Optimization of semiochemical monitoring for pea leaf weevil, *Sitona lineatus* (Coleoptera: Curculionidae), in the Prairie Provinces

by

Amanda St.Onge

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Department of Biological Sciences
University of Alberta

Abstract

The pea leaf weevil, *Sitona lineatus* Linnaeus (Coleoptera: Curculionidae) is an invasive pest of increasing concern to pulse producers in the Canadian Prairie Provinces. Pea leaf weevil larvae cause damage to field pea (*Pisum sativum)* and faba bean (*Vicia faba*) crops by feeding on root nodules which contain nitrogen-fixing *Rhizobium* bacteria. Larval feeding reduces the nitrogen balance of the pea and bean crops, causing a reduction in the number and quality of pods, as well as reducing the amount of fixed nitrogen available in the soil for future crops. Larval feeding is difficult to monitor but adult weevils are active aboveground, particularly during spring and fall dispersal to reproductive or overwintering sites, respectively. Both sexes of adults are attracted to semiochemicals, including the male-produced aggregation pheromone (4-methyl-3,5-heptanedione) and host plant volatiles ((*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, and linalool). The purpose of this research is to develop an optimal semiochemical trap, which reliably attracts and retains PLW, is related to PLW activity in fields, is cost effective, and can be used for monitoring pea leaf weevil in field pea crops in the Canadian Prairie Provinces.

Different combinations of semiochemicals at various release rates were tested in pitfall traps positioned at the edge of pea crops in southern Alberta. Weevils were attracted to aggregation pheromone lures in both the spring and fall activity periods; the addition of host plant volatiles to the pheromone lure sometimes enhanced weevil captures, especially in the fall. Of the various trap types tested to capture and retain weevils including various cone traps, sticky traps, unitraps, and pitfall traps, the pitfall traps were the most successful.

A secondary objective of this research was to investigate seasonal plasticity in pea leaf weevil response to semiochemicals. Male and female pea leaf weevil adults were tested individually in a 4-way olfactometer for their response to four natural odour sources: 1) five male pea leaf weevils; 2) five male pea leaf weevils on pea plants; 3) pea plants; or 4) a blank control. Weevils in three physiological states were tested in the olfactometer: newly eclosed, recently overwintered, and reproductively active. The response of pea leaf weevils in the olfactometer bioassays did not differ with weevil physiological state or sex. Weevils of all physiological states and both sexes responded preferentially to odours released by male pea leaf weevils.

The semiochemical traps developed here can be used to determine the presence of pea leaf weevil in its expanded range. These semiochemical traps are also useful to monitor the arrival of pea leaf weevil into a pea crop at the start of the season, to better time the application of foliar insecticides. Further research relating captures of PLW in the fall to weevil damage in the upcoming spring would provide pea producers with a method to predict upcoming damage.

Preface

This research is part of a collaborative work led by Dr. Maya Evenden of the University of Alberta and Dr. Hector Cárcamo of Agriculture and Agrifood Canada. I was responsible for data collection and analysis, literature review, and manuscript composition. Dr. Evenden, Dr. Cárcamo and Scott Meers of Alberta Agriculture and Forestry were involved with concept formation. Drs. Evenden and Cárcamo were also involved with manuscript composition and editing.

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1.1 Introduction

With a changing climate and ever-increasing population, global food security is increasingly important. Insect pests can wreak havoc on agricultural systems, but can also be effectively managed with modern, science-based approaches. Management practices based on the biology of the target pest can be effective while minimizing the impact on non-target species and the environment. This concept is known as integrated pest management (IPM) and is commonly used in agricultural systems today. Integrated pest management programs utilize information about the biology of the pest insect, its host plants and associated natural enemies to minimize non-target effects of management while reducing pest activity below economically damaging levels (Kogan 1998; Witzgall *et al.* 2010; Barzman *et al.* 2015). This usually results in a more holistic and sustainable approach to insect management than the simple liberal application of pesticide. Many IPM techniques have been developed in the past 50 years, especially after the negative consequences of unrestrained use of pesticides such as DDT were brought to light in the 1960s (Carson 1962; Kogan 1998; Barzman *et al.* 2015). Such techniques are particularly important in large monocultures that are susceptible to attack by invasive species (Altieri and Letourneu 1982). To maintain these food systems, it is important to identify potential insect pests, understand their biology, and implement an IPM program to manage pests before they disrupt food markets.

