult stage in Northern Utah (Price 2017) and *Sitona humeralis* in egg, larval and adult stage in Turkey (Gözüaçık at al. 2021). Adults feed on the foliage of leguminous plants making a characteristic notch on the leaves (Barratt and Byers 1992). Following mating, eggs are laid in the soil in the proximity of the primary host plants (Gözüaçık at al. 2021). Upon hatching, larvae burrow into the soil and feed on root nodules and roots resulting in reduced reproductive capacity of the plant and crop yield (Johnson and O'Keeffe 1981, Carcamo and Vankosky 2013). Pupation occurs in the soil (Roselle and Connin 1955) and the newly emerged weevils feed on leguminous host plants before seeking out overwintering sites. In New Zealand, *Sitona lepidus* have recorded two generations in some years (Gerard et al. 2010).

Alfalfa fields heavily infested with *Sitona* weevils have reduced stand establishment, altered water and nutrient uptake, secondary infection by soil borne pathogens, winter kill, and reduced quality and yield (Rim 2020). High densities of *Sitona* weevils have the potential to destroy crops with >90% nodule damage (Tahhan and Hariri 1982, Gözüaçik 2023). Among all *Sitona* weevils that feed on alfalfa as adults few can complete their life cycle on alfalfa. Only alfalfa curculio (Mast 1963) and the clover root curculio (Dickason et al. 1968) can complete their life cycle on alfalfa, as larvae feed on alfalfa roots and root nodules.

Alfalfa curculio (*Sitona lineellus* Bonsdorff (= *S. scissifrons* (Say))

Sitona lineellus is believed to be an introduced species, native to Europe and first recovered in Canada from Ontario (Campbell et al. 1989). Because of the widespread presence of this weevil in most Canadian provinces, however, many people suggest that alfalfa curculio is native to North America (Anderson 1997). *Sitona lineellus* (2.9 - 4.0 mm) is a small, brown or grey coloured weevil with a dark region that runs lengthwise at the centre of the elytra with broken stripes on either side. The bristles on the elytra of alfalfa curculio are short, erect and curved. The elytra of alfalfa curculio have small rounded white scales with slightly narrower brown scales. The eyes are strongly protuberant (Bright and Bouchard 2008).

The principal host plants of the alfalfa curculio are alfalfa, sweet clover (*Melilotus officinalis* L.) and red clover (Beirne 1971), although feeding on vetch has also been reported (Loan 1963). The adult feeds on the leaves, buds, flowers and stems with distinct notches on the leaf margin. Feeding can completely destroy alfalfa plants in the seedling stage. The larvae feed on root nodules and develop through two instars within the root nodules and two instars feeding externally on roots and nodules (Loan 1963). Adults overwinter in debris inside or near to fields. Adults disperse on the ground more than by flight (Loan 1963). Mating and oviposition happen in early spring and females lay an average of 100 - 150 eggs. Larvae hatch within 21 days and adult new generation weevils emerge by July to feed prior to overwintering (Loan 1963). Native parasites can destroy up to 42% of alfalfa curculio larvae (Beirne 1971).

Clover root weevil (*Sitona hispidulus* Fabricius)

Sitona hispidulus (clover root weevil), is native to Europe and an introduced pest to North America (Hatch 1971). The weevil damages several leguminous crops (clover and alfalfa) particularly under drought conditions (Beirne 1971). The clover root weevil is considered a serious pest in Illinois, Virginia, Kansas (Bigger 1930, Underhill et al. 1955, Marshall and Wilbur 1934) and the Maritime Provinces (Thompson and Willis 1967). Beirne (1971) recorded the presence of clover root weevil on alfalfa and clover in Ontario, Saskatchewan and British Columbia. Adult feeding results in crescent shaped notches on leaf edges or symmetric paired holes at the midrib. Adults feed mainly at night and move to the lower plant parts during the day (Marshall and Wilbur 1934). The damage is mainly caused by larvae feeding on the root nodules, secondary rootlets and by girdling the taproots (Marshall and Wilbur 1934, Thompson and Willis 1967, Rim et al. 2021). Adults rely on walking for dispersal (Underhill et al. 1955). Adults are about 3.2 - 4.0 mm long with characteristics of long bristles on the elytra and flat compound eyes (Bright and Bouchard 2008).

Clover root weevil has a univoltine life cycle (Underhill et al. 1955) and overwinter as adults (Bigger 1930, Beirne 1971). Eggs laid on the soil surface in the spring hatch within two weeks (Marshall and Wilbur 1934, Underhill et al. 1955, Rim et al. 2021), and larvae move downward to feed on root nodules. The later instar stages feed externally on the tap roots and can damage the main root system (Bigger 1930, Dintenfass and Brown 1986). Pupation occurs in the soil (Rim 2020, Marshall and Wilbur 1934).

Sweet clover weevil (*Sitona cylindricollis* Fåhraeus)

Sitona cylindricollis is an introduced pest to Canada from Europe, first reported from Montréal, Québec in 1927 (Brown 1940), then in Manitoba in 1939 when a heavy infestation occurred on sweet clover (Bird 1947). *Sitona cylindricollis* (3.5 - 5 mm) has flat and short bristles on the mottled elytra. The eyes are flat (Phillips and Barratt 2004). Similar to other

Sitona weevils, adults make u-shaped notches on the leaf margins when feeding and leave just the mid-rib in extreme cases (Bird 1947). Adults also feed on green seeds in the absence of foliage (Craig 1978). Larvae feed on root nodules and root hairs which facilitates root rot by *Rhizoctonia* spp. (Godfrey and Yeargan 1987). Sweet clover weevils only feed on alfalfa if there is a scarcity of sweet clover plants (Herron 1953, Bird 1947, Craig 1978, Cárcamo and Vankosky 2013).

Sweet clover weevils overwinter as sexually immature adults in or near the fields (Caesar 1936, Bird 1947, Beirne 1971). Adults emerge in early spring with peak emergence in May (Manitoba) (Bird 1947). Weevils disperse by flight or walking to locate host plants (Bird 1947). Following mating, females lay eggs in the soil (Bird 1947, Herron 1953). Egg hatch is temperature dependent and ranges from 7-10 days (Herron 1953). The larvae develop through four larval stages (Bird 1947, Craig 1978, Abdullaeva and Bobakulovna 2022). Larvae occur as deep as 25 cm in sandy soils and soil depth varies with larval development (Herron 1953, Beirne 1971). Pupation occurs in the top 2.5 cm of the soil (Manitoba) (Bird 1947). Sweet clover weevils do not do well under hot and dry weather conditions (Bird 1949). Sweet clover weevils are naturally affected by the pathogen, *Beauveria bassiana* (Balsamo) Vuillemin (Bird 1949). Parasitoid wasps (Hymenoptera: Braconidae), *Microctonus aethiops* (Nees), *Pygostolus falcatus* (Nees) and *Perilitus rutilus* (Nees) and a dipteran (Tachinidae), *Syntomogaster exigua* (Meigen) were released in Montana to control sweet clover weevil, only *Pygostolus falcatus* became established in low densities (Loan 1961).

Clover root curculio (*Sitona flavescens* Marsham (= *S. lepidus* Gyllenhal (= *S. obsoletus* Marsham)

Sitona flavescens is an introduced pest to North America (Bright 1994). The clover root curculio infests several forage crops including alfalfa, clover and other leguminous crops, but has a preference for white clover (*Trifolium repens*) (Johnson et al. 2004, Phillips and Barratt 2004, Thompson 1964). Similar to other *Sitona* weevils, the adults feed on leaves and the larvae feed below surface on root nodules (App and Manglitz 1972). Adults are ~3.6 - 5 mm in length. The elytra have flat, recumbent scales intermixed with narrow hair-like golden or reddish scales (Bright 1994, Bright and Bouchard 2008).

Pea leaf weevil (*Sitona lineatus* Linnaeus)

The pea leaf weevil is an introduced pest from Europe and North Africa (Vankosky et al. 2009), first noticed in southern Alberta in 1997 (Vankosky et al. 2009). Adult pea leaf weevils are ~ 3.2 - 4.5 mm long with dark longitudinal stripes on the straight elytra. Bristles on the elytra are almost flat and short. The eyes are moderately rounded. Pea leaf weevils are distinguished by the edge of the front coxa that touches the groove on the prosternum (Bright and Bouchard 2008).

Adult pea leaf weevils undergo two physiological stages during their univoltine life cycle (Jackson 1920, Landon et al. 1995). Adults emerge from overwintering locations and feed on perennial legumes before moving to primary host plants (field pea: *Pisum sativum* L. or faba bean: *Vicia faba* L.) (Hamon et al. 1987). At this point, weevils are reproductively mature and males release an aggregation pheromone (4-methyl-3,5-heptanedione) that attracts both sexes (Blight et al. 1984). Weevils mate and females lay eggs in soil. Larvae feed on the root nodules (Hamon et al. 1987, Hunter 2001) before pupation in the soil. The new generation weevils are sexually inactive and search for perennial legumes, including alfalfa, where they feed and overwinter until next spring (Carcamo and Vankosky 2011).

Pea leaf weevils respond to the aggregation pheromone in spring and fall (Blight and Wadhams 1987, Evenden et al. 2016, Bandeira et al. 2021). The attractiveness of the semiochemicals tested in the field confirmed baited pitfall traps capture more adult pea leaf weevils than unbaited pitfall traps (Reddy et al. 2018, St. Onge et al. 2018). The pheromone also attracts other *Sitona* weevils like sweet clover weevil (St. Onge et al. 2018) and lucerne weevil (*Sitona humeralis* Stephens) (Toth et al. 1998, Lohonyai et al. 2019).

1.3.4 Alfalfa weevil

The alfalfa weevil is the major consumer of alfalfa foliage wherever it is cultivated (Schaber et al. 1994). Both larvae and adults feed on alfalfa foliage during the pre- to the early-bloom stages, causing significant economic loss (Hamlin et al. 1949). Alfalfa weevils prefer alfalfa but will feed on clover, and rarely on vetch (Ellsbury et al. 1992, Bae et al. 2013). The major damage is caused by the larvae which are ~ 8 mm length and pale green in colour with a longitudinal white band on the dorsal side and a black head capsule when fully mature (Government of Saskatchewan 2024). Extensive larval feeding can result in a greyish look to the field (Government of Manitoba 2024). Alfalfa weevil adults (5 mm length) are brown with a dark brown stripe at the centre of the elytra.

1.4 Introduction of alfalfa weevil to Canada

Alfalfa weevil is of Eurasian origin, and was introduced to North America in three locations. Alfalfa weevils were initially introduced to Utah in 1904 and are referred to as the western strain (Titus 1911). The Egyptian strain of alfalfa weevils (Wehrle 1940), was introduced to Arizona in 1939, while the eastern strain (Poos and Bissell 1953) was found in Maryland in 1951. The alfalfa weevil strains are differentiated based on physiological, behavioural and ecological differences. The eastern and Egyptian strains pupate in cocoons attached to the alfalfa plant, whereas the western strain pupates off the plant in litter (Pellisier et al. 2017). The endosymbiont, *Wolbachia*, in the western strain protects weevils from parasitoids and prevents breeding with Egyptian and eastern strains (Salt and Van den Bosch 1967, Hsiao 1996). Eastern and western strain alfalfa weevils aestivate away from the field individually while the Egyptian strain aestivate in groups under bark or in crevices (Böttger et al. 2013). In Canada, alfalfa weevil first appeared in southeastern Alberta and southwestern Saskatchewan after migrating from Montana (USA) in 1954 but now occurs across Canada (Hobbs et al. 1959, Soroka et al. 2020a). Within a period of about 13 years, alfalfa weevil went from being a minor pest to a significant pest (Soroka et al. 2020a). The range expansion of alfalfa weevil depends on the suitability of the environment (Soroka et al. 2020a).

1.5 Alfalfa weevil biology

Overwintering adult alfalfa weevil become active with the emergence of new alfalfa shoots in spring. Adults feed for a few days, mate, and oviposit on alfalfa stems in groups of 5 - 20 (Pellissier et al. 2017). In about two weeks, eggs hatch and larvae feed on foliage (Soroka et al. 2020a). Early-stage larvae (first and second instar) feed on terminal buds and newly emerged shoots causing pin hole damage (Godfrey et al. 2005). Later larval instars (third and fourth instar) skeletonize leaves leading to reduced plant vigour and biomass (Hoff et al. 2002).

Feeding damage is mostly observed in mid - late June in the Prairie Provinces (Schaber et al. 1994). Mature larvae move off the plant to the ground for pupation in plant litter or debris (Harcourt et al. 1977). Upon emergence, the new generation adults feed for a brief period, after which they overwinter until spring in or near alfalfa fields (Hobbs et al. 1959, Manitoba Agriculture 2018). In New York, Prokopy and Gyrisco (1965) noticed summer migration of alfalfa weevils to the border, woods or the sheltered areas from mid-June to mid-August. Prokopy et al. (1967) observed more aestivating weevils at the field edge with natural cover and straw and aestivating adults in woods adjacent to alfalfa fields were mostly in soil underneath litter.

Alfalfa weevil development varies with temperature and can be predicted using degree day models. Soroka et al. (2020b) compare different degree day models from different regions, including the Harcourt (Harcourt 1981), the North Dakota (Beauzay et al. 2013) and the Guppy-Mukerji models (Guppy and Mukherji 1974). According to the accepted Harcourt (base temperature = 9°C) and North Dakota (base temperature = 8.9°C) models, the thermal constant for egg hatch is 155 and 167 degree days, respectively. The accumulated degree days for adult emergence is 454 and >452 respectively (Soroka et al. 2020b).

1.6 Integrated Pest Management of alfalfa weevil

1.6.1 Monitoring

Various sampling techniques have been developed to assess alfalfa weevil within and outside alfalfa fields (Soroka et al. 2020a, Hilburn 1985). Emergence cages assess overwintering location of alfalfa weevils (Soroka et al. 2020a, Godfrey et al. 1986, Roberts et al. 1978). Soil sampling is another method to monitor the overwintering adult alfalfa weevils (Armbrust et al. 1969, Prokopy et al. 1967). Pitfall traps can be used to monitor both density and diversity of weevils in alfalfa fields (Kowalski 1975, Hatten et al. 2010) and have been employed in insect movement studies (Pausch et al. 1979). Modified pitfall traps with barriers can monitor movement of ground-dwelling arthropods moving in and out of the field (Hilburn 1985). The most commonly used monitoring tool for alfalfa weevil is sweep net sampling to capture larvae and adults present on the foliage of plants at least 15 - 25 cm tall (Harper et al. 1993, Soroka et al. 2020a). Sweep net sampling is used to monitor alfalfa weevil larvae and to trigger insecticide control using an economic threshold of 20 - 25 third or fourth instar in one 90° or straight sweep samples or 35 - 50% of damaged foliage tips (Government of Alberta 2024, Aasen and Bjorge 2009).

Semiochemicals are signalling chemicals that carry information between organisms (Dicke and Sabelis 1988). These compounds have the potential for development into effective surveillance tools for pest control (Suckling 2015). There are no known pheromones used in intraspecific chemical communication in alfalfa weevils but putative pheromone components have been identified in hexane extracts of alfalfa weevils (Hedin et al. 1988). The compounds obtained from alfalfa weevil extracts were identified as the aldehydes, (*Z*)-3,3-dimethylcyclohexane $\alpha^{1,\beta}$-ethanol and (*Z*)- and (*E*)-3,3-dimethylcyclohexane- $\Delta^{1,\alpha}$-acetaldehyde. These are known pheromone components of the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) (Hedin et al. 1988), but no behavioural activity by alfalfa weevils to these compounds has been demonstrated. Alfalfa weevils have been captured in traps baited with the pea leaf weevil aggregation pheromone (Quinn et al. 1999). Alfalfa weevils are attracted to volatile compounds emitted by fresh alfalfa foliage (Byrne and Steinhauer 1966, Meyer and Raffensperger 1974), but kairomonal lures have not been developed to date.

1.6.2 Control

In order to avoid economic losses caused by alfalfa weevil, farmers use different Integrated Pest Management (IPM) tactics including cultural, biological, and chemical methods (Onstad and Shoemaker 1984). The most effective cultural method is to harvest at early bloom when alfalfa is grown for hay (Herreid et al. 2024, Government of Saskatchewan 2024). This, however, is not a viable option for control of alfalfa weevil in alfalfa grown for seed.

Organophosphate and pyrethroid insecticides are commonly used for alfalfa weevil control (Schaber et al. 1994, Seidenglanz et al. 2010, Pellisier et al. 2017). Alfalfa weevil has evolved resistance to several insecticides. Insecticide resistance in alfalfa weevil was first observed for heptachlor (organochlorine insecticide) in Virginia, in the 1960s (Bishop 1964). Recently, alfalfa weevil has developed insecticide resistance to pyrethroid insecticides, especially those containing lambda-cyhalothrin and zeta-cypermethrin (Rodbell and Wanner 2021, Rodbell et al. 2023). Excessive use of chemical control measures can also cause ecological and environmental issues. As alfalfa grown for seed relies on bee pollination for crop production, growers cultivating alfalfa for seed face additional challenges to protect pollinators from harmful chemicals while maintaining a high-yielding alfalfa crop (Jabbour and Noy 2020). The commonly used insecticides in alfalfa fields in Alberta are lambda-cyhalothrin (Matador® 120E, Syngenta; Silencer® 120EC, ADAMA Canada), deltamethrin (Decis® 5EC, Bayer), Chlorantraniliprole (Coragen®, FMC of Canada Limited) (Alberta's Crop Protection Guide 2024).

Predation and parasitism by natural enemies of alfalfa weevil can play a crucial role in pest management. Parasitoids of alfalfa weevil include *Bathyplectes curculionis* (Thomson) (Radcliffe and Flanders 1998, Levi-Mourrao et al. 2022), *Bathyplectes anurus* (Thomson) (Hymenoptera: Ichneumonidae) (Maund and Hsiao 1991), *Oomyzus incertus* (Ratzeburg) (Hymenoptera: Eulophidae) (Shrestha et al. 2018) and *Microctonus colesi* Drea (Hymenoptera: Braconidae) (Drea et al. 1972) of which *B. curculionis* and *O. incertus* are found in Alberta (Soroka et al. 2020a; Soroka and Otani 2011, Hiarcourt 1990). *Bathyplectes curculionis* was introduced to North America from Europe and successfully established in Utah (Chamberlin 1926). It was later observed in southern Alberta reducing weevil populations (Hobbs et al. 1959, Rand et al. 2018). *Bathyplectes curculionis* (0.5 - 1 cm) are black in colour as adults with yellow colouration on the ventral side of the abdomen (Soroka et al. 2020a). Adults emerge to start parasitising alfalfa weevil larvae after mating by ovipositing a single egg in alfalfa weevil larvae in spring (Chamberlin 1926). Only one generation of the parasitoid has been detected in Alberta (Soroka et al. 2020a). Another introduced parasitoid, *Oomyzus incertus*, believed to be from Europe (Chamberlin 1926), is a tiny black metallic chalcid wasp. A single wasp lays multiple eggs in a single alfalfa weevil larva. Radcliffe and Flanders (1998) observed that *O. incertus* can have 3 - 4 generations in a year, but its voltinism in Alberta is unknown (Soroka et al. 2020a). Generalist predators like coccinellids, and nabids (Shrestha et al. 2021) and the entomopathogen *Entamophthora phytonomi* (Harcourt and Guppy 1991) also reduce population of alfalfa weevil. The parasitoids prefer mostly the early instars over the late instar larvae (Ouayogode and Davis 1981). Population outbreaks of alfalfa weevil were recorded in Ontario in response to dry weather. High humidity after spring rainfall led to conditions favourable for entomopathogens (Harcourt and Guppy 1991). A study by Shrestha et al. (2018) demonstrated the potential of *Bacillus thuringiensis galleriae* strain SDS-502 in managing alfalfa weevil without affecting the parasitism by *Bathyplectes* spp. in Montana.

1.7 Thesis objectives

This study assesses alfalfa weevil abundance and presence of overwintered (May-June) and new generation (July to September) alfalfa weevil in alfalfa fields grown for seed in southern Alberta. I test various monitoring tools to determine where alfalfa weevils are located throughout the season and if they move in and out of alfalfa fields in the adult stage. I also look for the location of overwintering alfalfa weevil adults inside or away from alfalfa crop. Captured alfalfa weevils are separated by sex. The alfalfa weevil abundance for differently aged alfalfa stands were compared. The study also assessed the relationship between captured overwintered adult alfalfa weevils and the subsequent larval population. The attractiveness of putative semiochemicals to alfalfa weevil adults was tested in one season. The abundance of alfalfa weevils is correlated with weather parameters including temperature, relative humidity and precipitation and compared to larval population density. The abundance and diversity of other weevils (*Sitona* species) is assessed in by-catch of sweep net samples.