The first step in developing an IPM program is to correctly identify the insect species and to determine whether the population is pestiferous and causes economic damage to crops. The ability to accurately and sensitively monitor potential pest species, to determine their location and abundance, is essential to IPM; in an IPM program, management only occurs if insect populations are causing economic damage to crops (Kogan 1998; Barzman *et al.* 2015). Semiochemical monitoring traps are useful tools often used in IPM programs to monitor populations of specific insect pests (Bjostad *et al.* 1993; Baker 2008; Witzgall *et al.* 2010). Semiochemicals, or "message-bearing" chemicals, carry an important biological signal for an organism (Law and Regnier 1971). For example, sex and aggregation pheromones signal the locations of potential mates or mating aggregations. Alarm pheromones signal danger to conspecifics and host plant volatiles can be exploited by herbivores for host location (Landolt

1997). Semiochemicals are important for driving insect behaviour, and by manipulating these semiochemicals, humans can alter insect activity (Bjostad *et al.* 1993; Law and Regnier 1971; Witzgall *et al.* 2010). Semiochemical-baited monitoring traps have been developed in many managed systems to monitor and detect specific insects. Semiochemical-baited monitoring traps are especially effective in monitoring pest populations at low density such as newly invasive species or those dispersed across the landscape (Witzgall *et al.* 2010).

Semiochemical monitoring traps are often baited with pheromones of the target insect (Landolt 1997; Baker 2008; Witzgall *et al.* 2010). Pheromones, which are used by organisms for intraspecific communication, are highly species-specific signals; traps baited with pheromone lures should primarily attract the target species (Law and Regnier 1971). Sex pheromones, which are released by a signalling sex and responded to by a single receiving sex, are common amongst moths (Lepidoptera) and are the most commonly used semiochemicals as insect trap lures (Landolt 1997; Agelopoulos *et al.* 1999). Aggregation pheromones signal the locations of conspecifics, usually to both male and female receivers, for the purpose of forming mating or overwintering aggregations (Landolt 1997; Agelopoulos *et al.* 1999). Aggregation pheromones are commonly used for intraspecific communication in beetles, especially weevils and bark beetles (Coleoptera: Curculionidae) and have also been incorporated into trap lures for use in IPM (Landolt 1997; Agelopoulos *et al.* 1999; Law and Regnier 1971).

Host plant volatiles are important semiochemical signals used by phytophagous insects for host location (Bjstad *et al.* 1993; Landolt and Phillips 1997; Szendrei and Rodriguez-Saona 2010). Host plant volatiles act as kairomones that insects detect and benefit from at a cost to the plant; i.e. when host plant volatiles are utilized by insects to locate feeding or oviposition sites (Brown *et al.* 1970). Synthetic copies of host plant volatiles used as lures usually attract a broader range of insects over closer distances than pheromone lures; host plant volailes are generally less specific-signals than pheromones (Landolt and Phillips 1997; Szendrei and Rodriguez-Saona 2010). Host plant volatiles and pheromones can act synergistically to attract insects and therefore combined lures that incorporate both signals can be the most effective (Dickens 1986; Dickens 1989; Oehlschlager *et al.* 1993; Landolt 1997; Landolt and Phillips 1997; Rochat *et al.* 2000; Reddy and Guerrero 2004; Said *et al.* 2011). Semiochemicals can make effective trap lures, but it is important to identify the optimal semiochemical(s) to be used as a lure for a given target insect (Agelopoulos *et al.* 1999).