2. Materials and Methods

2.1 Study sites

Adult alfalfa weevils were monitored at 18 irrigated fields in total in alfalfa grown for seed fields between Brooks and Rosemary, Alberta (50.5657° N, 111.8978° W) over 3 years of study (Table 2-1). Monitoring occurred in five fields in 2021 and 2022, and eight fields in 2023 (Figure 2-1). Ten sites were initially selected in 2023, but two first year fields were subsequently removed due to alfalfa winterkill. An additional five fields were selected for a separate baited pitfall trap experiment to test putatively attractive semiochemicals to alfalfa weevils in 2022. All sites selected were separated by a minimum distance of 500 m. Alfalfa stand age varied from first year to fourth year fields (Table 2-1). In 2023, field age was incorporated into the study design to test for an effect of stand age on weevil abundance.

2.2 Weevil sampling

Various trap types were set up in the field in early spring (April/May) and sample collection started in May of each year (Table 2-2). All samples were collected weekly (2021, 2022) and biweekly (2023) from 0800-1845 h on collection days. Various trap types were deployed (Figure 2-2) to assess abundance and location of alfalfa weevil adults throughout the growing season from May to September of each study year. We divided the growing season into samples conducted on the overwintered (May-June) and new generation (July-September) of alfalfa weevil adults. The overwintered generation of alfalfa weevil adults are darker, have worn scales over time, whereas new generation adult weevils are light brown (Figure 2-3).

2.2.1 Emergence cages

To determine if adult alfalfa weevils overwintered in or adjacent to alfalfa fields, emergence cages were set up, with alfalfa emergence in 2021-2023 (Table 2-2). Open-bottom black emergence cages (60 cm x 60 cm x 60 cm, MegaView Science co. ltd) fitted with a collection bottle (Figure 2-2) were placed on top of vegetation to collect overwintered weevils emerging from the soil. The base of each cage was secured with nails and the sides were covered with soil. In 2021 and 2022, four emergence cages were positioned at each of four sites. Two of the four cages were positioned 50 m apart just outside the field over non-crop vegetation. The other two cages were positioned in the interior of the field, 50 m from the field edge directly over alfalfa plants (Figure 2-4). To increase the intensity of sampling in 2023, 3 - 5 emergence traps were set up at each of ten field sites, in a similar manner to cages in the previous years to compare emergence from locations within and outside the field. Cages were left in the field from 22 April to 14 May 2021; 26 April to 18 May 2022; and 24 April-10 May 2023.

2.2.2 Trapping techniques

A number of trap types were used to capture adult alfalfa weevils to assess the position of weevils in and out of the field throughout the season. In the first year of this study, trapping transects were erected along the edge of each site, 1 - 3 m inside the field on 6 May 2021. Traps along the transects were separated by 25 m and consisted of two double-sided yellow sticky cards (Silvandersson Sweden) to detect direction of movement into and out of the field, two pitfall traps to capture walking insects, and one malaise trap to capture flying weevils (One Townes style malaise trap (MegaView Science co. Ltd)) (Figure 2-2). On 21 May 2021, two additional pitfall traps were positioned in the interior of the field (50-150 m from the edge), adjacent to leafcutter bee shelters at each site to compare capture of weevils at the field edge and interior. Yellow sticky cards (10 x 25 cm) were positioned on either side of wooden stakes ~1 m above the ground. Both malaise traps and yellow sticky cards were removed from the transect on 22 July 2021, they had not captured any alfalfa weevil adults. The trap transects were set up in a similar manner in 2022 (26 April) and 2023 (24 April), but consisted only of two pitfall traps positioned 50 m apart at each the edge and interior of the field (2022) and two directional pitfall traps (2023) positioned at the edge and interior of the field (Figure 2-4).

2.2.3 Pitfall traps

Pitfall traps consisted of a solo cup (473 mL plastic cup) with a liner, holding ~200 mL ethylene glycol-based antifreeze (WinterProofTM Water System Antifreeze). Solo cups were positioned so that the lip of the cup was level with the ground. The traps were protected from rain by white coroplast (15 cm x 15 cm) (EM plastic Hi-Core corrugated polypropylene sheet, Home Depot) anchored to the soil with nails (10-15 cm long) (Figure 2-2). Directional pitfall traps were used in 2023 to assess the direction of movement of alfalfa weevils. Each directional pitfall trap consisted of two 473 mL plastic cups positioned on either side of a black zig-zag coroplast plastic sheet (Figure 2-2). The zig-zag plastic sheet was positioned between the cups in the soil with ~20 cm of the sheet protruding above the soil surface to corral weevils that approach the trap from each direction. Pitfall traps were emptied and antifreeze was replenished at each trap check, weekly in 2021 and 2022 and biweekly in 2023 (Table 2-2). Collected specimens were stored in antifreeze at 4°C until processing. Samples were filtered through cheesecloth and alfalfa weevils and non-target weevils were stored in 70% alcohol.

In 2022, water-filled pan traps (Figure 2-2) were added to the trap transect on 21 July to target migrating new generation weevils that orient to water in other parts of their range (Byrne and Steinhauer 1966). Traps consisted of plastic containers (32 cm x 22 cm x 9 cm) that were painted yellow (Spray Rust Paint Gloss Yellow, Home Depot). Containers were modified with a slit (~12 cm x 1 cm), 6 cm from the base of the container on one side to prevent overflow. Traps were filled with water (~1500 mL) in the field along with a few drops of dish soap to break the water surface tension. The traps were placed on the trap transect on either side of pitfall traps placed at the field border. Collected specimens were sorted and stored as described for the pitfall traps. The trap samples were collected weekly from 13 May - 15 September 2022 (Table 2-2).

2.2.4 Sweep net sampling

In addition to collections of adult alfalfa weevils in the various trap types, we used sweep samples to monitor alfalfa weevil abundance (Harper et al. 1993, Soroka et al. 2020a). At each site in all three study years, sweep samples (Figure 2-2) were taken throughout the season (May-September) on a weekly (2021, 2022) or biweekly basis (2023) (Table 2-2). At each site and collection period, 200, 180° sweeps were conducted with a standard sweep (38 cm diameter) net following an established protocol (Cárcamo and Mori, unpublished data) (Figure 2-5). Sweep samples were initiated (n = 50) at 10 m into the field perpendicular to the field edge. Subsequent sweeps (n = 50) were taken while walking along a 45° angle for 25 m, and then parallel to the field edge (n = 50) for another 25 m. The final 50 sweeps were taken while walking back to the field edge at a 45° angle. Sweep net samples were placed in labelled bags for transport in refrigerated containers to the university for storage at -20°C until processing. Alfalfa weevil adults, other weevil adults and alfalfa weevil larvae were separated from the rest of the material and preserved in 70% ethanol. *Sitona* weevils were later identified (Bright and Bouchard 2008) and alfalfa weevils were separated by sex (Pienkowski et al. 1969) (Figure 2-6).

2.2.5 Soil samples

Soil samples were collected to determine if alfalfa weevil adults inhabit the soil during dormant periods. In 2021, soil samples were collected weekly at each field site to target the new generation weevils from 22 July to 15 September. We used a trowel to collect the top 2.5 cm of soil from five samples at each of four field locations (Figure 2-5). Soil from each site was pooled and stored in a single bag for each collection week (Table 2-3). In 2022, two ~3 L soil samples were collected adjacent to each pitfall trap on the edge and interior of the field using a soil auger (18 cm x 9.5 cm auger, AMS samplers, USA) (Table 2-3). Samples were collected in early spring (26 April and 4 May 2022) to target the overwintered adult weevils and late summer (11, 18, 24 August and 9 September) to target new generation adults (Table 2-2). Soil samples from the field interior and edge were kept separately in labelled plastic bags. In 2023, soil samples were collected using a trowel, as in 2021, but five samples were taken around each of the pitfall traps (Figure 2-4) positioned at the edge and interior of the field in early spring (24 April and 9 May) and late summer (16, 29 August and 9 September) (Table 2-2). As in 2022, soil samples from the field interior were kept separately from edge samples (Table 2-3). Samples from all three years were sieved through 2 mm sieves (U.S.A. Fisherbrand test sieves) to remove alfalfa weevil adults. Alfalfa weevils were collected and stored in 70% alcohol.

2.3 Test of attractiveness of putative semiochemicals

A separate experiment was conducted in 2022 to test the attractiveness of putative semiochemicals to overwintered and new generation alfalfa weevil adults (Hedin et al. 1988, Quinn et al. 1999). Five field sites were selected for the baited pitfall experiment. Pitfall traps constructed from solo cups were positioned in fields on 27 April 2022, 25 m apart along the transect 1 m into the field. The traps consisted of a single solo cup (473 mL plastic cup) with a liner, holding ~200 mL ethylene glycol-based antifreeze (WinterProofTM Water System Antifreeze). Solo cups were positioned so that the lip of the cup was level with the ground. The traps were protected from rain by white coroplast (15 cm x 15 cm) (EM plastic Hi-Core corrugated polypropylene sheet, Home Depot) anchored to soil with nails (10 - 15 cm long). Lures were attached to the coroplast cover using stickpins colour coded by treatment so that the lure hung above the cup opening. Trap treatments consisted of: 1) a grey rubber septum loaded with 10 mg of pea leaf weevil aggregation pheromone (4-methyl-3,5-heptanedione) (Lot 121.841 K3-06.0, ChemTica Internacional, Heredia, Costa Rica); 2) a grey rubber septum loaded with 10 mg of a 1:1 ratio of (*Z*)-3,3-dimethylcyclohexane- δ1, α – acetaldehyde : (*E*)-3,3-dimethylcyclohexane- δ1, α-acetaldehyde (ChemTica Internacional, Heredia, Costa Rica) in 100 µl hexane; and 3) a grey rubber septum loaded with 100 µl of hexane as a control. Traps were baited with new lures on 26 July 2022 to target the new generation weevils. All the treatments were replicated twice in each field. Trap catch was collected weekly from 9 May to 11 September 2022 (Table 2-2).

2.4 Weather parameters

HOBO weather stations (HOBO MX2300 Series Data Logger) measured minimum and maximum temperature at each field site in 2021 and 2022. Weather stations were positioned 2 m above the ground at the field edge in the proximity of the trap transect. In 2021-2023, weather data including the average, minimum and maximum temperature, average relative humidity and precipitation was obtained from Agriculture and Irrigation, Alberta Climate Information Service (ACIS) Brooks station (ACIS 2023). All analyses were done using the ACIS Brooks station weather data as some of the data was missing from the HOBO weather stations.

2.5 Data analyses

Data were analyzed using R v. 4.1.1 2021.08.10 (R Core Development Team 2021). The data document was cleaned using dplyr in R studio (Wickham et al. 2020). Data were separately analysed for each sample collection type for each weevil generation. Collection weeks in each of the three years were converted to Julian weeks for analysis. Generalized mixed models (lmer) with a negative binomial distribution were developed for most data sets with site specified as a random effect. Before analyses, data were assessed visually for normality using q-q plots and statistically using the Shapiro Wilk's test. The homogeneity of variance and over-dispersion of the models were tested in Dharma R package (Hartig 2018). Model simplification was conducted using ANOVA hypothesis testing with a threshold set at $p < 0.05$ until the most parsimonious model remained. Models with the lowest AIC (Akaike Information Criterion) were chosen and were compared with corresponding null models to ensure the explanatory power of the model. A Tukey's post hoc test using 'multcomp' R package was conducted to compare among treatments in significant models (Tukey, 1977). Graphs were constructed using estimated marginal means calculated from the models. Samples that had no alfalfa weevils were not analysed, which included the ineffective trap types initially tested (sticky traps, malaise traps).

2.5.1 Emergence cages

A generalized linear mixed effects model fitted with a Poisson distribution with trap location as the predictor variable and site as a random effect to test for the presence of adult alfalfa weevils inside and outside of the field overwintered in 2022 and 2023. Analysis was not done for the data in 2021 as only one alfalfa weevil was collected from 16 emergence cages.

2.5.2 Trapping techniques

Sticky card, malaise traps and water traps captured only few or no alfalfa weevil adults, and analyses were not conducted for those trap types. Analyses were also not conducted for weevil capture in pitfall traps placed at the inside and at the border of the field as few alfalfa weevil and non-target weevil adults were captured in all the experimental years. In 2023, directionality in the movement of alfalfa weevils was studied using directional pitfall traps, but analyses were not conducted due to low weevil capture.

2.5.3 Sweep net sampling

To assess the capture of alfalfa weevil and non-target weevil adults in sweep samples over time, captured weevils were compared in each year with site as the random intercept and month as the fixed variable. Weevils collected from May, June, July, August and September were compared. The number of samples collected from September were, however less than the rest of the growing season as the crops were harvested by mid- September. The valid model fit is robust enough to handle the unbalanced samples by month. Similar steps were followed for the comparison of non-target weevil adults collected from sweep nets with month as the fixed and site as the random variable in each year of study.

Alfalfa weevil adults from sweep samples were analysed with sex as the predictor and site, Julian week as random effects separately for weevils in both generations. To observe if the capture of overwintered adult weevils predicts the subsequent larval population density, a Kendall’s tau correlation test was conducted (Kendall 1938). Sweep samples from 2021, 2022 and 2023 were used for the Kendall's tau correlation test. To test the effect of alfalfa stand age on alfalfa weevil adult and larval populations in 2023, a generalized mixed model with a negative binomial distribution was used. Stand age was the fixed independent variable and site was included as a random effect. Due to limited sample size, however, especially for the first year stands with only two observations, further studies with increased sample size have to be done.

2.5.4 Soil sampling

Analysis was not conducted for the alfalfa weevils collected from soil samples inside and outside the alfalfa field as few weevils were captured in the study years (2022 and 2023). In 2021, soil samples were collected along the sweep net sample transect so it was not possible to compare samples from within or outside the field.

2.5.5 Test of attractiveness of putative semiochemicals

A generalized linear mixed effects model with a negative binomial distribution tested the effect of putative semiochemicals to attract alfalfa weevil adults and non-target weevil adults with lure type as the independent variables and site as random variable.

2.5.6 Weather parameters

In order to study the effect of weather parameters (average temperature, average relative humidity and precipitation as predictors) on adult alfalfa weevil abundance in sweep samples, a generalized linear mixed effects model with a negative binomial distribution was used with site and week included as random effects.

Table 2-1. Alfalfa fields sampled for adult alfalfa weevils (2021-2023).

Year	Field	Latitude: Longitude	Alfalfa Variety	Alfalfa Stand Age (Years)	Insecticide (Pesticide Group[1])	Application Date
2021	A	50.58299: -111.79903	Unknown	Unknown	Unknown	Unknown
2021	B	50.64344: -111.7647	Vision	3	Cormoran (4,15) Voliam Express (3,28)	16 June 23 August
2021	C	50.714534: -112.06793	Instinct	3	Assail (4)	16 June
2021	D	50.73576: -112.04647	Haygrazer	3	Silencer (3) Coragen (28)	18 June, 16 August 18 June
2021	E	50.7543: -112.1170	Unknown	3	Beleaf (29) Coragen (28) Decis (3) Matador (3)	21 June 21 June 13 July 9 August
2022	A	50.58299, -111.799033	Unknown	Unknown	Unknown	Unknown
2022	B	50.64344, -111.7647	Vision	4	Cygon (1B) Decis (3)	22 June 24 August

Year	Field	Latitude: Longitude	Alfalfa Variety	Alfalfa Stand Age (Years)	Insecticide (Pesticide Group[1])	Application Date
2022	F	50.597452, -111.799043	Instinct	2	Cygon (1B) Voliam Express (3,28) Decis (3)	21 June 29 June 26 August
2022	G	50.58742, -111.82661	Unknown	Unknown	Unknown	Unknown
2022	H	50.724923, -112.093523	Hybriforce 4400	4	Coragen (28) Matador (3) Decis (3)	27 June 27 June 12 August
2023	I	50.631978, -111.761835	Safeguard	2	Unknown	Unknown
2023	J	50.480996, -111.77451	Instinct	3	Silencer (3)	8 May 15 July
2023	K	50.724923, -112.093523	FG52M150	2	Assail (4) Coragen (28) Decis (3)	5 June 12 June 12 June
2023	L	50.72554, -112.10249	Instinct	2	Assail (4) Coragen (28) Decis (3)	5 June 12 June 12 June
2023	M	50.742997, -112.018607	Planet	3	Matador (3) Assail (4)	12 June 21 June

Year	Field	Latitude: Longitude	Alfalfa Variety	Alfalfa Stand Age (Years)	Insecticide (Pesticide Group[1])	Application Date
2023	N	50.761685, -112.105502	Dakota (North star)	3	Matador (3) Assail (4)	12 June 21 June
2023	O	50.773730, -112.116911	Unknown	1	Matador (3) Coragen (28) Decis (3)	13 June 23 June 10 August
2023	P	50.809096, -112.104596	Rebound AA	1	Silencer (3) Decis (3)	11 June, 26 June 12 August

[1]Pesticide Groups in: *The Blue Book: Alberta's Crop Protection Guide*

Table 2-2. Dates of assessment using various sampling techniques for adult alfalfa weevils assessed in alfalfa fields (2021-2023).

Year	Field	Sampling Type	Collection Dates Overwintered generation weevils	Collection Dates New generation weevils
2021	A	Emergence cage	14 May	Not sampled
		Sticky trap	14, 21, 28, May; 3, 10 June	Not sampled
		Malaise trap	14, 21, 28, May; 3, 10 June	Not sampled
		Pitfall trap	14, 21, 28, May; 3, 10, 18, 25 June	9, 16, 23, 30 July; 6, 12, 19, 27 August; 7, 15 September
		Sweep net samples	28 May; 3, 10, 18, 25 June	9, 16, 23, 30 July; 6, 12, 19, 27 August; 7, 15 September
		Soil samples	Not sampled	23, 30 July; 6, 12, 19, 27 August; 7, 15 September
2021	B	Emergence cage	14 May	Not sampled
		Sticky trap	14, 21, 28, May; 3, 10 June	Not sampled
		Malaise trap	14, 21, 28, May; 3, 10 June	Not sampled
		Pitfall trap	14, 21, 28, May; 3, 10, 18, 25 June	9, 16, 23, 30 July; 6, 12, 19, 27 August; 7, 15 September
		Sweep net samples	28 May; 3, 10, 18, 25 June	9, 16, 23, 30 July; 6, 12, 19, 27 August; 7 September
		Soil samples	Not sampled	23, 30 July; 6, 12, 19, 27 August; 7, 15 September
2021	C	Sticky trap	13, 20, 27 May; 4, 11 June	Not sampled
		Malaise trap	13, 20, 27 May; 4, 11 June	Not sampled
		Pitfall trap	13, 20, 27 May; 4, 11, 17, 24 June	8, 15, 22, 29 July; 5, 13, 20, 26 August; 8, 14 September
		Sweep net samples	27 May; 4, 11, 17, 24 June	8, 15, 22, 29 July; 5, 13, 20, 26 August; 14 September
		Soil samples	Not sampled	

Year	Field	Sampling Type	Collection Dates Overwintered generation weevils	Collection Dates New generation weevils
				22, 29 July; 5, 13, 20, 26 August; 8, 14 September
2021	D	Emergence cage	14 May	Not sampled
		Sticky trap	13, 20, 27 May; 4, 11 June	Not sampled
		Malaise trap	13, 20, 27 May; 4, 11 June	Not sampled
		Pitfall trap	13, 20, 27 May; 4, 11, 17, 24 June	8, 15, 22, 29 July; 5, 13, 20, 26 August; 14 September
		Sweep net samples	27 May; 4, 11, 17, 24 June	8, 15, 22, 29 July; 5, 13, 20, 26 August; 14 September
		Soil samples	Not sampled	22, 29 July; 5, 13, 20, 26 August; 14 September
2021	E	Emergence cage	14 May	Not sampled
		Sticky trap	13, 20, 27 May; 4, 11 June	Not sampled
		Malaise trap	13, 20, 27 May; 4, 11 June	Not sampled
		Pitfall trap	13, 20, 27 May; 4, 11, 17, 24 June	8, 15, 22, 29 July; 5, 13, 20, 26 August; 14 September
		Sweep net samples	27 May; 4, 11, 17, 24 June	8, 15, 22, 29 July; 5, 13, 20, 26 August; 14 September
		Soil samples	Not sampled	22, 29 July; 5, 13, 20, 26 August; 14 September
2022	A	Emergence cage	18 May	Not sampled
		Pitfall trap	4, 12, 18, 25 May; 2, 8, 23, 30 June	13, 20, 27 July; 3, 11, 18 August; 9 September
		Sweep net samples	25 May; 2, 8, 30 June	13, 20, 28 July; 3, 11, 18, 25 August
		Soil samples	26 April; 4 May	11, 18, 24 August; 9 September
2022	B	Emergence cage	18 May	Not sampled
		Water trap	Not sampled	3, 11,18, 24 August; 9 September
		Pitfall trap	4, 12, 18, 25 May; 2, 8, 23, 30 June	13, 20, 27 July; 3, 11, 18, 24 August; 9 September
		Sweep net samples	18, 25 May; 2, 8, 30 June	13, 20, 28 July; 3, 11, 18, 25 August; 9 September