Insect response to semiochemicals can vary depending on many endogenous and exogenous factors. Endogenous factors include the physiology of the responding insect, such as its age, reproductive or feeding status (Gadenne *et al.* 2016). Exogenous factors include temperature or photoperiod (Caro and Bateson 1986; Leroy *et al.* 1999; Anton *et al.* 2007), or the blend of background volatiles present in the environment (Rusch *et al.* 2016). To respond to a semiochemical, an insect must first detect the odorant by having it bind to the relevant olfactory receptor neurons (Wadhams *et al.* 1982; Dickens 1990; Mustaparta 2002; Park *et al.* 2013). A neural signal is then sent to the antennal lobe, where it is integrated and transmitted via projection interneurons to the mushroom bodies of the protocerbrum (Fahrbach 2006). Depending on how this information is integrated along with other information from the insect's internal and external environment, action potentials may be generated in motor neurons to elicit a behavioural response. Thus, an insect's response to a semiochemical may be variable depending on other sensory inputs during chemoreception. Neuromodulation also occurs with development which allows insects to be differentially responsive to chemical cues throughout their lifecycle (Fahrbach 2006; Anton *et al.* 2007). Because of the plasticity of insect response to semiochemicals, semiochemical-based monitoring tools may be attractive to different subsets of the population at different times of year and under different environmental conditions (McNeil 1991).

Semiochemicals are important biological signals for insects and can be exploited for use in IPM (Bjostad *et al.* 1993; Baker 2008; Witzgall *et al.* 2010). The most basic use of semiochemicals in IPM is to monitor pest populations (Bjostad *et al.* 1993; Baker 2008; Witzgall *et al.* 2010). Semiochemical-baited monitoring traps can determine an insect pest's presence in an area or they can be further developed to monitor insect activity or population density (Jones *et al.* 2009; Miluch *et al.* 2013; Mori *et al.* 2014). Monitoring insect populations and activity can be useful information for producers, who may use trap captures to guide management decisions, such as when to seed or apply insecticide. Semiochemicals can also be used to manipulate or control insect populations (Bjostad *et al.* 1993; Baker 2008; Vankosky *et al.* 2009; Witzgall *et al.* 2010). For example, semiochemical baits can be combined with a mortality agent for mass-trapping or attract-and-kill strategies (Smit *et al.* 1997; Smith 1998; El-Sayed *et al.* 2006; El-Sayed *et al.* 2009). Smart *et al.* (1994) describe a push-pull trap cropping system using PLW pheromone to attract PLW to the trap crop and neem oil as a feeding deterrent to push PLW pheromone from the main pea crop. Pheromone sources can also be manipulated within a field to disrupt the mate finding behaviour of the target insects (Pickett *et al.* 2014; Mori and Evenden (2014)). Semiochemical-based management tools are particularly useful when the target pest occurs at low densities, such as during the eradication of an invasive species (El-Sayed *et al.* 2006; El-Sayed *et al.* 2009; Witzgall *et al.* 2010). Because insect behaviour is heavily tied to olfaction, semiochemicals can be used to monitor or manipulate insect behaviour in an IPM program.

There are a number of notable examples for which semiochemical-based IPM strategies have been successfully employed to manage weevil pests. Monitoring traps baited with pheromone and synthetic cotton volatiles were crucial in the eradication program targeting the invasive cotton boll weevil, *Anthonomis grandis* (Coleoptera: Curculionidae), in the southern USA (Smith 1998). The identification of the four-component male-produced boll weevil aggregation pheromone (Tumlinson *et al.* 1969) and of cotton plant volatiles that synergize weevil attraction to pheromone (Dickens 1986; Dickens 1989), led to the development of semiochemical-baited monitoring traps to detect weevil activity and guide pesticide application. Semiochemical traps were also used to confirm the efficacy of pesticide applications (Smith 1998). Semiochemical-based mass-trapping has been a successful IPM tactic for control of American palm weevil, (*Rhynchophorus palmarum* (Coleoptera: Curculionidae)), an economically important pest of oil and coconut palms in Central and Southern America and a vector of red ring disease (Baker 2008; Witzgall *et al.* 2010). The identification of the male-produced pheromone rhynchophorol ((4*S*)-2-methyl-(5*E*)-hepten-4-ol) (Rochat *et al.* 1991) led to the development of a mass-trapping tactic (Oehlschlager *et al.* 2002). Host plant volatiles also enhance the attraction of palm weevils to pheromone-baited traps (Oehlschlager *et al.* 1993; Rochat *et al.* 2000). Mass-trapping with pheromone traps reduced the number of red-ring infested trees by 80% in the first year of trapping and by 99.5% after nine years (Oehlschlager *et al.* 2002). This program has since been adopted across Central and South America (Baker 2008). It has also been adapted for red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), a pest of palms in the Middle East (Abbas *et al.* 2005). A semiochemical-based

IPM program aided control of sweetpotato weevil (*Cylas* spp.; Coleoptera: Curculionidae*)*, a worldwide pest of sweet potato (Smit *et al.* 1997). The female-produced sex pheromone ((*Z*)-3-dodecen-1-ol(*E*)-2-butenoate) of *C. formicarius* (Heath *et al.* 1986), is used as a semiochemical trapping lure (Heath *et al.* 1991; Jansson *et al.* 1991). Pheromone-baited traps have been used to monitor invasive populations of *C. formicarius* populations in Africa (Parker *et al.* 1992) and to mass-trap males and reduce *C. formicarius* populations in Asia (Smit *et al.* 1997; Hwang and Hung 1991; Pillai *et al.* 1993).

The pea leaf weevil (PLW), *Sitona lineatus* L. (Coleoptera: Curculionidae) is an invasive pest of field pea (*Pisum sativum*) and faba bean (*Vicia faba*) that is expanding its range into Canada's main regions of pulse production. This species was described from Mediterranean Europe and northern Africa but invasive populations are present in pea-producing regions of northern Europe, Africa, Asia and North America (Vankosky *et al.* 2009). In North America, PLW was first reported in British Columbia in 1930 (Downes 1938), the northwestern USA in the 1930s and the northeastern USA in the 1980s (Hoebecke and Wheeler 1985), and in Florida in 2002 (Bloem *et al.* 2002). The first report of the PLW in the Canadian Prairie Provinces was in 1995, near Swift Current, Saskatchewan (Pepper 1999). In 1997, the PLW was reported in Alberta near Lethbridge (Vankosky *et al.* 2009). For the past two decades, PLW range has expanded to the east and north in the Prairie Provinces (Cárcamo and Meers 2007; Vankosky *et al.* 2009). Prior to 2014, damaging PLW populations were located south of Highway 1 and west of Highway 2 in Alberta. However, in 2016, damaging populations were located considerably further north, near Athabasca, Alberta and in 2017, damaging populations are expected along Highway 2 near Red Deer, Alberta (Stamm 2016). Canadian pulse producers would benefit from the development of a reliable, accurate system to monitor the ongoing PLW invasion in the Prairie Provinces.