Year	Field	Sampling Type	Collection Dates Overwintered generation weevils	Collection Dates New generation weevils
		Soil samples	26 April; 4 May	11, 18, 24 August; 9 September
2022	F	Emergence cage	18 May	Not sampled
		Water trap	Not sampled	24 July; 3, 11,18, 24 August; 9 September
		Pitfall trap	4, 12, 18, 25 May; 2, 8, 23, 30 June	13, 20, 27 July; 3, 11, 18, 24 August; 9 September
		Sweep net samples	25 May; 2, 8, 30 June	13, 20, 28 July; 3, 11, 18, 25 August; 9 September
		Soil samples	26 April; 4 May	11, 18, 24 August; 9 September
2022	G	Water trap	Not sampled	24 July; 3, 11,18, 24 August; 9 September
		Pitfall trap	4, 12, 18, 25 May; 2, 8, 22, 30 June	13, 20, 27 July; 3, 11, 18, 25 August; 9 September
		Sweep net samples	25 May; 2, 8, 22, 30 June	13, 20, 28 July; 3, 11, 18, 25 August; 9 September
		Soil samples	4 May	11, 18, 25 August; 9 September
2022	H	Emergence cage	19 May	Not sampled
		Water trap	Not sampled	24 July; 4, 11,17, 25 August; 8 September
		Pitfall trap	5, 12, 19, 26 May; 2, 9, 23, 29 June	14, 21, 27 July; 4, 18, 25 August; 8 September
		Sweep net samples	26 May; 2, 9, 29 June	14, 21, 27 July; 4, 11, 18, 25 August; 9 September
		Soil samples	27 April; 5 May	11, 18, 25 August; 8 September
2022	1	Baited pitfall	12, 19 May; 2, 9, 23, 29 June	14, 21, 27 July; 4, 18, 25 August; 8 September
2022	2	Baited pitfall	12, 19 May; 2, 9, 23, 29 June	14, 21, 27 July; 4, 11, 18, 25 August; 9 September
2022	3	Baited pitfall	12, 19 May; 2, 9, 22, 29 June	14, 21, 27 July; 4, 11, 18, 25 August; 9 September
2022	4	Baited pitfall	12, 19 May; 2, 9, 22, 29 June	14, 21, 27 July; 4, 11, 18, 25 August; 9 September

Year	Field	Sampling Type	Collection Dates Overwintered generation weevils	Collection Dates New generation weevils
2022	5	Baited pitfall	12, 19 May; 2, 9, 22, 29 June	14, 21, 27 July; 4, 11, 18, 25 August; 9 September
2023	I	Emergence cage	10 May	Not sampled
		Pitfall trap	10, 24 May; 7, 21 June	5, 19 July; 2, 17, 29 August; 9 September
		Sweep net samples	10 May; 6, 21 June	5, 19 July; 2, 17, 29 August; 9 September
		Soil samples	26 April; 4 May	11, 18, 24 August; 9 September
2023	J	Emergence cage	10 May	Not sampled
		Pitfall trap	10, 24 May; 7, 21 June	5, 19 July; 2, 17, 29 August; 9 September
		Sweep net samples	10, 24 May; 21 June	5, 19 July; 2, 17, 29 August
		Soil samples	26 April; 4 May	11, 18, 24 August; 9 September
2023	K	Emergence cage	10 May	Not sampled
		Pitfall trap	9, 23 May; 6, 20 June	4, 18 July; 2, 16, 29 August; 9 September
		Sweep net samples	9, 24 May; 6, 20 June	4, 18 July; 2, 16, 29 August, 9 September
		Soil samples	26 April; 4 May	11, 18, 24 August; 9 September
2023	L	Emergence cage	9 May	Not sampled
		Pitfall trap	9, 23 May; 6, 20 June	4, 18 July; 2, 16, 29 August; 9 September
		Sweep net samples	9, 23 May; 6, 20 June	4, 18 July; 2, 16, 29 August; 9 September
		Soil samples	4 May	11, 18, 25 August; 9 September

Year	Field	Sampling Type	Collection Dates Overwintered generation weevils	Collection Dates New generation weevils
2023	M	Emergence cage	9 May	Not sampled
		Pitfall trap	9, 24 May; 6, 20 June	4, 18 July; 2, 16, 29 August
		Sweep net samples	9, 24 May; 6, 20 June	4, 18 July; 2, 16, 29 August
		Soil samples	27 April; 5 May	11, 18, 25 August; 8 September
2023	N	Emergence cage	9 May	Not sampled
		Pitfall trap	9, 23 May; 6, 20 June	4, 18 July; 2, 16, 29 August; 9 September
		Sweep net samples	9, 23 May; 6, 20 June	4, 18 July; 2, 16, 29 August; 9 September
		Soil samples	27 April; 5 May	11, 18, 25 August; 8 September
2023	O	Emergence cage	9 May	Not sampled
		Pitfall trap	9, 23 May; 6, 20 June	4, 18 July; 2, 16, 29 August; 9 September
		Sweep net samples	9, 23 May; 6, 20 June	4, 18 July; 2, 16, 29 August; 9 September
		Soil samples	27 April; 5 May	11, 18, 25 August; 8 September
2023	P	Emergence cage	9 May	Not sampled
		Pitfall trap	9, 23 May; 6, 20 June	4, 18 July; 2, 16, 29 August; 9 September
		Sweep net samples	9, 23 May; 6, 20 June	4, 18 July; 2, 16, 29 August; 9 September
		Soil samples	27 April; 5 May	11, 18, 25 August; 8 September

Table 2-3. Details on soil samples collected from each experimental site (2021-2023).

Year	Field	Weevil generation	Volume of collected sample (L)	
			Border	Interior
2021	A	New	Not measured	Not measured
	B	New	Not measured	Not measured
	C	New	Not measured	Not measured
	D	New	Not measured	Not measured
	E	New	Not measured	Not measured
2022	A	Overwintered	6.4	5.75
		New	2.417	2.675
	B	Overwintered	6.25	6.5
		New	2.775	3.04
	F	Overwintered	6	6.15
		New	3.068	2.83
	G	Overwintered	3.2	3.9
		New	8.3	2.787
	H	Overwintered	5.65	5.3
		New	3.042	3.08
2023	I	Overwintered	2.35	2.5
		New	1.92	1.4
	J	Overwintered	2.05	2.4
		New	2	2
	K	Overwintered	2.55	2.75
		New	2.33	2.25
	L	Overwintered	2.55	2.7
		New	2	2.25
	M	Overwintered	1.95	3.3

Year	Field	Weevil generation	Volume of collected sample (L)	
			Border	Interior
		New	2	1.125
	N	Overwintered	3	3.1
		New	1.48	2.08
	O	Spring	2.2	2.05
		New	1.53	1.65
	P	Spring	2.85	3.3
		New	1.75	2

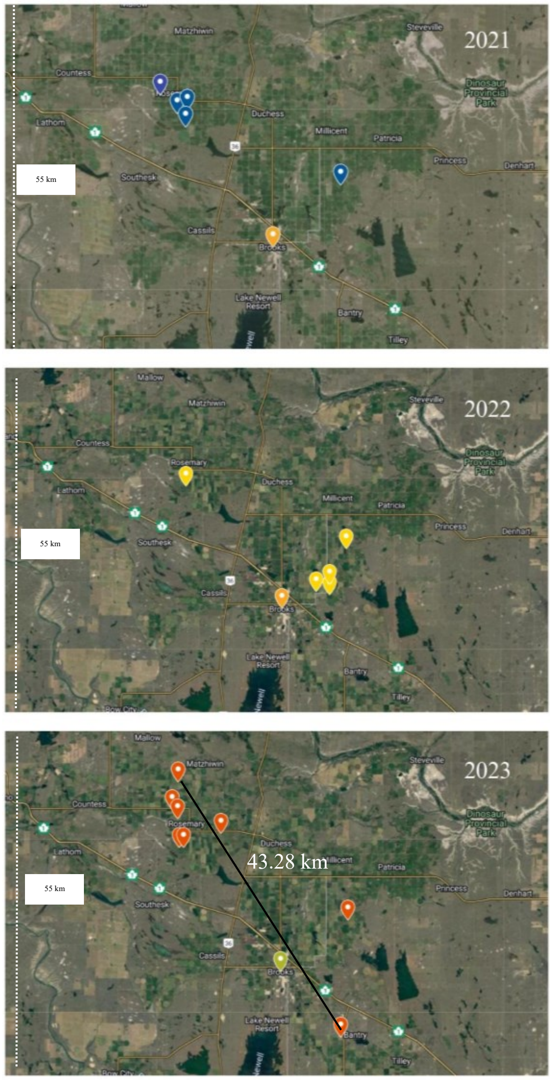

Figure 2-1. Maps depicting location of experimental sites in 2021-2023. Sites consisted of commercial alfalfa fields grown for seed located between Brooks and Rosemary, AB. Site locations are indicated by blue, yellow and red markers in 2021, 2022, and 2023, respectively. Created using Google Maps. Brooks is labelled using orange (2021, 2022) and green (2023) colour pins.

Figure 2-2. Collection methods used for capturing alfalfa weevil (2021 - 2023): A) Emergence cages; B) Solo pitfall traps (2021, 2022); C) Directional pitfall traps (2023); D) Sweep net samples; E) Soil samples; F) Malaise traps (2021); G) Sticky traps (2021); and H) Water traps (2022). Malaise and sticky traps were discontinued in 2021 due to lack of capture.

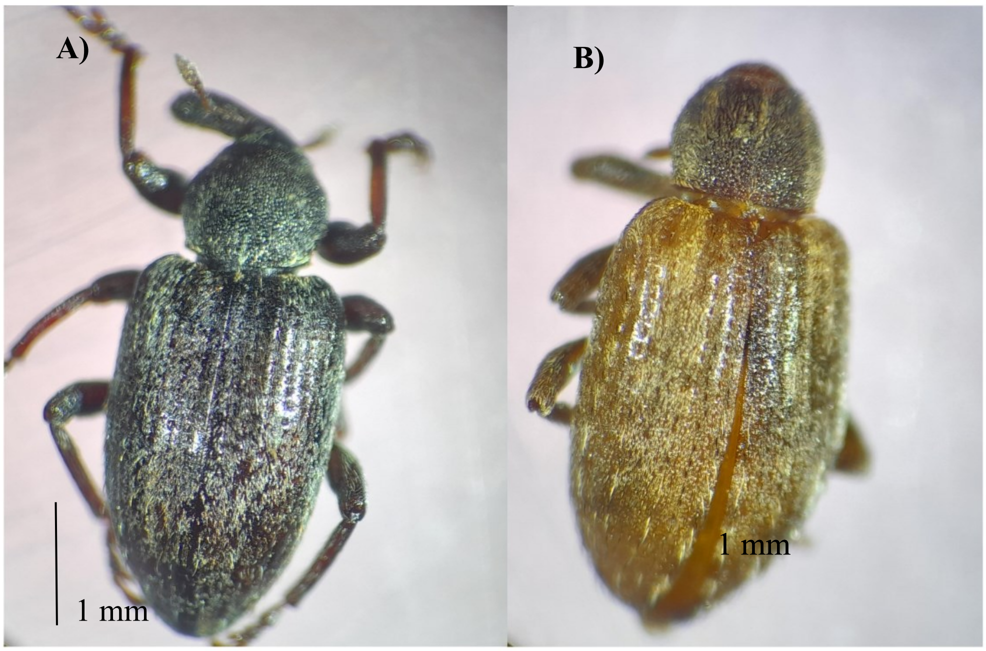

Figure 2-3. Overwintered (A) and new generation (B) alfalfa weevil adults. The old overwintered alfalfa weevil adults have dark colouration and scale wear. The new generation alfalfa weevil adults are brownish colour and get darker over time.

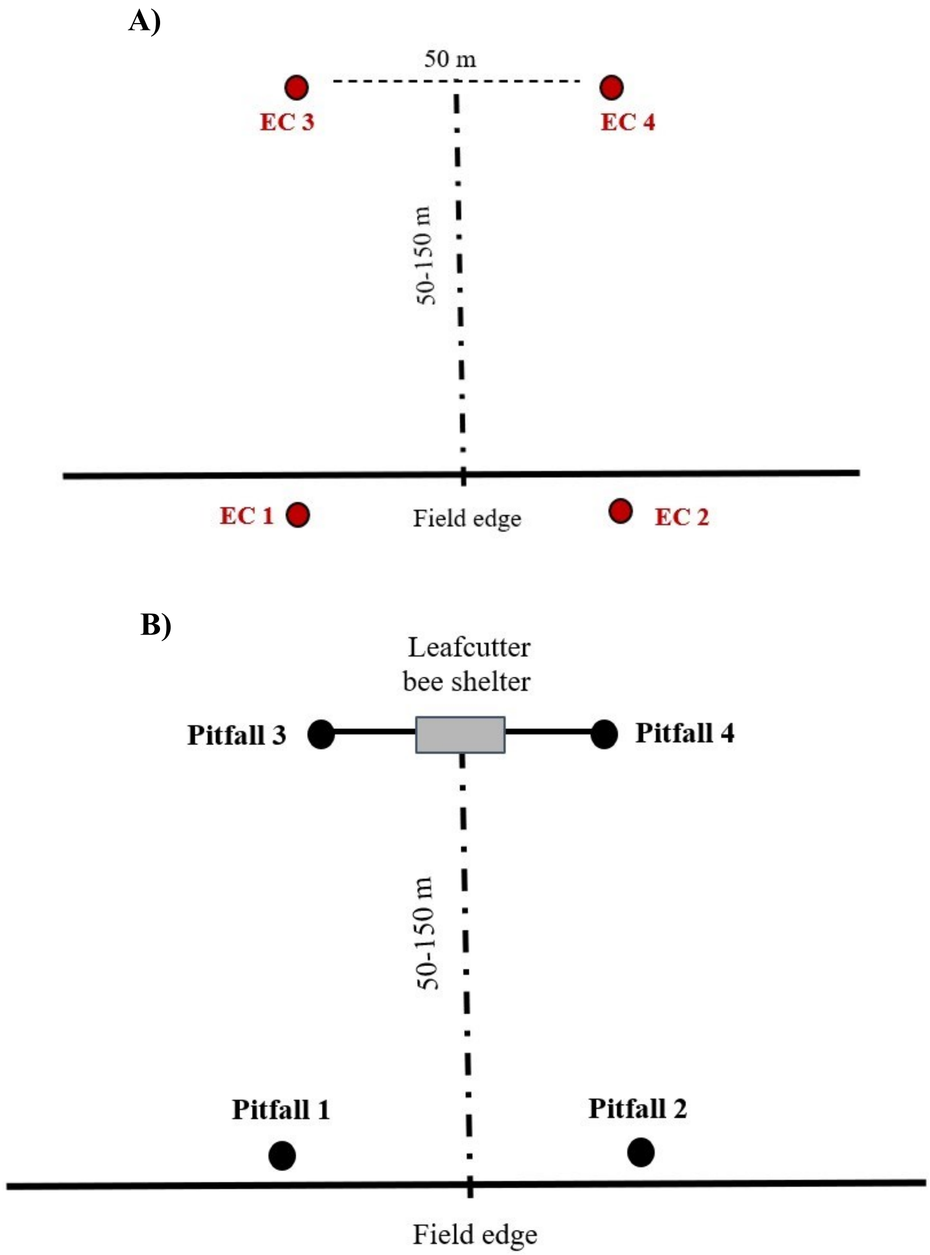

Figure 2-4. Location of (A) emergence cages and (B) pitfall traps in alfalfa seed fields (2021 - 2023). Position of emergence cages is denoted by red circles and pitfall traps by black circles. Solo pitfall traps were used in 2021 and 2022. In 2023, directional pitfall traps were used that consisted of two plastic cups separated by a black plastic barrier in zig zag manner to distinguish capture of weevils moving from both directions.

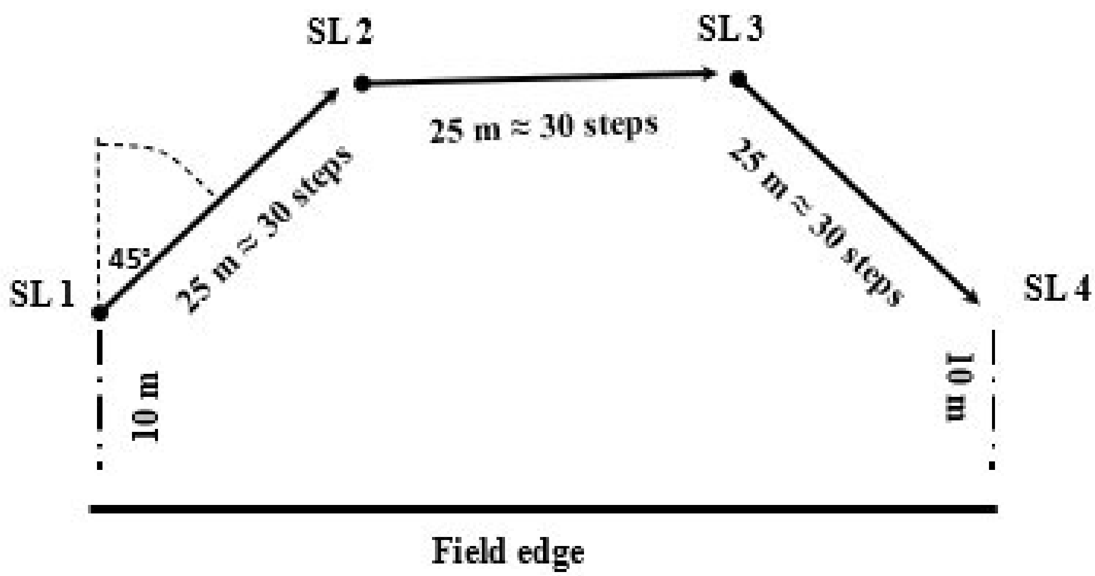

Figure 2-5. Collection transect for sweep net samples. Sweep net samples were collected using a standard sweep net from four locations in each field: SL1, SL2, SL3 and SL4. Fifty sweeps were conducted at each location for 200 sweeps per alfalfa field. Soil samples in the first year (2021) were collected from each sweep location (SL) with a total of 20 samples from a site.

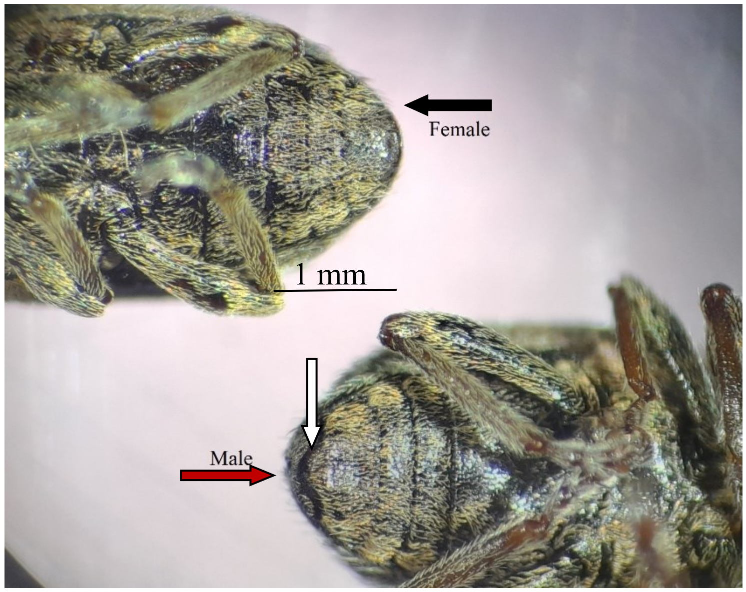

Figure 2-6. Ventral side of adult alfalfa weevil male and female under a dissecting microscope (6X). The last abdominal tergite of the female (top) has a smooth rounded abdominal sternite (black arrow). The male (bottom) has a caudal projection (white arrow) and the abdominal tergite extends downward covering the abdomen (red arrow).

3. Results

3.1 Trapping techniques

I evaluated a variety of capture methods to monitor adult alfalfa weevils in alfalfa grown for seed fields in southern Alberta over three field seasons. In the process of method evaluation, non-target arthropods were captured and non-target *Sitona* weevils were also identified and enumerated and weevil specimens captured in sweep net samples were separated by sex.