Pea leaf weevils are most damaging in the larval stage, but the adults are more active and easy to target for monitoring. Pea leaf weevil larvae cause damage by feeding on the root nodules of pea or faba bean plants (Jackson 1920). These root nodules are specialized structures that house the nitrogen-fixing bacterium *Rhizobium leguminosarum* (Doyle and Luckow 2003). By feeding on the root nodules, PLW larvae reduce the amount of nitrogen available to the host plant, causing a reduction in the number and nitrogen content of pods and therefore, a reduction in yield (Hunter 2001; El-Dessouki 1971). The amount of fixed nitrogen available in the soil for future crops to uptake is also reduced (Corre-Hellou and Crozat 2005). Larval feeding activity occurs in June and July in Canada and peaks when pea plants are flowering (Jackson 1920). After completing five instars, larvae will pupate in the soil. Adult PLW emerge in July and August, when they feed on most available green legume plants including volunteer pea or faba bean seedlings in harvested fields or perennial legumes, such as alfalfa (*Medicago sativa)* or clover *(Trifolium* spp.) (Jackson 1920; Fisher and O'Keeffe 1979; Landon *et al.* 1997). Adult PLW do not undergo a true diapause and may continue to feed on primary and secondary hosts during winter if conditions are suitable (Schotzko and O'Keeffe 1986). Adult reproductive development occurs from the time of eclosion in July or August until the following March. During this time, female PLW are expected to require feeding on primary hosts, pea or faba bean, in order for oogenesis to occur (Schotzko and O'Keeffe 1986). In the spring, PLW adults emerge from overwintering sites, presumably near secondary host plants, and disperse to their primary host plants to form mating aggregations (Jackson 1920; Fisher and O'Keeffe 1979). Mating occurs on the primary host plants starting in March or April followed by oviposition in the soil (Jackson 1920). Pea leaf weevils mate multiple times (personal observations), similar to the related clover root weevil (*Sitona lepidus)* (Gerard *et al.* 2005) and each female PLW is capable of upwards of 3000 eggs in their lifetime (Schotzko and O'Keeffe 1988). Jackson (1920) reported that eggs hatch in approximately three weeks in the United Kingdom. Egg development is dependent on temperature, with eggs requiring only 6.3 ± 0.5 days to hatch at 29°C and 70 ± 2.5 days at 8°C (Lerin 2004). Eggs are also susceptible to low humidity, and desiccated eggs will not hatch (Jackson 1920; Fisher 1977). Upon hatching, larvae will burrow into the soil in search of *Rhizobium* –containing root nodules on pea or faba bean plants. Although the larvae are the most damaging stage, PLW adults are more active and therefore easier to target for monitoring or control (Jackson 1920; Vankosky *et al.* 2009).

Pea leaf weevil adults disperse two times per year: in the spring when locating reproductive sites and during the fall while searching for suitable overwintering sites. Both sexes of PLW are attracted to the male-produced pheromone (4-methyl-3,5-heptanedione) and to host plant volatiles during the spring (Blight *et al.* 1984; Blight and Wadhams 1987) Nielsen and Jensen 1993; Landon *et al.* 1997) and fall dispersal periods (Evenden *et al.* 2016). Semiochemical traps baited with synthetic pheromone or with synthetic pheromone and bean volatiles (linalool, (*Z)*-3-hexenol, and (*Z)*-3-hexenyl acetate) attract both male and female PLW in the spring in pea fields in the United Kingdom and Denmark (Blight *et al.* 1984; Nielsen and Jensen 1993). Adult PLW are most responsive to host plant odours, especially (*Z*)-3-hexenyl acetate, during the two dispersal periods in both behavioural an electrophysiological analyses (Landon *et al.* 1997). In both olfactometer and electroantennogram assays, PLW response to (*Z*)-3-hexenyl acetate is negligible during the winter, indicating that PLW adults have a plastic response to semiochemical cues. Interestingly, this seasonal plasticity includes an initial attraction to (*Z*)-3-hexenyl acetate, followed by a period of negligible response, then a period of increased attraction with attraction decreasing towards the end of the imaginal life (Landon *et al.* 1997). Adult PLW are long-lived, and are in different physiological states during the two main periods of their adult activity (Jackson 1920; Schotzko and O'Keeffe 1986). It is unknown if PLW response to aggregation pheromone is also plastic between seasons of activity. Evenden *et al.* (2016) were the first to demonstrate that PLW respond to pheromone-baited traps during the fall as well as the spring dispersal period. It is unknown, however, if PLW response to aggregation pheromone decreases during the winter months or towards the end of the adult life, similar to PLW response to (*Z*)-3-hexenyl acetate (Landon *et al.* 1997). In addition, little is known about differential responses of male and female PLW to host or conspecific-produced semiochemicals (Szendrei *et al.* 2010).

Pea leaf weevils are suitable candidates for a semiochemical-based monitoring program because they are long-lived and highly active, allowing a wide window in which to target monitoring of these insects. Pea leaf weevil adults walk and fly to colonize fields (Fisher and O'Keeffe 1979; Nielsen and Jensen 1993), and although their flight capacity is unknown, PLW are able to move at least between fields within a crop rotation (Kokorin 1966; Harmon *et al.* 1987). The invasion of PLW in the Prairie Provinces is ongoing, and PLW are present in low densities in some areas (Alberta Agriculture and Forestry 2017). Since first reported in the Prairie Provinces, the geographic spread and population densities of PLW have changed drastically from year to year (Vankosky *et al.* 2009; Alberta Agriculture and Forestry 2017). In some areas, pea producers are choosing to use thiamethoxam-coated pea seed to combat PLW damage (Cárcamo *et al.* 2012). Semiochemical-monitoring traps may be useful to assess 1) the presence of PLW in its expanded range in the Prairie Provinces; and 2) densities of PLW where populations are established, which would be useful information for pea producers making management decisions.

Pea leaf weevils are attracted to semiochemicals during both periods of adult activity: when they are reproductively active in the spring (Blight *et al.* 1984; Nielsen and Jensen 1993; Landon *et al.* 1997) and just prior to overwintering in the fall (Landon *et al.* 1997; Evenden *et al.* 2016). Semiochemical-based monitoring of PLW in the spring may allow pulse producers to better time the seeding of their crops, as delaying seeding could protect crops from PLW damage (Dore and Meynard 1995; Quinn *et al.* 1999; Vankosky *et al.* 2009). Pulse producers sometimes use foliar insecticidal sprays to decrease adult weevil populations and prevent oviposition. To be effective, however, insecticidal sprays must be applied as soon as adult PLW are present in the crop (Bardner *et al.* 1983; Ester and Jeuring 1992; Steene *et al.* 1999). Semiochemical traps could monitor PLW arrival into fields before crops emerge and allow pulse producers to apply foliar insecticides in a timely manner (Vankosky *et al.* 2009). Semiochemical-based monitoring in the fall may be even more useful for pulse producers in the Prairie Provinces. Estimating population size of PLW entering overwintering would give producers time to decide whether it is necessary to use insecticide-coated seeds the following spring (Vankosky *et al.* 2009). For semiochemical traps to be useful in an IPM program, a relationship between PLW captures in traps and population density or yield loss due to PLW must be established (Bjostad *et al.* 1993; Nielsen and Jensen 1993; Vankosky *et al.* 2009).

Blight *et al.* (1984) and Nielsen and Jensen (1993) demonstrated that traps baited with aggregation pheromone or with pheromone and bean volatiles were attractive to PLW in fallow or faba bean fields in Europe. Quinn *et al.* (1999) demonstrated that similar pheromone-baited traps were also attractive to PLW in field pea crops in Washington and Idaho. Evenden *et al.* (2016) were the first to test this semiochemical trapping system in pea crops in the Prairie Provinces and the first to demonstrate that these traps also attract and capture PLW in the fall.

1.2 Thesis Objectives:

The objective of this research is to develop a semiochemical-monitoring system for the pea leaf weevil in the Prairie Provinces. Aggregation pheromone (4-methyl-3,5-heptanedione) and host plant volatiles (linalool, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate are effective semiochemical lures in cone traps for monitoring the springtime PLW flight in European farms

(Blight *et al.* 1984; Nielsen and Jensen 1993). A pilot study conducted in 2011 and 2012 found these semiochemicals attractive to PLW in Alberta in both the spring and fall flight periods (Evenden *et al.* 2016). Here, I further evaluate these semiochemical traps, by manipulating release dosage and release device as well as trap type to identify an optimal semiochemical trap for monitoring PLW. An optimal semiochemical trap will reliably attract and capture PLW, be cost-effective, and- the number of weevils ~~PLW~~ captured~~s in these traps~~ will be related to PLW activity in pea fields. I compare the efficacy of PLW aggregation pheromone alone and in combination with bean host volatiles at various release rates released from several release devices. Adult feeding damage and larval density are assessed at sites with semiochemical-baited traps to determine if adult capture in semiochemical-baited traps reflects feeding damage. A thorough study of various trap types was performed to determine an appropriate trapping system to attract and retain adult PLW in the Prairie Provinces.