3.1.1 Emergence cages

To assess the location of overwintering adult alfalfa weevils, emergence cages were positioned inside and outside of alfalfa fields in the early spring of 2021-2023. In 2021, emergence cages placed inside and outside the field captured only one weevil in the 16 emergence cages placed in 4 fields. In 2022, a total of 42 alfalfa weevil adults emerged into 16 cages positioned in 4 fields. In 2022, more weevil adults were captured in emergence cages placed outside the field 4.38 ± 2.61 (SE) over non-alfalfa vegetation than in cages positioned in the field interior directly over alfalfa plants 0.88 ± 0.64 (SE) ($\chi^2 = 15.662$, df = 1, $p < 0.001$). A similar pattern was observed in 2023 when 42 cages were positioned at 10 sites. In 2023, more adult weevils emerged into cages positioned outside the field 3.52 ± 1.66 (SE) than into those in the field interior 0.76 ± 0.28 (SE) ($\chi^2 = 30.857$, df = 1, $p < 0.001$) (Figure 3-1). These findings suggest that alfalfa weevil adults overwinter both within and outside alfalfa fields grown for seed, but may prefer to overwinter away from alfalfa fields.

3.1.2 Pitfall traps

Pitfall traps captured few adult alfalfa weevils (average of < 1 adult weevil per site) and trap capture could not be analyzed statistically. Throughout the 2021 field season (n = 5 sites), averages of only 0.18 ± 0.47 SE and 0.15 ± 0.04 SE adult alfalfa weevils were collected from traps placed inside (144 samples) and at the edge (164 samples) of the field, respectively. In 2022 (n = 5 sites), slightly more adult alfalfa weevils were captured in pitfall traps positioned in the field interior (0.38 ± 0.08 SE) than in those at the field edge (0.29 ± 0.05 SE) from five sites. In 2023 (n = 8 sites), the sampling effort using pitfall traps was increased with directional pitfall traps (2 cups per trap). Despite this increased sample effort, directional pitfall traps captured few weevils in the interior (0.28 ± 0.05 SE) or edge of the field (0.25 ± 0.07 SE) (Figure 3-2). The traps placed at the edge of the field were compared for directionality of movement (Figure 3-3). There was a slight trend for more inward movement (0.19 ± 0.06 SE) of overwintered alfalfa weevil adults than those moving outward (0.08 ± 0.03 SE) in 2023 (Figure 3-3). Directional pitfall traps were not more effective than normal pitfall traps in capturing alfalfa weevil adults. As the number of adult alfalfa weevils captured in pitfall traps was low, pitfall traps are not recommended for adult weevil monitoring.

Pitfall traps captured slightly more non-target weevil adults than alfalfa weevils. Trap position in the field affected the number of non-target weevils captured in pitfall traps. Pitfall traps located at the edge of the field captured more non-target weevils with an average of 0.38 ± 0.06 (SE) (n = 5 fields, 2021), 1.28 ± 0.31 (SE) (n = 5 fields, 2022) and 0.44 ± 0.27 (SE) (n = 8 fields, 2023). Interior traps captured only 0.09 ± 0.03 (SE) (n = 5 fields, 2021), 0.39 ± 0.09 (SE) (n = 5 fields, 2022) and 0.08 ± 0.02 (SE) (n = 8 fields, 2023) weevils (Figure 3-4). Although the pitfall traps captured non-target weevils, these traps are not recommended for monitoring weevil species in alfalfa in southern Alberta.

3.1.3 Sweep net sampling

Sweep net samples were collected at weekly intervals from five sites in 2021 and 2022 and from eight sites at biweekly intervals in 2023 (Table 2-2). In 2021 (n = 5 fields), significantly more alfalfa weevil adults were collected from June (7.00 ± 1.61 SE), July (18.85 ± 4.07 SE), and August (1.063 ± 3.08 SE), compared to May (0.60 ± 0.40 SE) and September (1.50 ± 0.73 SE) (χ^2 = 42.735, df = 4, p <0.001). On the other hand, in 2022 (n = 5 fields), significantly more alfalfa weevil adults were collected in May (11.67 ± 7.04 SE), June (82.13 ± 46.26 SE), July (53.20 ± 22.17 SE), August (12.50 ± 3.25 SE) and compared to September (0.75 ± 0.75 SE) (χ^2 = 30.13, df = 4, p <0.001). A similar trend was observed in 2023 (n = 8 fields) with more capture of alfalfa weevils in July (101.63 ± 31.67 SE), followed by June (37.13 ± 12.16 SE), August (24.13 ± 9.76 SE), May (19.63 ± 7.38 SE), and September (1.67 ± 0.76 SE) (χ^2 = 41.777, df = 4, p <0.001) (Figure 3-5).

Figure 3-6 shows non-target weevil species (adults) collected as by-catch of sweep samples. In 2021, a greater number of non-target weevils were collected with an average of 41.68 ± 11.12 SE in August followed by 19.65 ± 10.57 SE in July, 14.85 ± 4.52 SE in June, 4.25 ± 1.99 SE in September and 1.00 ± 0.63 SE in May (χ^2 = 21.545, df = 4, p <0.001). In 2022 (χ^2 = 16.152, df = 4, p = 0.003), the number of non-target weevils collected was 37 ± 20.13 SE (May), 102 ± 50.56 SE (June), 43.40 ± 30.02 SE (July), 31.95 ± 8.49 SE (August) and 2.25 ± 1.11 SE (September). On the other hand, an equal number of non-target weevils (χ^2 = 3.66, df = 4, p = 0.453) was collected from May (48 ± 19.76 SE), June (23.87 ± 8.66 SE), July (19.56 ± 13.74 SE), August (32.75 ± 15.29 SE) and September (4.33 ± 2.36 SE) in 2023.

Alfalfa weevil adults captured in sweep net samples were separated by sex. In samples of the overwintered generation in 2021, significantly (χ^2 = 4.326, df = 1, p = 0.038) more females were captured than males. A similar proportion of overwintered males and females were captured, however, in 2022 (χ^2 = 3.224, df = 1, p = 0.073) and 2023 (χ2 = 0.456, df = 1, p = 0.499) (Figure 3-7). Equal numbers of new generation male and female weevils were sampled in sweep nets in 2021 (χ^2 = 1.633, df = 1, p = 0.201), 2022 (χ^2 = 0.018, df = 1, p = 0.894) and 2023 (χ2 = 0.039, df = 1, p = 0.844) (Figure 3-7).

Across the three years of study, there was a significant relationship between the number of alfalfa weevils captured in sweep net samples in the early season and subsequent larval populations at the same sites (T = 131, df = 16, p < 0.001) (Figure 3-8). Sweep net sampling was the most effective sampling tool tested, as it captured both adult and larval alfalfa weevils and sampled more target insects than any other sample technique studied.

I assessed the effect of alfalfa stand age on adult alfalfa weevils captured in sweep net samples in 2023 when variably aged sites were monitored. Sites included 2 first year fields (young), 3 second year fields (intermediate) and 3 third year fields (mature). The total season-long number of adult alfalfa weevils captured in sweep net samples did not vary with the age of the alfalfa stand (χ^2 = 4.591, df = 2, p = 0.101). The number of weevils captured, however, increased with stand age. The estimated marginal means derived from the generalized linear mixed effects model with a negative binomial error distribution for season-long capture was 1.44 ± 0.91 SE, 2.68 ± 0.76 SE and 3.96 ± 0.75 SE adult alfalfa weevils for young, intermediate and mature stands, respectively. Analysis of overwintered adults captured from differently aged alfalfa stands showed a significant difference ($\chi^2 = 14.675$, df = 2, p <0.001) with the lowest alfalfa weevil capture in young alfalfa stands (estimated means of 1.63 ± 0.45 SE) compared to the intermediate (3.38 ± 0.39 SE) and the mature (3.82 ± 0.39 SE) alfalfa stands (Figure 3-9). The number of alfalfa weevil larvae captured in sweep net samples did not differ with alfalfa stand age ($\chi^2 = 2.397$, df = 2, p = 0.302). There was a trend, however, for relatively more alfalfa weevil larvae in mature stands (3.05 ± 1.75 SE) when compared to young (1.30 ± 1.84 SE) and intermediate (1.39 ± 1.75 SE) stands (Figure 3-9).

Sitona weevil adults were collected as by-catch in sweep net samples at similar levels in 2021 ($\chi^2 = 111.44$, df = 2, p <0.001), 2022 ($\chi^2 =78.15$, df = 2, p <0.001) and 2023 ($\chi^2 = 125.17$, df = 2, p <0.001) (Figure 3-10). A total of 6200 weevils over three years was counted from the by-catch of sweep samples of which 5916 weevils were identified as alfalfa curculio (*Sitona lineellus* Bonsdorff), 94 weevils were pea leaf weevil (*Sitona lineatus* L.) and the remaining were unidentified non-*Sitona* weevils (other weevils) (Figure 3-10). Alfalfa curculio adults were present throughout the alfalfa growing season. Sweep net samples of alfalfa curculio adults were almost equivalent to the total alfalfa weevil count (6255) in the same samples, indicating that sweep net sampling is an effective tool to sample both species in alfalfa grown for seed in Alberta.

3.1.4 Soil samples

Soil samples were collected in early spring (2022, 2023) and fall (2021, 2022, 2023) to determine if weevils overwintered in the soil within or outside of the field. Although alfalfa weevil adults were retrieved in all years and in both seasons, too few were captured to conduct statistical analyses. Most weevils (3.5 ± 5.53 SE per five sites) were retrieved *via* soil sampling in fall 2021, which could be because of the larger volume of soil sampled that year using a shovel (Table 3-1). In 2022, few weevils were captured in soil samples in the spring, 0.22 ± 0.13 (SE) from five fields. There was a trend for more weevil capture in soil samples taken in the interior of the field than at the edge in spring 2022. Fall soil sampling in 2022 also rendered low capture of adult alfalfa weevils 0.17 ± 0.05 (SE) from five fields, with a slight trend to more capture in the field interior (Figure 3-11). Few adult weevils were retrieved in soil samples taken in the spring (0.025 ± 0.03 SE, from 8 sites) or fall (0.16 ± 0.07 SE, from 8 sites) of 2023. In

2021, the samples were collected adjacent to where sweep locations were collected (Figure 2-5) and then pooled together. In 2022 and 2023, soil samples collected inside and at the edge of fields were similarly low at both locations (Figure 3-11). Soil sampling revealed the presence of alfalfa weevils in the soil going into and coming out of overwintering, but the number of weevils retrieved was insufficient to definitively determine overwintering location or seasonal movements.

3.2 Test of attractiveness of putative semiochemicals

The pitfall traps baited with pea leaf weevil aggregation pheromone, boll weevil pheromone and a hexane (control) were variably attractive to alfalfa weevils in the experiment conducted in 2022. Numerically more alfalfa weevil adults were captured in pitfall traps baited with the pea leaf weevil pheromone and boll weevil pheromone than the control traps (Figure 3-12), but there was no significant difference in the alfalfa weevil capture across trap type (χ^2 = 4.384, df = 2, p = 0.112). Pitfall traps baited with pea leaf weevil pheromone captured 1.089 ± 0.16 SE alfalfa weevil adults whereas 0.909 ± 0.27 SE and 0.743 ± 0.15 SE alfalfa weevils were captured from traps baited with boll weevil pheromone and unbaited traps respectively.

The results for non-target weevils were parallel to the results of alfalfa weevils with no significant effect of semiochemical baits on non-target weevil capture (χ^2 = 4.270, df = 2, p = 0.118). There were 0.911 ± 0.28 SE non-target weevils captured in traps baited with pea leaf weevil pheromone, 0.618 ± 0.14 SE from boll weevil pheromone baited traps and 0.514 ± 0.15 SE weevils captured in the control traps (Figure 3-12). As the result is only from a single year, more studies are required to confirm the results.

3.3 Unsuccessful capture methods for adult alfalfa weevils

Several of the capture methods assessed in this study caught zero or few adult alfalfa weevils, and were therefore assessed for only one season. The double-sided yellow sticky traps tested in 2021 failed to capture any alfalfa weevils either entering or leaving the field. Only eight non-target weevils were captured on the sticky traps. Sticky traps were removed from the field by 22 July 2021 and were not further assessed in the study. The malaise traps were not effective in capturing adult alfalfa weevils. Although no alfalfa weevils were captured in malaise traps, 87 non-target weevils were recovered. Malaise trap capture was assessed in the 2021 season only.

As previous work has shown that new generation weevils orient to water sources (Byrne and Steinhauer 1966), I assessed adult alfalfa weevil capture in water traps placed at the field border in fall 2022. Few adults were captured in water traps in July 1 ± 0.32 SE (5 samples), August 0.268 ± 0.08 SE (56 samples) or September 0.25 ± 0.25 SE (4 samples), so no statistical analysis was conducted (Figure 3-13). Water traps also captured few non-target weevils, as only an average of 0.4 ± 0.24 SE (July), 0.43 ±0.23 SE (August), and 1.25 ± 1.25 SE (September) were captured (Figure 3-13). Water traps were considered ineffective at attracting and capturing adult alfalfa weevils and were not tested in 2023.

3.4 Weather parameters

The effect of weather (weekly average temperature, relative humidity and precipitation) on the abundance of alfalfa weevil adults in sweep samples was assessed in all three study years. Adult alfalfa weevil capture in sweep net samples was affected by weekly average temperature and relative humidity. Weekly average temperature affected adult alfalfa weevil counts positively in 2021 ($\chi^2 = 11.959$, df = 1, $p < 0.001$), whereas a negative relationship was observed in 2022 ($\chi^2 = 5.099$, df = 1, $p = 0.024$) (Figure 3-14, Figure 3-15). There was also a significant positive relationship between alfalfa weevil adult capture in sweep net samples and average weekly relative humidity in 2021 ($\chi^2 = 7.841$, df = 1, $p = 0.005$) and 2022 ($\chi^2 = 14.157$, df = 1, $p < 0.001$) (Figure 3-15). On the other hand, weekly average precipitation had no effect on the adult weevil abundance in the three years of experiment (Table 3-1). Average weekly temperature ($\chi^2 = 0.108$, df = 1, $p = 0.742$) and relative humidity ($\chi^2 = 2.963$, df = 1, $p = 0.085$) did not have any effect on alfalfa weevil capture in 2023.

Table 3-1. Number of alfalfa weevil adults captured from soil samples (2021-2023).

Year	Field	Season	Number of sample period	Average number of alfalfa weevils captured	
				Border	Interior
2021	A	Fall	8	-	2.125
	B	Fall	7	-	0.571
	C	Fall	8	-	6.5
	D	Fall	6	-	8.33
	E	Fall	7	-	0.429
2022	A	Spring	2	0	0.5
		Fall	3	0	1.667
	B	Spring	2	0	0
		Fall	4	0	0
	F	Spring	2	0	0
		Fall	4	0	0.25
	G	Spring	1	0	0
		Fall	4	0.5	0
	H	Spring	2	0.5	1
		Fall	3	1	0.33
2023	I	Spring	2	0	0
		Fall	3	0	0
	J	Spring	2	0	0
		Fall	2	0	0
	K	Spring	2	0	0
		Fall	3	0	0
	L	Spring	2	0	0
		Fall	3	0.667	0.33
	M	Spring	2	0	0
		Fall	2	0	0.5

Year	**Field**	**Season**	**Number of sample period**	**Average number of alfalfa weevils captured**	
				Border	Interior
	N	Spring	2	0.5	0
		Fall	3	0	0.667
	O	Spring	2	0	0
		Fall	3	0.33	0
	P	Spring	2	0	0
		Fall	3	0	0

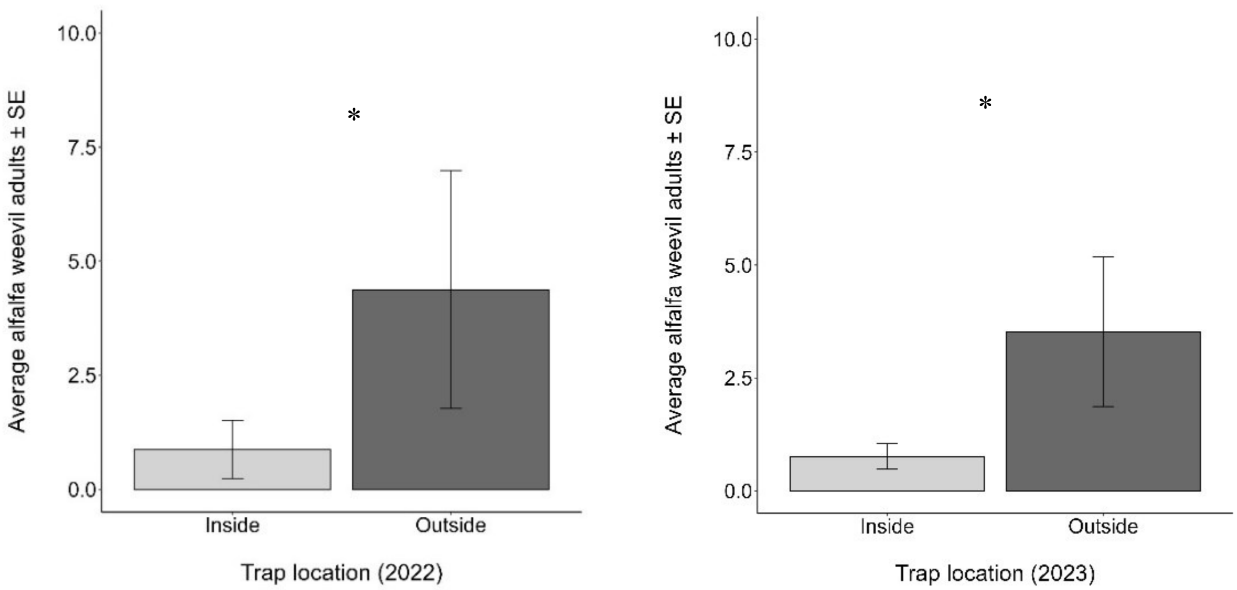

Figure 3-1. Average number of alfalfa weevil adults collected from emergence cages placed in alfalfa fields grown for seed in southern Alberta (2022-2023). Only one alfalfa weevil adult was collected from 16 emergence cages in 2021 and hence not analysed. The alfalfa weevils collected from emergence cages placed outside as well as inside the field were collected and compared in the early spring to capture emergence of overwintered weevils from the soil. Error bars denote standard error. The asterisk (*) sign above the graph denotes the significant differences ($p < 0.05$).

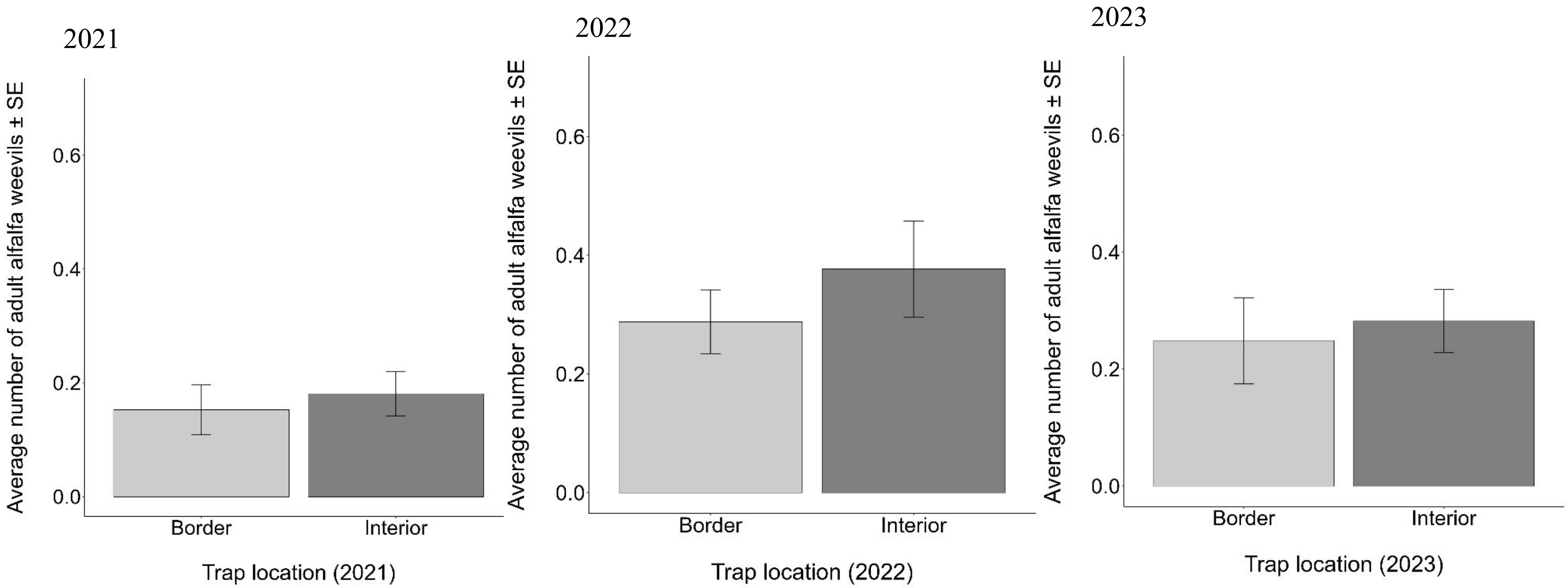

Figure 3-2. Average number of alfalfa weevil adults collected from pitfall traps in alfalfa fields grown for seed from southern Alberta (2021-2023). The samples were collected from five fields each in 2021 and 2022 and eight fields in 2023. The alfalfa weevils collected from pitfall traps placed inside and at the border of the field were compared. Error bars denote standard error.

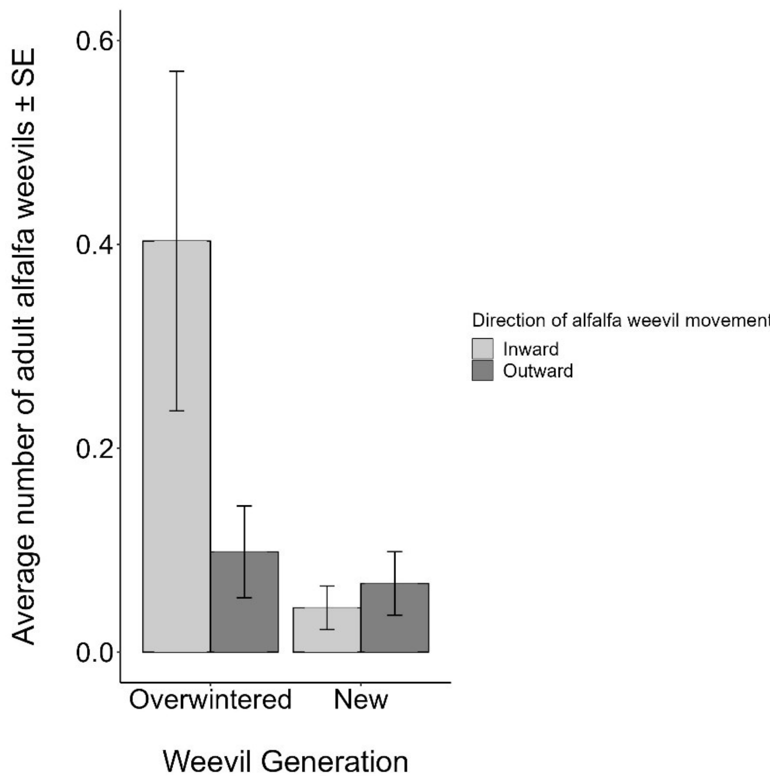

Figure 3-3. Average number of alfalfa weevils collected from directional pitfall traps placed at the edge of alfalfa fields grown for seed in southern Alberta (2023). The alfalfa weevils collected from directional pitfall traps placed at the edge of the field to compare the movement (inward or outward) of overwintered alfalfa weevils captured in May-June and new generation weevils captured in July-September. The error bars denote standard error.

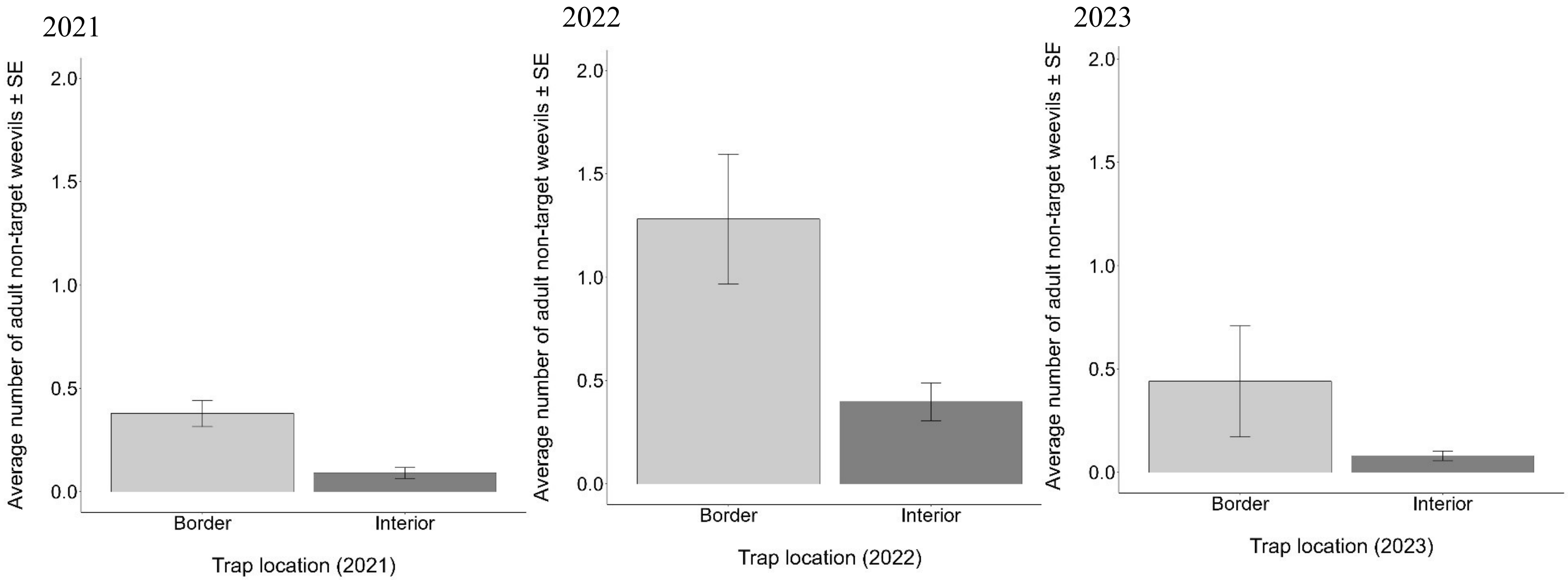

Figure 3-4. Average number of non-target weevils collected from pitfall traps in alfalfa fields grown for seed from southern Alberta (2021-2023). The samples were collected from five fields each in 2021 and 2022 and eight fields in 2023. The non-target weevils collected from pitfall traps placed inside and at the border of the field were compared. The error bars denote standard error.

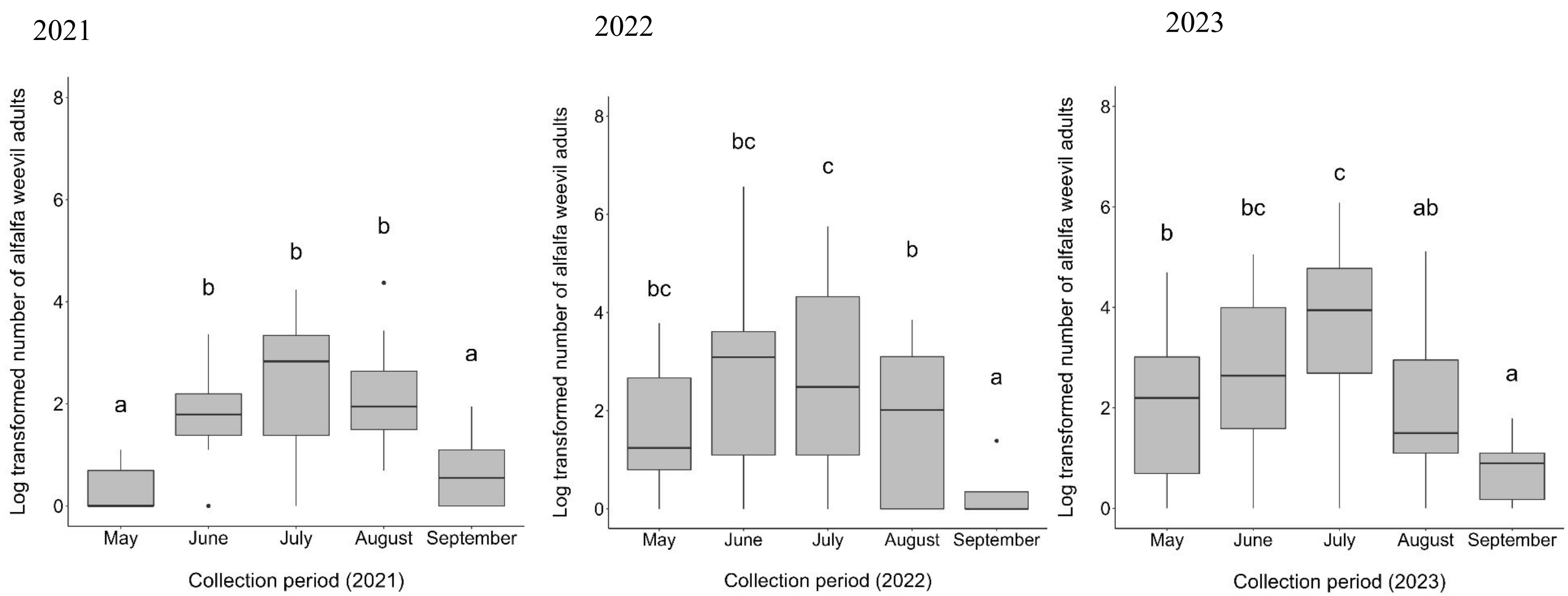

Figure 3-5. Box-and-whisker plots of total alfalfa weevil adults (log transformed) collected from sweep net samples. The central line of the box represents median and the top and bottom boxes represent the data within first and third quartiles, respectively. Whiskers indicate the maximum and minimum value. Different letters above the boxes indicate statistical difference between months in each test year (2021, 2022, 2023) obtained from Tukey's post hoc test ($p<0.05$).

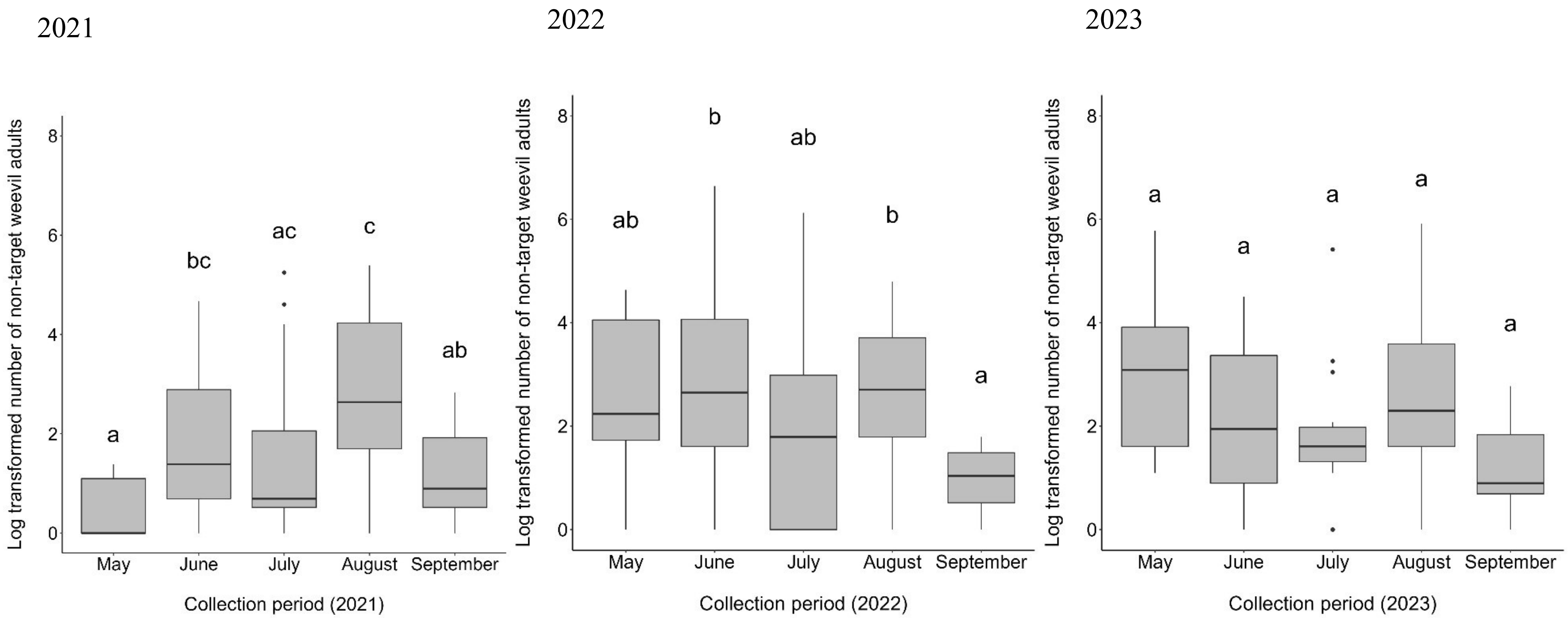

Figure 3-6. Box-and-whisker plots of total non-target weevil adults collected from sweep net samples (log transformed). The central line of the box represents median and the top and bottom boxes represent the data within first and third quartiles, respectively. Whiskers indicate the maximum and minimum value. Different letters above the boxes indicate statistical difference between months in each test year (2021, 2022, 2023) obtained from Tukey's post hoc test ($p<0.05$).

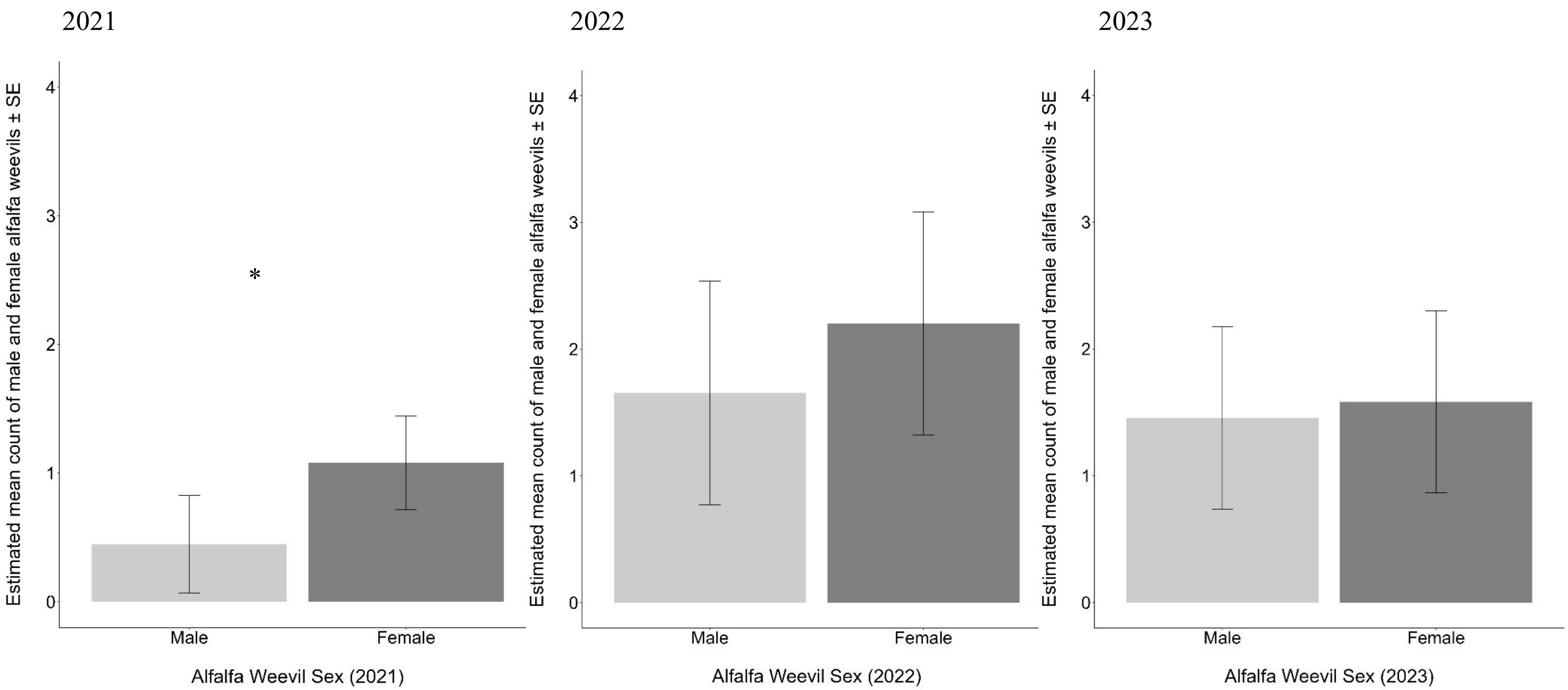

Figure 3-7. Average (estimated marginal means) number of male and female overwintered alfalfa weevils collected from sweep net samples from alfalfa fields grown for seed in southern Alberta (2022-2023). The estimated marginal means is derived from the generalized linear mixed effects model with negative binomial distribution. The error bars denote standard error. The asterisk (*) sign above the graph denotes the significant differences ($p < 0.05$) within sample year (2021, 2022, 2023).

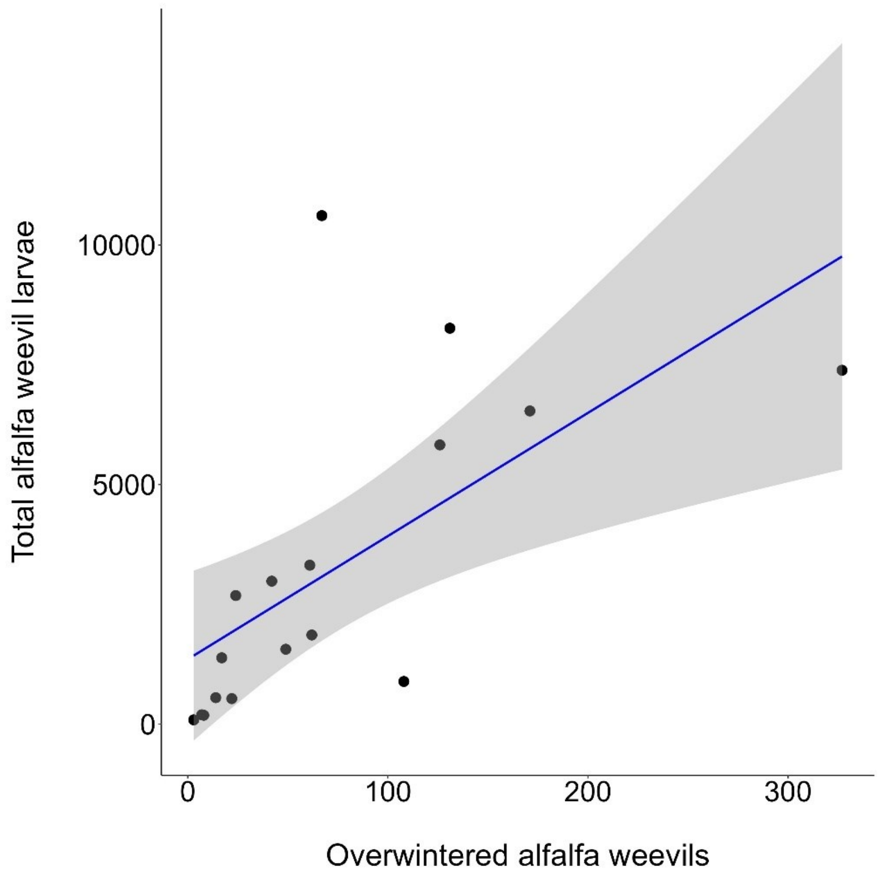

Figure 3-8. Scatter plot showing relationship between the overwintered adults and larvae captured during the growing season from sweep net sampling. The figure illustrates the trend between the overwintered adults and the subsequent larval population from 17 alfalfa fields grown for seed. The Kendall's Tau correlation test showed a significant ($p < 0.001$) relationship between the adults (x-axis) emerged from the overwintering sites and the larval population captured throughout the growing season (y-axis).

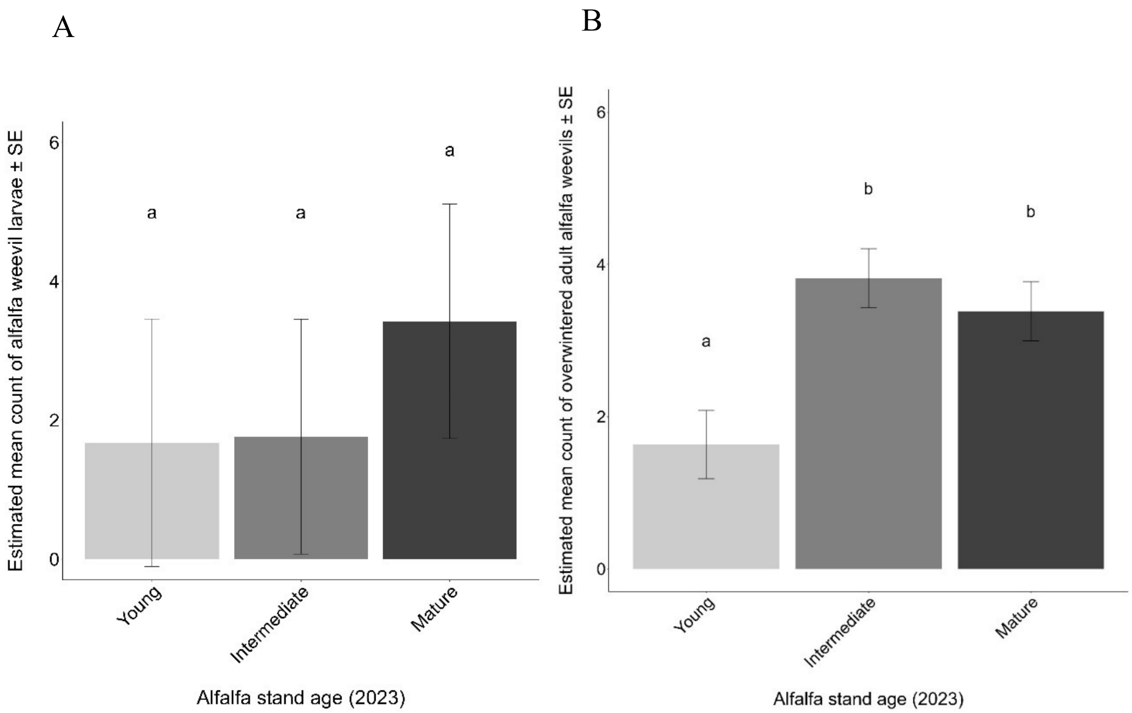

Figure 3-9. Average (estimated marginal means) number of alfalfa weevil larvae (A) collected from May to September and overwintered alfalfa weevil adults (B) collected from May-June using sweep nets from alfalfa seed fields of varying age. Alfalfa stand age (x-axis) varied from 1-3 years (Young stand = first year stand, Intermediate = second year stand, mature = third year stand). The estimated marginal means are derived from the generalized linear mixed effects model with negative binomial distribution. The error bars denote standard error. Different letters above the boxes indicate statistical difference between stand age from Tukey's post hoc test ($p<0.05$).

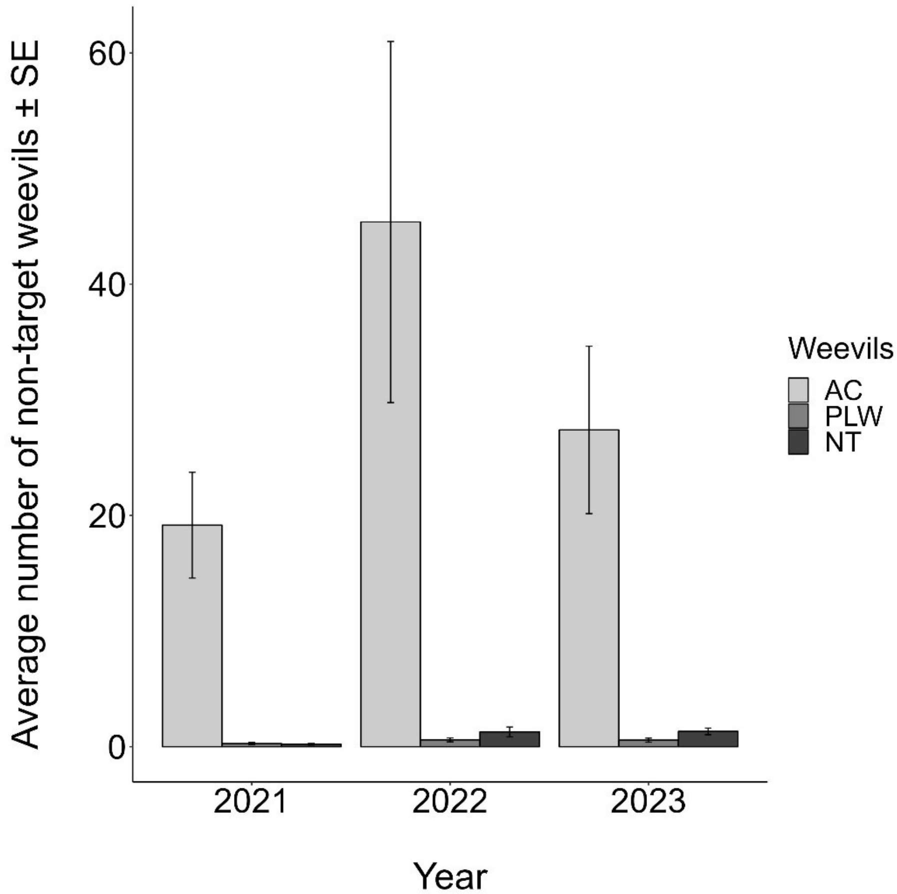

Figure 3-10. Average number of identified *Sitona* species and other non-target weevils collected from sweep sample by-catch from alfalfa fields grown for seed in southern Alberta (2021 - 2023). Most by-catch consisted of alfalfa curculio (AC). A few pea leaf weevils (PLW) were also identified. There was a small number of non-*Sitona* species (unidentified) (NT) captured as by-catch. The error bars denote standard error.

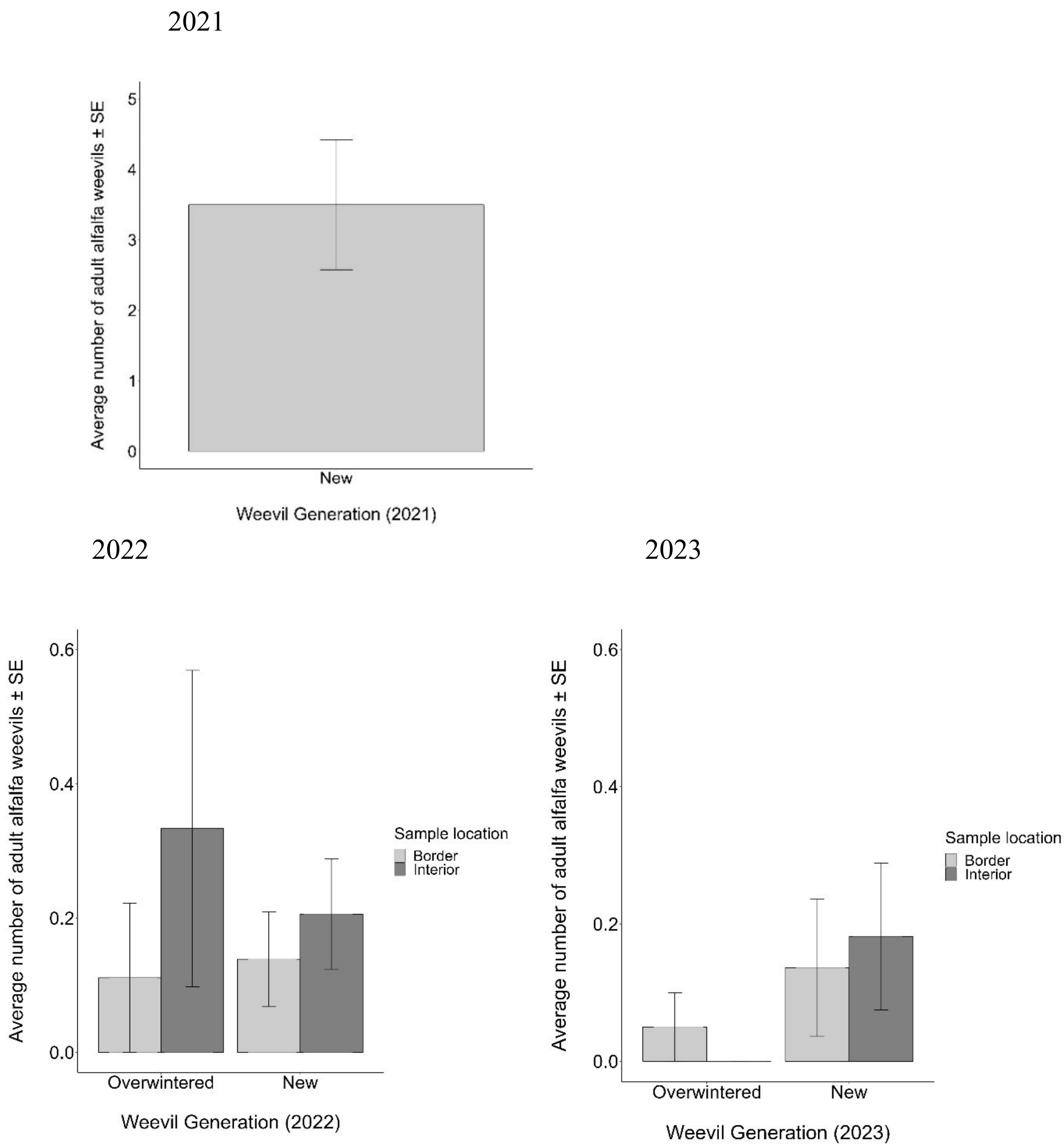

Figure 3-11. Average number of alfalfa weevils collected from soil samples from alfalfa fields grown for seed from southern Alberta (2021-2023). In 2022 and 2023, soil samples were collected from inside and at the edge of the field in early spring (April, May) and late summer (August-September). The error bars denote standard error.

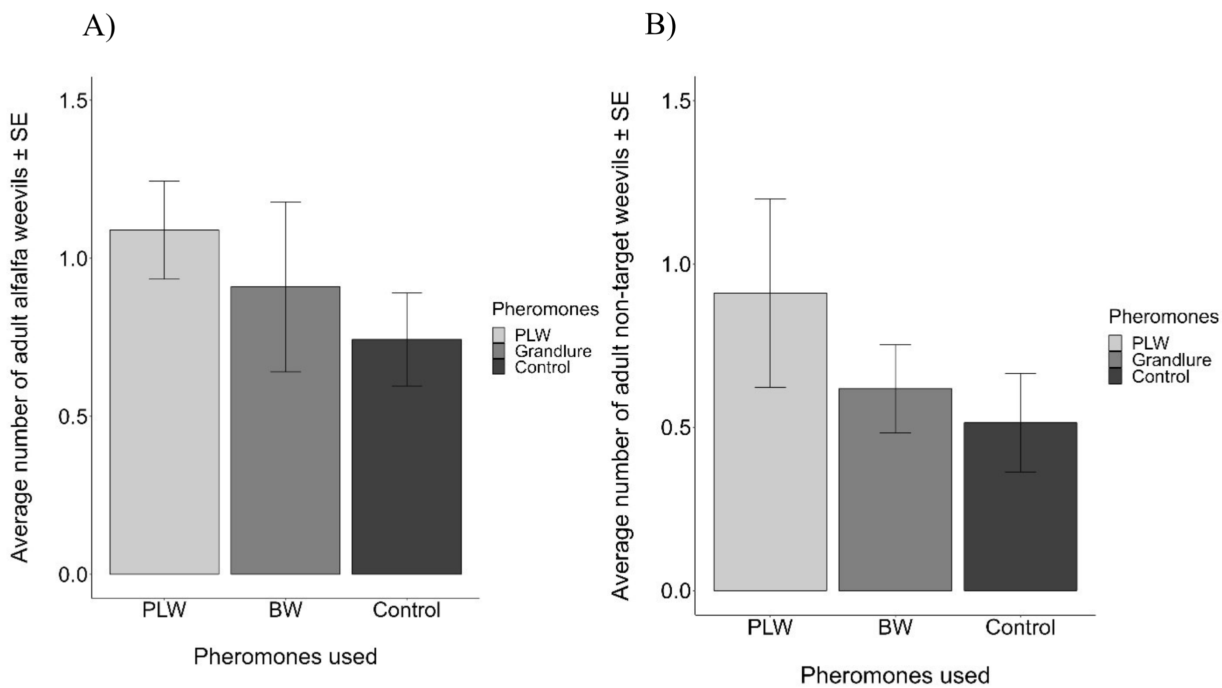

Figure 3-12. Average number of alfalfa weevil (A) and non-target weevil (B) adults collected from baited pitfall traps placed at the border of alfalfa fields in 2022. The pitfall traps were baited with lures containing pea leaf weevil aggregation pheromone (PLW), boll weevil pheromone (BW) and the solvent hexane control. The experiment was conducted in 2022. The error bars denote standard error.

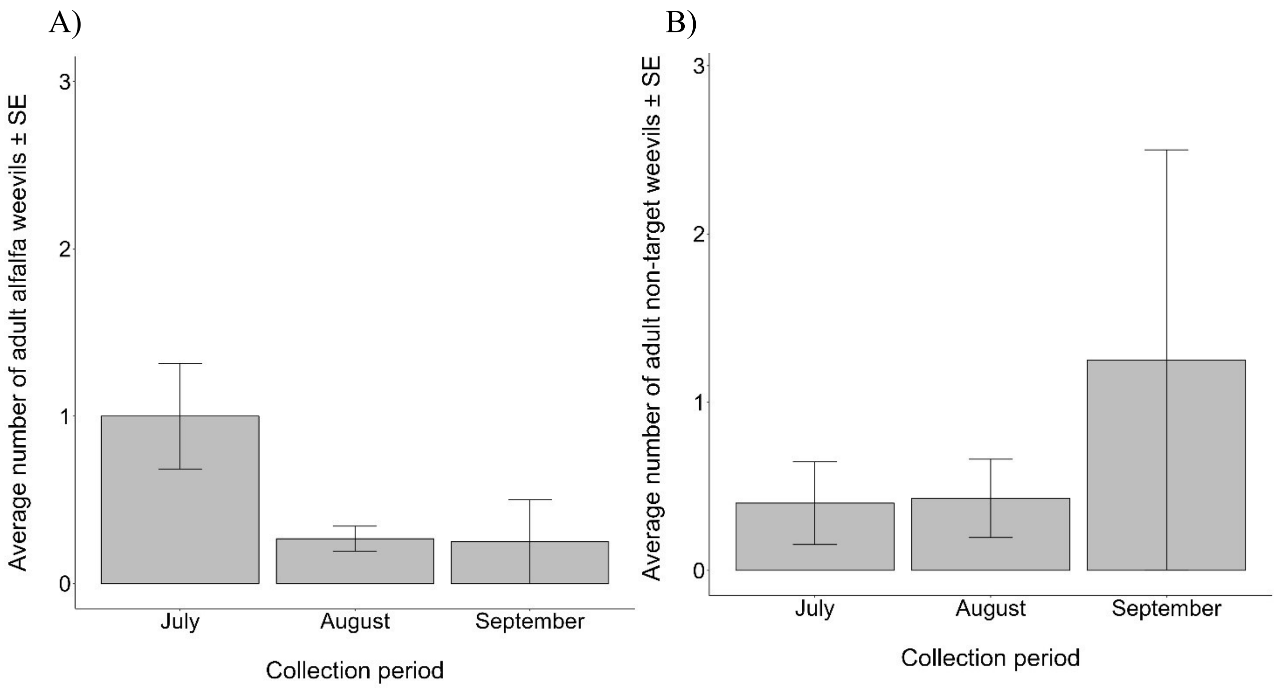

Figure 3-13. Average number of alfalfa weevil (A) and non-target weevils (B) adults collected from water traps placed at the border of alfalfa fields on either side of the pitfall traps (n = 4 fields). The experiment was conducted in 2022. The samples were collected every week from July to September. The error bars denote standard error.

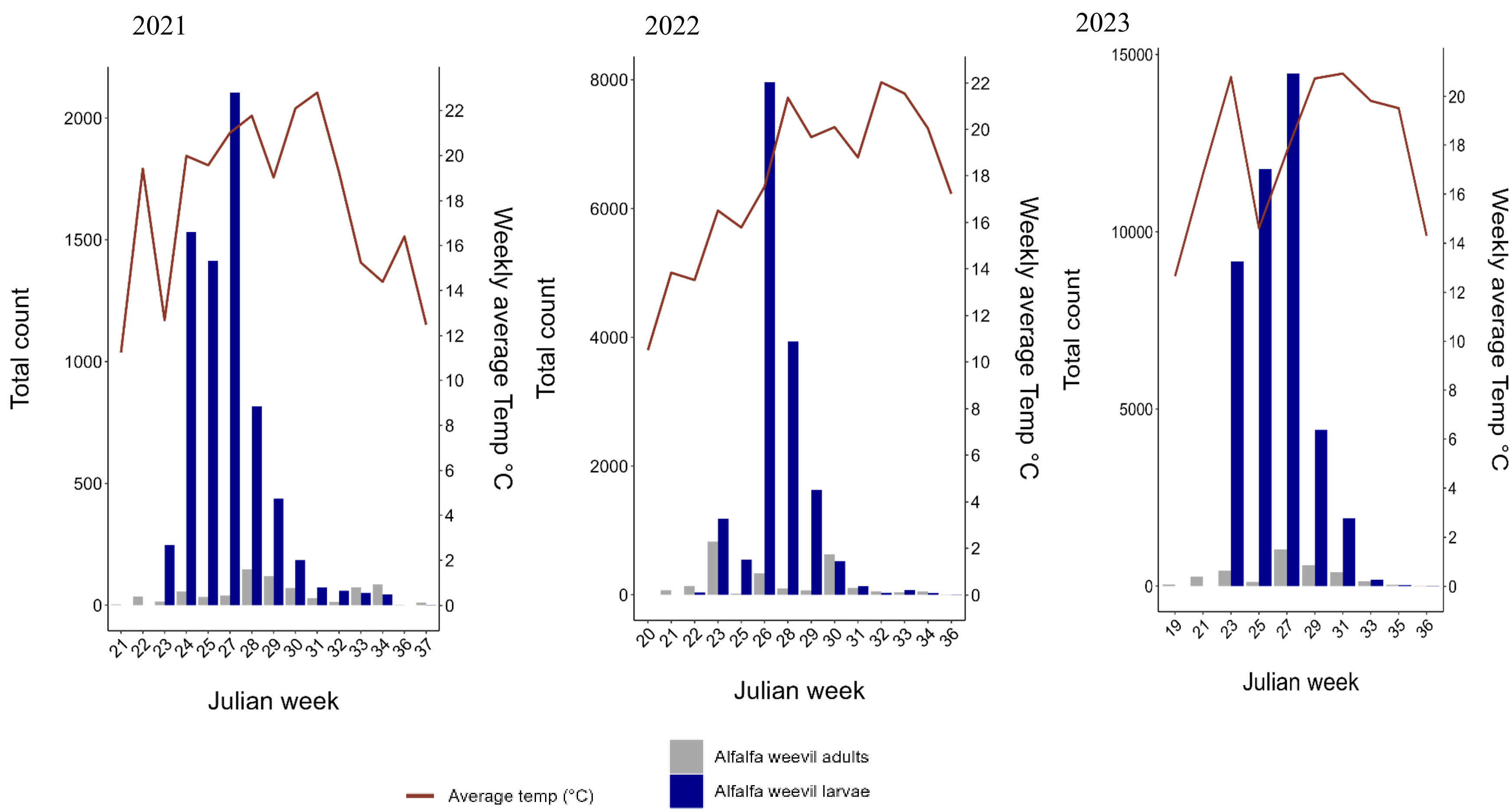

Figure 3-14. Graph showing the average temperature (line) with total alfalfa weevil adult and larval counts (bar) on each sampling week (2021 - 2023).

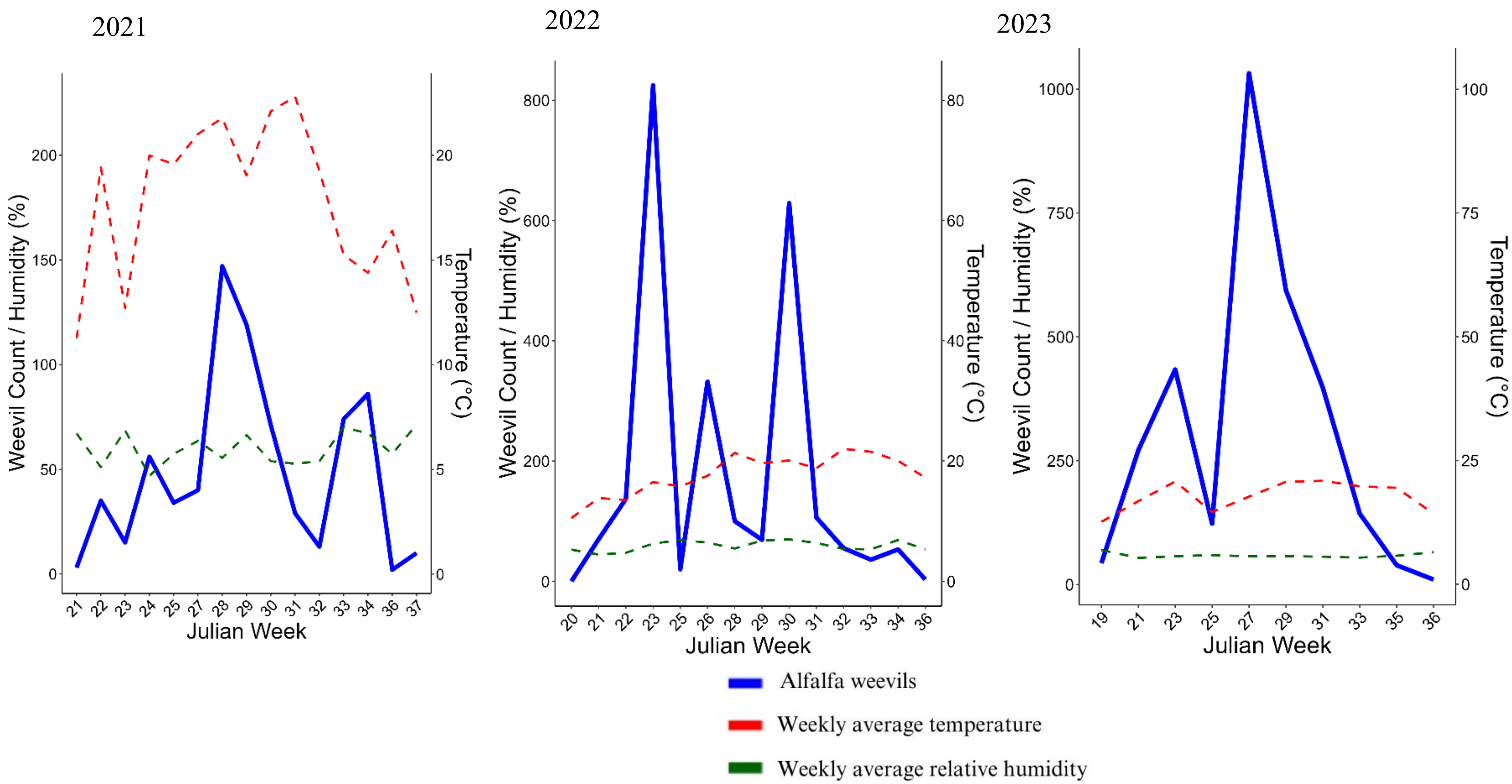

Figure 3-15. Graph showing the total alfalfa weevil adults (blue) from sweep samples over three years, weekly average temperature (red) and weekly average relative humidity (green) (2021 - 2023).

4. Discussion

Alfalfa weevil (*Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) is the key insect pest of alfalfa world wide (Schaber et al. 1994). Both larval and adult alfalfa weevils feed on foliage throughout the growing season (Pellisier et al. 2017). Overwintered adult alfalfa weevils feed for a short period before ovipositing in alfalfa stems in the spring (Soroka et al. 2020b). Larvae develop through four instars while feeding on alfalfa leaves and new generation adults emerge in late summer and continue to feed before winter (Hobbs et al. 1959). As alfalfa is a significant forage crop around the world and is cultivated on nearly 3.06 million hectares of land in the Canadian Prairie Provinces (He and cash 2009, Ren et al. 2021, Statistics Canada 2021), alfalfa weevil monitoring and management is of paramount importance. In this thesis, I compare different potential monitoring techniques to assess alfalfa weevil adult distribution and abundance in alfalfa grown for seed in Alberta. Alfalfa weevil has developed resistance to insecticides, especially synthetic pyrethroids with lambda cyhalothrin as the active ingredient (Rodbell and Wanner 2021, Rodbell et al. 2023). Insecticide resistance not only complicates integrated pest management of this important pest, but it also raises questions about gene flow as a result of dispersal of individuals within and between populations (Bessette et al. 2022). Hence, my study looks at assessing distribution and abundance of alfalfa weevil adults throughout the growing season.

Assessment of various sample techniques in this study revealed that sweep-net sampling is the best method to capture adult alfalfa weevils throughout the growing season. As sweep nets are already an established tool to monitor alfalfa weevil larval density and development (Hoff et al. 2002), additional sampling for adults should be easily adopted by alfalfa producers and managers to get a better idea on emerging overwintered alfalfa weevil adults the following year. Early larval instars of alfalfa weevil are effectively collected using a bucket shaking method (Hoff et al. 2002). Current economic decision making for larval control is based on 20 larvae per sweep that triggers management action (Aasen and Bjorge 2009). Sweep net samples in my study captured a total of 6,255 adult alfalfa weevils, only 238 alfalfa weevils in pitfall traps, 21 in water traps and no weevils from sticky card and malaise traps. Sweep nets are easy to use (Hoff et al. 2002) and provide data on alfalfa weevil adult and larval populations as well as natural enemies, *Bathyplectes curculionis* (Thomson), *B. anurus*, *M. colesi*, and *O. incertus* that reduces alfalfa weevil density in Alberta (Soroka et al. 2020a, Soroka and Otani 2011).

Sweep net samples captured variable numbers of alfalfa weevil adults across the growing season. More adults were sampled from the new generation in 2021, whereas more overwintered adult weevils were captured in 2022 and 2023. This could be due to a combination of weather parameters, and population being affected by parasitoids or insecticides. Most growers who participated in our study did multiple insecticide applications beginning in mid-June to target alfalfa weevils in the larval stage (Table 2-1). Management decisions, however, depend on threshold levels of alfalfa weevil, lygus bugs and alfalfa plant bugs. Insecticide sprays would likely affect the population density of the subsequent new generation adults sampled (Appendix Figure A1, Figure A2). As most weevils were sampled in the month of June and July in my study, this might indicate the ineffectiveness of insecticides for reducing alfalfa weevil adult populations. The current study also demonstrated a relationship between overwintered adult alfalfa weevils and subsequent larval populations suggesting that early monitoring of alfalfa weevil adults can help forecast larval density (Barbosa et al. 2012) The population is, however, also affected by temperature and a combination of other weather parameters which could be predicted using degree day models (Crozier and Dwyer 2006, Soroka et al. 2020b).

My three-year study demonstrated the influence of temperature and relative humidity on alfalfa weevil adult abundance measured with sweep net samples. Weekly average temperature had a significant effect on the alfalfa weevil adult population in 2021 and 2022 but with a negative effect on adult weevil population in 2022. This observation could be due to the variation in timing of temperature peak that could have affected the survival rate, and possibly predation and parasitism by natural enemies. An effect of weekly average humidity on alfalfa weevil adults was observed in 2021 and 2022 weevil populations. Alfalfa weevil have a linear increase in developmental rate in response to mean daily temperature above the threshold level of 10°C (Schaber et al. 1994) with a maximum rate of development at 30 - 32°C (Guppy and Mukerji 1974). Warm temperatures also enhance oviposition by female alfalfa weevils (Lecato and Pienkowski 1970, Hsieh and Armbrust 1974). The weather conditions combined with irrigation in alfalfa fields could affect the microclimate and influence the alfalfa weevil population (Alford et al. 2018, Braem et al. 2023). Both sexes of alfalfa weevil were captured in sweep net samples throughout the growing season. In my study, there was a female-biased sex ratio in the overwintered generation in 2021. This is in contrast to cabbage seed pod weevil (*Ceutorhynchus assimilis* Paykull) in which male weevils emerge from overwintering before females (Ulmer and Dosdall 2006). More females in spring coincides with the alfalfa shoot emergence and the availability of oviposition sites.

Humidity levels also impact alfalfa weevil adult biology and ecology. A study conducted in New York revealed that egg hatch and development of immatures are most successful under high humidity (Koehler and Gyrisco 1961). Although Alberta is much drier than other parts of the alfalfa weevil range, my study shows a positive relationship between relative humidity and adult alfalfa weevil capture in sweep net samples. Humidity also influences foraging behaviour of adult weevils. Adult weevils orient to foliage better under humid conditions (Meyer and Raffensperger 1974) and prefer humid conditions for foraging in general (Springer and Pienkowski 1969). The humidity in the microenvironment could also be affected by the irrigation schedule followed by the growers in the selected fields. Irrigation was initiated in May and June to support the crop growth. Once the crop starts flowering, growers irrigate the field for an inch every week followed by ~2 cm inch in August to induce seed ripening (Retzlaff, personal communication, August 22, 2024). New generation alfalfa weevil adults can aggregate near large water bodies in late summer suggesting they are attracted to water (Simpson and Welborn 1965). I captured few new generation adults, however, in water traps placed in the field in late summer. My analysis from sweep net sample data did not show a significant effect of precipitation on adult alfalfa weevil adult abundance, but there was more total precipitation for the month of June in 2022 (81.9 mm in 12 days) than for either 2021 (12.8 mm in 6 days) or 2023 (15.9 mm in 8 days). The study by Levi-Mourao et al. (2021) in Spain found that temperature and humidity affect egg hatch and survival, where the egg developmental time decreased with the temperature and low relative humidity. Low temperature combined with low humidity is lethal to prepupae and pupae of alfalfa weevils (Koehler and Gyrisco 1961). Similarly, adults are less active at low temperatures compared to warm conditions in Maryland (Manglitz and App 1957).

Environmental conditions can also affect natural enemy populations and therefore indirectly impact alfalfa weevil populations. Harcourt and Guppy (1991) report that natural control of alfalfa weevil by the fungus *Zoophthora phytonomi* (Arthur) is favoured by increased precipitation and high humidity in eastern Ontario. High precipitation in spring enhances conidial production by *Zoophthora* and affects new generation alfalfa weevil larvae and pupae (Harcourt and Guppy 1991). The parasitoids, *Bathyplectes curculionis* and *Oomyzus incertus* reduce alfalfa weevil in southern Alberta (Soroka et al. 2020a, Soroka and Otani 2011). Reid (2022) recorded 0 - 90% parasitism on alfalfa weevil larvae across sites in Alberta. According to Radcliffe and Flanders (1998), alfalfa weevil number is reduced naturally in areas where the different parasitoids of alfalfa weevil are established controlling the fall population by parasitizing larval and pupal stages in North America. Studies also shows the effective searching behaviour in *B. curculionis* at a temperature range between 10 - 30°C (Barney et al. 1977) that favours their attack on alfalfa weevils.

Alfalfa grown for seed is managed as a perennial crop and seed is harvested on an annual basis (Jing et al. 2020). The 2023 sample year included sites with variously aged alfalfa stands. The number of overwintered alfalfa weevil adults captured in sweep net samples gradually increased with the stand age. On the other hand, the total number of larvae and adult alfalfa weevils captured throughout the growing season was not affected by the stand age, suggesting that overwintered adult alfalfa weevils orient to mature stands but population growth is supported in all stands. The absence of overwintered alfalfa weevil adults in emergence cages in first year alfalfa stands of 2023 suggests a delayed field colonization of alfalfa stands by overwintered adult alfalfa weevils. This also implies that alfalfa weevil adults prefer established alfalfa fields for overwintering. A recent study (Pellisier et al. 2022) in Wyoming that examined the influence of landscape composition and insecticide application on subsequent alfalfa weevil population density, also detected no relationship between alfalfa stand age and season-long weevil density. Foraging by the alfalfa weevil parasitoid, *Bathyplectes curculionis*, is affected by alfalfa weevil density and distance between the fields, regardless of the stand age (Evans 2018, Pellisier et al. 2022). Natural enemies may have a greater impact on populations in fields with more weevils early in the season. As a result, equal numbers of alfalfa weevil adults across differently aged alfalfa stands occur later in the season.

In this study, the second most successful monitoring tool for alfalfa weevils was pitfall traps. Pitfall traps are used to trap ground dwelling insects that disperse primarily by walking (Hohbein and Conwey 2018, Brown and Matthews 2016). I tested two types of pitfall traps including the traditional solo pitfall trap (Evenden et al. 2016) in the first two years of the study and added a directional pitfall trap that used two solo cups separated by a barrier to determine the direction of insect movement before entering the trap in 2023 (Hilburn 1985). I did not capture many alfalfa weevil adults in either the solo or directional pitfall traps. Pitfall traps are most effective to monitor large, ground-dwelling insects (Engel et al. 2017). Small insects or 'trap-shy' insects usually are not captured reliably in pitfall traps (Ulyshen et al. 2005). The small size and foliage foraging habits of the alfalfa weevil preclude reliable monitoring using pitfall traps for this insect. In my study, there was no difference in alfalfa weevil adult capture in pitfall traps positioned at the edge or inside the field, but as so few weevils were captured it is hard to determine if those numbers reflect population distribution as a whole (Pausch et al. 1979). The pitfall traps in my experiment captured several species of predaceous carabid beetles that might also have affected the alfalfa weevil adults collected in the traps (Barney and Armbrust 1980, Rand 2017). In addition, the capture area of the two cups in the directional pitfall traps may not be adequate to study the directionality of weevil movement. Linear pitfall traps could be an alternative that would sample a larger area of potential weevil movement (Pausch et al. 1979).

In order to increase the efficacy of pitfall traps, I tested semiochemicals that have previously been shown to be produced by or attractive to alfalfa weevil adults. Baited pitfall traps captured few alfalfa weevil adults in my study but numerically more were recovered from traps baited with the pea leaf weevil aggregation pheromone (4-methyl-3,5-heptanedione). Quinn et al (1999) also reported the presence of alfalfa weevils in traps baited with the pea leaf weevil pheromone but did not illustrate attraction through comparison with capture in unbaited traps. Studies have shown the attraction of other *Sitona* weevils like sweet clover weevil (St. Onge et al. 2018) and lucerne weevil (*Sitona humeralis* Stephens) (Toth et al. 1998, Lohonyai et al. 2019) to the pea leaf weevil pheromone. My findings support the presence of *Sitona* spp. to traps baited with the pea leaf weevil pheromone in alfalfa. Alfalfa weevils were also captured in traps baited with the putative pheromone components: ((*Z*)-3,3-dimethylcyclohexane- δ1, α – acetaldehyde: (*E*)-3,3-dimethylcyclohexane- δ1, α-acetaldehyde) in a 1:1 ratio (Hedin et al. 1988), however in low numbers. Capture may be increased if components are presented in the same ratio (2:1) that were identified in hexane washes of adult alfalfa weevils (Hedin et al. 2018). These compounds make up the pheromone composition of the cotton boll weevil (*Anthonomis grandis* Boh) and also attract other weevils including sugar beet weevil (*Bothynoderes punctiventris* Germar

(Coleoptera: Curculionidae)) (Toth et al. 2007), and milkweed stem weevil (*Rhyssomatus* spp. (Coleoptera: Curculiondae)) (Suh and Westbrook 2011). The attractiveness of the semiochemicals tested in the field showed that baited pitfall traps captured numerically more adult weevils than unbaited pitfall traps (Reddy et al. 2018, St. Onge et al. 2018). In the current research, 290 alfalfa weevils were captured in the variously baited pitfall traps compared to 109 alfalfa weevils in unbaited traps of 2022. This result indicates the need to conduct more studies on semiochemical attraction of alfalfa weevils including detailed behavioural studies in olfactometers to measure individual weevil response and movement towards various semiochemicals (Lohonyai et al. 2019). Volatile organic compounds released from alfalfa attract alfalfa weevils in an olfactometer assay (Byrne and Steinhauer 1966) and in the field (Lee et al. 2012) studies, and should also be investigated as potential baits for monitoring and management tools.

The inclusion of traps that capture insects in flight (sticky cards, malaise traps and water traps) in this study revealed that alfalfa weevils do not disperse by flight in southern Alberta. These findings are in contrast to early studies that reported alfalfa weevil dispersal by flight at heights up to 7 m above the ground depending on wind, however using intercept flight traps (3.5 x 5 ft) rather than small-sized sticky traps as in the current study (Prokopy and Gyrisco 1965, Sherburne et al. 1969). These studies were conducted in New York (Prokopy and Gyrisco 1965, Sherburne et al. 1969) where different alfalfa weevil strains dominate. Eastern and western strain alfalfa weevil adults aestivate away from the field individually while the Egyptian strain weevil adults aestivate in groups under bark or in crevices (Böttger et al. 2013). Alfalfa weevils prefer humid habitats (Byrne and Steinhauer 1966, Springer and Peinkowski 1969) and aggregate near large water bodies during dispersal away from fields in the fall (Simpson and Welborn 1975). Weevils did not orient to water traps in our study late in the season in 2022. This might indicate that weevils do not leave the field during summer aestivation, as occurs in other parts of the weevils' invasive range (Prokopy et al. 1967). Alternatively, perhaps a larger water body is needed to attract alfalfa weevils late in the season.

In order to determine if overwintering alfalfa weevil adults are located in the soil within or outside of alfalfa fields, soil samples were collected early in the spring (2022 and 2023) and late in the summer/fall (2021-2023). Although alfalfa weevil adults were retrieved in all years and in both seasons, too few were captured to conduct statistical analyses. More alfalfa weevil adults were captured in the first year (2021) compared to the second (2022) and third year (2023). This could be due to a larger volume of soil sampled in 2021 or more weevils became dislodged from plants during sweep sampling in 2021 that was conducted only within the alfalfa field. Many weevils will "play dead" and drop to the ground when disturbed (Bjork and Davis 1984). The recovery of adult weevils in the early spring and late summer/fall supports the emergence cage results. The overwintering alfalfa weevils might be in the top 2 to 3 cm of soil, or debris (Tysowsky and Dorsey 1970). Prokopy et al. (1967) found new generation alfalfa weevils in the top 2 cm of the soil beneath the litter during summer aestivation. Tysowsky and Dorsey (1970) also found adult alfalfa weevils in the top 2 cm of soil during the overwinter period (December to March) in addition to the summer aestivation period (June to September) in Virginia. Results from the emergence cages found more weevils overwintered outside the alfalfa field, although some overwinter in the field.

Sitona spp. bycatch was identified from the sweep net samples taken in 2021-2023. Alfalfa curculio and pea leaf weevil represented the majority of the by-catch. Only 94 pea leaf weevils were caught compared to 5916 alfalfa curculio weevils over the three-year study. The pea leaf weevil uses alfalfa as a secondary host for adult feeding in late fall to early spring (Hamon et al. 1987), whereas alfalfa curculio feeds on alfalfa throughout its life cycle (Loan 1963). Larvae of alfalfa curculio consume the root nodules and associated rhizobia bacteria which makes it a more important pest of alfalfa than the pea leaf weevil (Loan 1963, Mast 1963). Interestingly, other *Sitona* weevil species that are known from alfalfa including sweet clover weevil (*Sitona cylindricollis*) (Sanaei et al. 2015) and clover root curculio (*Sitona hispidulus*) (Price, 2017) were not recovered in my study. By-catch of *Sitona* species in pitfall traps was numerically higher in traps placed at the edge of fields, as compared to the interior even though no significant difference was observed. This finding suggests that *Sitona* weevils move into alfalfa in the spring (Willsey et al. 2019).

Field irrigation and weather conditions may also affect the *Sitona* species sampled. Irrigation can create either a favourable condition by improving the microenvironment or adverse condition due to excessive soil moisture that affects the eggs and larvae. Excessive moisture can also induce colonization of fungal pathogens**.** A study by Johnson et al. (2010), showed that moisture enhanced egg hatch of *Sitona lepidus* and eggs develop faster at 25 ºC than 10 ºC and in slightly acidic soils of pH 5 (Johnson et al. 2010). In a study by Almogdad et al. (2020) in Lithuania, pea leaf weevil abundance was high when the average temperature was 1.6ºC above the normal temperature. The presence of alfalfa curculio in field with alfalfa weevil highlights the need to study the interaction of weevil pests on alfalfa grown for seed.

5. Conclusion

Alfalfa (*Medicago sativa* L.) (Fabaceae), known as the queen of forages, is one of the major perennial legumes grown for hay and seed (Attram et al. 2016). Alfalfa is considered as an essential component of livestock and poultry diet (Firdaous et al. 2017, Boucher et al. 2023). Alfalfa is consumed by humans mostly as sprouts (Mielmann 2013). Alfalfa is mainly grown in arid and semi-arid regions of Alberta, Saskatchewan, and Manitoba on nearly 3.06 million hectares (Sim and Meers 2016, Statistics Canada 2021). Alberta alone produces nearly 2,964 tonnes of alfalfa seed annually (AFIN 2012, Statistics Canada 2021). Apart from growing alfalfa for forage and seed, alfalfa is additionally incorporated in crop rotation to enhance the soil quality through nitrogen fixation (Summers 1998). Alfalfa is highly dependent on bees for pollination and provides nectar in return (Pankiw et al. 1956, Bosch and Kemp 2005).

Being an economically significant crop, the beneficial and detrimental insects on alfalfa need to be studied in order to take necessary decisions for crop protection. Alfalfa weevils are developing insecticide resistance, which is making management difficult (Rodbell and Wanner 2021, Rodbell et al. 2023). In this thesis, I compare monitoring tools for alfalfa weevil adults to assess their location throughout the growing season over a period of three years (2021-2023).

Five fields with established alfalfa stands grown for seed each were selected in 2021 and 2022 and eight fields in 2023 between Rosemary and Brooks in southern Alberta. Among all the sample techniques tested, sweep net sampling is most promising for alfalfa weevil monitoring as adult and larval alfalfa weevils and other pest (*Sitona*) weevils are captured with this technique. Relative humidity and temperature (2021) were positively related to alfalfa weevil adults in sweep net samples but more alfalfa weevil adults were collected in the fall in 2022 and 2023. Both types of pitfall traps tested were ineffective at capturing adult alfalfa weevils, although baiting traps with semiochemicals increased capture of adult alfalfa weevils and deserves more study. Linear pitfall traps utilizing 1 m length of galvanised metal guttering could be used as an alternative that would sample a larger area of potential weevil movement (Pausch et al. 1979). Also, baited pitfall trap capture may be increased if components of boll weevil pheromone are used in the same ratio (2:1) that were identified in hexane washes of adult alfalfa weevils (Hedin et al. 2018).

Emergence cage and soil sampling of alfalfa weevils in early spring and late summer/fall indicates that weevils are present in the soil both inside and outside of the field to overwinter. Overwintering alfalfa weevils might be in the top 2 - 3 cm of soil or in debris on top of the soil which is supported by previous studies in other parts of its range (Prokopy et al. 1967 (New York), Tysowsky and Dorsey 1970 (Virginia)). Post-overwintering adult alfalfa weevils seem to prefer to infest mature stands over young stands but subsequent larval and season-long adult populations are similar across alfalfa stands of varying age.

Most of the *Sitona* weevil bycatch was retrieved in sweep net samples. Most of the non-target weevils captured were alfalfa curculio that uses alfalfa as a primary reproductive host. Pea leaf weevils that use alfalfa as a secondary host were captured in lower numbers. The presence of alfalfa curculio is of greater concern than pea leaf weevil in alfalfa due to larval consumption of root nodules. The presence of alfalfa weevil (above ground feeders) and *Sitona lineellus* (below ground feeder) can weaken the alfalfa plants increasing the stress. Alfalfa curculio is understudied in Alberta, and more research is required to determine its potential to develop as another major curculio pest as well as the interaction effect with other alfalfa pests in alfalfa fields grown for seed in southern Alberta.

Hence, my study showed the effective capture method for alfalfa weevils as sweep net sampling for monitoring both larval and adult stages of alfalfa weevil from alfalfa fields grown for seed in southern Alberta. This also indicated the presence of alfalfa weevil on plants more than in the soil. Also, the study reveals the preferred overwintering location of alfalfa weevil adults outside the field. The study also showed the preference of alfalfa weevils towards the mature crop stands for colonization.

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Appendix

Table A1. Statistical models with response, random, and fixed variables with test statistics, degrees of freedom, and p-values. The significant p-values are bolded. AW stands for alfalfa weevils and NT for non-target weevils.

Experiment	**Weevil Generation/ Year**	**Model Type**	**Model**	**Test Statistics**	**df**	**p-value**
Emergence cage	Overwintered/2022	GLMM (Poisson, Log link)	AW[1] adults ~ Trap location + (1| Site)	χ^2 =15.662	1	**< 0.001**
Emergence cage	Overwintered /2023	GLMM (Poisson, Log link)	AW adults ~ Trap location + (1| Site)	χ^2 =30.857	1	**< 0.001**
Sweep sample	2021	GLMM (Negative binomial)	AW adults ~ Month + (1| Site)	χ^2 =42.735	4	**<0.001**
Sweep sample	2022	GLMM (Negative binomial)	AW adults ~ Month + (1| Site)	χ^2 =30.13	4	**<0.001**
Sweep sample	2023	GLMM (Negative binomial)	AW adults ~ Month + (1| Site)	χ^2 = 41.777	4	**<0.001**

[1] Alfalfa Weevil

Experiment	**Weevil Generation/ Year**	**Model Type**	**Model**	**Test Statistics**	**df**	**p-value**
Sweep sample	2021	GLMM (Negative binomial)	NT [2]adults ~ Month + (1| Site)	$\chi^2 = 21.545$	4	**<0.001**
Sweep sample	2022	GLMM (Negative binomial)	NT adults ~ Month + (1| Site)	$\chi^2 = 16.152$	4	**0.003**
Sweep sample	2023	GLMM (Negative binomial)	NT adults ~ Month + (1|Month/JulianWeek) + (1| Site)	$\chi^2 = 3.665$	4	0.453
Sexing (Sweep sample)	Overwintere d /2021	GLMM (Negative binomial)	AW count ~ Sex + (1|Site)	$\chi^2 = 4.326$	1	**0.038**
Sexing (Sweep sample)	New/2021	GLMM (Negative binomial)	AW count ~ Sex + (1|JulianWeek) + (1|Site)	$\chi^2 = 1.633$	1	0.201
Sexing (Sweep sample)	Overwintere d /2022	GLMM (Negative binomial)	AW count ~ Sex + (1|JulianWeek) + (1|Site)	$\chi^2 = 3.224$	1	0.073
Sexing (Sweep sample)	New/2022	GLMM (Negative binomial)	AW count ~ Sex + (1|JulianWeek) + (1|Site)	$\chi^2 = 0.018$	1	0.894

[2] Non-target weevils

Experiment	Weevil Generation/ Year	Model Type	Model	Test Statistics	df	p-value
Sexing (Sweep sample)	Overwintere d /2023	GLMM (Negative binomial)	AW count ~ Sex + (1|JulianWeek) + (1|Site)	$\chi^2 = 0.456$	1	0.499
Sexing (Sweep sample)	New/2023	GLMM (Negative binomial)	AW count ~ Sex + (1|JulianWeek) + (1|Site)	$\chi^2 = 0.039$	1	0.844
AW adult-larval relationship (Sweep samples)	2021-2023	Kendall's rank correlation test	Season-long AW larvae ~ Overwintered AW adults	$\chi^2 = 131$	16	**<0.001**
Stand age (Sweep sample)	2023	GLMM (Negative binomial)	AW adults ~ Stand age + (1|Site)	$\chi^2 = 4.591$	2	0.101
Stand age (Sweep sample)	Overwintere d /2023	GLMM (Negative binomial)	AW adults ~ Stand age + (1|Site)	$\chi^2 = 14.675$	2	**<0.001**
Stand age (Sweep sample)	2023	GLMM (Negative binomial)	AW larvae ~ Stand age + (1|JulianWeek) + (1|Site)	$\chi^2 = 2.397$	2	0.302
Non-target weevils (sweep sample)	2021	GLMM (Negative binomial)	NT weevil count ~ Weevils + (1|Site)	$\chi^2 = 111.44$	2	**<0.001**

Experiment	**Weevil Generation/ Year**	**Model Type**	**Model**	**Test Statistics**	**df**	**p-value**
Non-target weevils (sweep sample)	2022	GLMM (Negative binomial)	NT weevil count ~ Weevils + (1|Site)	$\chi^2 = 78.15$	2	**<0.001**
Non-target weevils (sweep sample)	2023	GLMM (Negative binomial)	NT weevil count ~ Weevils + (1|JulianWeek) + (1|Site)	$\chi^2 = 125.17$	2	**<0.001**
Baited pitfall experiment	2022	GLMM (Negative binomial)	AW adults ~ Pheromone + (1|Site)	$\chi^2 = 4.384$	2	0.112
Baited pitfall experiment	2022	GLMM (Negative binomial)	NT adults ~ Pheromone + (1|Site)	$\chi^2 = 4.270$	2	0.118
Weather parameters	2021	GLMM (Negative binomial)	AW adults ~			
			Weekly average temperature +	$\chi^2 = 11.959$	1	< **0.001**
			Weekly average relative humidity +	$\chi^2 = 7.841$	1	**0.005**
			Weekly average precipitation + (1|Site)	$\chi^2 = 0.221$	1	0.638
Weather parameters	2022	GLMM (Negative binomial)	AW adults ~			
			Weekly average temperature +	$\chi^2 = 5.099$	1	**0.024**
			Weekly average relative humidity +	$\chi^2 = 14.157$	1	< **0.001**
			Weekly average precipitation +	$\chi^2 = 2.946$	1	0.086

Experiment	Weevil Generation/ Year	Model Type	Model	Test Statistics	df	p-value
			(1|Site)			
Weather parameters	2023	GLMM (Negative binomial)	AW adults ~			
			Weekly average temperature +	$\chi^2 = 0.108$	1	0.742
			Weekly average relative humidity +	$\chi^2 = 2.963$	1	0.085
			Weekly average precipitation + (1|JulianWeek) + (1|Site)	$\chi^2 = 1.354$	1	0.245

Table A2. Number of alfalfa weevil adult and larval capture from emergence cage and sweep net sampling

Year	Field	Sampling Type	Collection Dates	Alfalfa weevil adults	Alfalfa weevil larvae
2021	A	Sweep net samples	28 May	0	0
			3 June	8	0
			10 June	5	149
			18 June	4	538
			25 June	0	383
			9 July	11	214
			16 July	68	77
			23 July	29	7
			30 July	27	7
			6 August	4	7
			12 August	1	2
			19 August	3	0
			27 August	3	0
			7 September	0	0
2021	B	Sweep net samples	28 May	0	0
			3 June	3	0
			10 June	0	14
			18 June	2	3
			25 June	3	5
			9 July	0	25
			16 July	1	25
			23 July	0	15
			30 July	0	47
			6 August	1	29
			12 August	0	11
			19 August	7	7
			27 August	13	4
			7 September	0	0
2021	C	Sweep net samples	27 May	1	0
			4 June	12	0
			11 June	4	24
			17 June	24	263
			24 June	21	208

Year	Field	Sampling Type	Collection Dates	Alfalfa weevil adults	Alfalfa weevil larvae
			8 July	13	821
			15 July	20	304
			22 July	28	169
			29 July	23	48
			5 August	6	12
			13 August	4	6
			20 August	8	4
			26 August	22	1
			8 September	2	0
			14 September	2	0
2021	D	Sweep net samples	27 May	2	0
			4 June	7	0
			11 June	3	36
			17 June	22	625
			24 June	8	770
			8 July	15	895
			15 July	51	371
			22 July	38	211
			29 July	4	44
			5 August	5	14
			13 August	6	17
			26 August	26	0
			14 September	2	1
2021	E	Sweep net samples	27 May	0	0
			4 June	5	0
			11 June	3	24
			17 June	4	103
			24 June	2	48
			8 July	1	149
			15 July	7	40
			22 July	24	36
			29 July	17	40
			5 August	13	11
			13 August	2	23
			20 August	56	39

Year	Field	Sampling Type	Collection Dates	Alfalfa weevil adults	Alfalfa weevil larvae
			26 August	22	39
			14 September	6	0
2022	A	Emergence cage	21 May	7	Not sampled
		Sweep net samples	25 May	1	0
			8 June	22	0
			30 June	31	36
			13 July	7	1542
			20 July	2	1309
			28 July	14	236
			3 August	314	136
			11 August	21	42
			18 August	14	14
			25 August	2	1
			9 September	0	1
2022	B	Emergence cage	18 May	0	Not sampled
		Sweep net samples	18 May	0	0
			25 May	3	0
			2 June	2	0
			8 June	2	2
			30 June	0	149
			13 July	1	39
			20 July	0	1
			28 July	3	4
			3 August	0	1
			11 August	0	0
			17 August	0	0
			25 August	0	0
			9 September	0	0
2022	F	Emergence cage	18 May	4	Not sampled
		Sweep net samples	25 May	21	0
			2 June	29	0
			8 June	56	29
			30 June	2	390

Year	Field	Sampling Type	Collection Dates	Alfalfa weevil adults	Alfalfa weevil larvae
			13 July	1	337
			20 July	11	71
			28 July	104	41
			3 August	11	9
			11 August	2	12
			18 August	6	0
			25 August	7	1
			9 September	0	0
2022	G	Emergence cage	Not sampled	Not sampled	Not sampled
		Sweep net samples	25 May	2	0
			2 June	0	0
			8 June	26	14
			22 June	20	545
			30	1	188
			13 July	2	366
			20 July	7	308
			29 July	149	121
			3 August	46	16
			11 August	39	1
			18 August	22	3
			25 August	11	1
			9 September	0	0
2022	H	Emergence cage	19 May	31	Not sampled
		Sweep net samples	26 May	43	0
			2 June	84	35
			9 June	710	1102
			29 June	322	5692
			14 July	94	1881
			21 July	37	1010
			27 July	59	217
			4 August	28	63
			11 August	0	6
			18 August	6	69
			25 August	35	28
			9 September	3	4

Year	Field	Sampling Type	Collection Dates	Alfalfa weevil adults	Alfalfa weevil larvae
2023	I	Emergence cage	10 May	1	Not sampled
		Sweep net samples	10 May	1	0
			6 June	2	57
			21 June	0	3
			5 July	2	28
			18	0	1
			2 August	0	0
			17	0	0
			29	2	0
			9 September	0	0
2023	J	Emergence cage	10 May	4	Not sampled
		Sweep net samples	10 May	4	0
			24 May	34	0
			21 June	29	5920
			5 July	437	3506
			18 July	263	794
			2 August	155	393
			17 August	113	0
			29 August	22	0
2023	K	Emergence cage	10 May	4	Not sampled
		Sweep net samples	9 May	1	0
			24 May	66	0
			6 June	94	1237
			20 June	10	1850
			4 July	83	2654
			18 July	27	569
			2 August	30	137
			16 August	10	80
			29 August	8	9
			9 September	5	0
2023	L	Emergence cage	9 May	2	Not sampled
		Sweep net samples	9 May	13	0
			23 May	108	0
			6 June	155	2729
			20 June	51	866

Year	**Field**	**Sampling Type**	**Collection Dates**	**Alfalfa weevil adults**	**Alfalfa weevil larvae**
			4 July	305	2229
			18 July	116	1184
			2 August	165	345
			16 August	5	29
			29 August	2	4
			9 September	2	0
2023	M	Emergence cage	9 May	63	Not sampled
		Sweep net samples	9 May	8	0
			24 May	4	0
			6 June	107	2286
			20 June	12	1352
			4 July	31	3615
			18 July	95	729
			2 August	24	209
			16 August	6	60
			29 August	3	12
2023	N	Emergence cage	9 May	17	Not sampled
		Sweep net samples	9 May	0	0
			23 May	16	0
			6 June	35	0
			20 June	56	1597
			4 July	19	1269
			18 July	123	1351
			2 August	69	938
			16 August	17	663
			29 August	3	10
			9 September	1	0
2023	O	Emergence cage	9 May	0	Not sampled
		Sweep net samples	9 May	0	0
			23 May	16	0
			6 June	7	1177
			20 June	1	421
			4 July	37	840
			18 July	13	142
			2 August	3	102

Year	Field	Sampling Type	Collection Dates	Alfalfa weevil adults	Alfalfa weevil larvae
			16 August	4	0
			29 August	1	1
			9 September	2	1
2023	P	Emergence cage	9 May	0	Not sampled
		Sweep net samples	9 May	0	0
			23 May	8	0
			6 June	13	79
			20 June	1	87
			4 July	14	241
			18 July	11	51
			2 August	3	68
			16 August	2	0
			29 August	0	1
			9 September	1	5

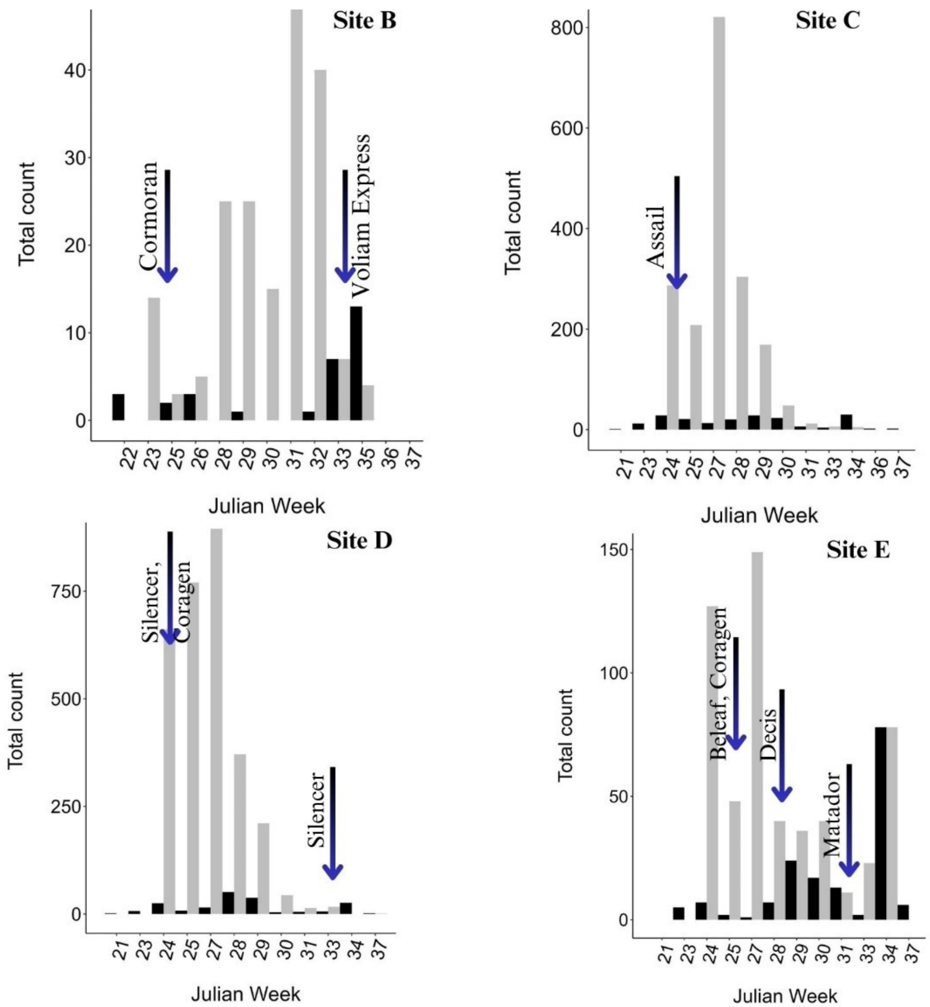

Figure A1. Bar plot showing the total alfalfa weevil adults (black) and alfalfa weevil larvae (grey) from sweep samples (2021). The graph shows the insecticide application week (arrow) labelled with the insecticides applied.

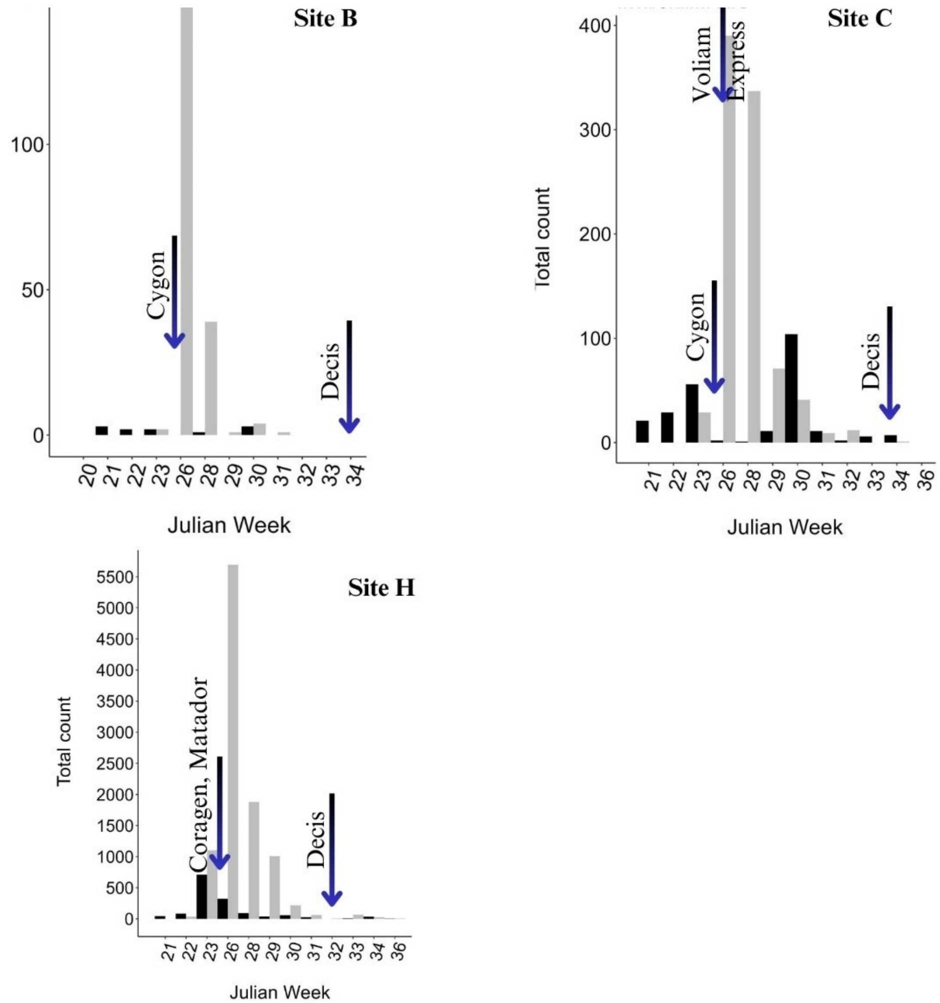

Figure A2. Bar plot showing the total alfalfa weevil adults (black) and alfalfa weevil larvae (grey) from sweep samples (2022). The graph shows the insecticide application week (arrow) labelled with the insecticides applied.