An additional objective of this research is to test for plasticity in response to semiochemical cues between sexes and throughout the long adult life stage. Semiochemical-trapping experiments are performed at the same sites at the start and end of the growing season, to monitor both the spring and fall PLW dispersal periods and to compare PLW behaviour between periods of dispersal. The sex ratio of PLW adults captured in semiochemical-baited traps during the two dispersal periods is compared among the variously baited semiochemical traps and between trapping periods. Pea leaf weevil response to the same semiochemicals is also studied under controlled conditions in a four-way olfactometer bioassay. Olfactometer bioassays are performed on PLW in three different physiological states: i) recently overwintered and sexually immature; ii) overwintered and sexually mature; and iii) newly eclosed and sexually immature.

Overall, this research aims to further the development of a PLW monitoring tool for pulse producers in the Prairie Provinces, by development of a semiochemical lure and trap and thorough understanding the effect of PLW sex and physiology on response to semiochemicals and captures in semiochemical traps.

1.3 Chapter 1 References

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Chapter 2: Evaluation of pea leaf weevil response to semiochemicals in the lab and field

2.1 Introduction:

The pea leaf weevil (PLW), *Sitona lineatus* L. (Coleoptera: Curculionidae) is an invasive pest of field pea (*Pisum sativum*) and faba bean (*Vicia faba*) that is expanding its range into Canada's main regions of pea production. Canada's peas are produced in the Prairie Provinces (Pulse Canada 2016), where the PLW was first reported in 1995 near Swift Current, Saskatchewan (Pepper 1999). Pea leaf weevil is continuing to expand its range north and east into the Prairie Provinces (Alberta Agriculture and Forestry 2017). Prior to 2014, damaging populations in Alberta were contained south of Highway 1, but in 2014, high levels of damage occurred northeast of Calgary. Pea leaf weevil damage was reported as far north as Athabasca, AB in 2016. In the 2017 growing season, high PLW densities are expected along highway 2 as far north as Red Deer, AB, an area which has not previously used insecticide-treated seed to target PLW (Alberta Agriculture and Forestry 2017).

Vankosky *et al.* (2009) proposed that PLW range expansion into western Canada occurred due to suitable climatic conditions for PLW reproduction and the increased acreage of pulse crops in this area. Climate projection models by Olfert *et al.* (2012) predict continued expansion of PLW into the Prairie Provinces as an effect of global warming. The PLW range expansion is a grave concern for Canadian producers of field pea and faba bean which are reproductive hosts for the PLW (Jackson 1920).

Field pea is an important pulse crop in Canada, with an average of 2.3 million hectares seeded or 4.5 to 5 million tonnes of peas produced per year in Saskatchewan, Alberta, and Manitoba (Pulse Canada 2016). In 2015, $4.2 billion worth of peas was exported from Canada (Pulse Canada 2016). Faba beans are an increasingly popular pulse crop in the Prairie Provinces, with approximately 32 000 hectares seeded in Alberta in 2014 (Alberta Agriculture and Forestry 2015). In Saskatchewan, faba beans were grown on approximately 25 000 hectares in 2015, a substantial increase from the 3400 and 7700 hectares grown in 2013 and 2014, respectively (Fleury and Barker 2016). Both pea and faba bean crops, in Alberta and in Saskatchewan, are at risk to PLW damage.

Pea leaf weevil adults feed aboveground on legumes, causing characteristic feeding notches at the margins of leaves (Jackson 1920). While sexually immature, PLW adults feed on green leaves of most legumes but prefer their reproductive host plants, pea or faba beans, when mating (Landon *et al.* 1995; Landon *et al.* 1997). Adults prefer to feed on new, tender leaves and their foliar feeding results in characteristic U-shaped notches at the margins of plant leaves (Jackson 1920; Landon *et al.* 1995). Defoliation of pea seedlings weakens the plant, but PLW damage depends highly on the timing of weevil attack relative to seeding, and pea plants can recover from low levels of damage with compensatory growth (Dore and Meynard 1995; Williams *et al.* 1995). If PLW attack levels are intense while pea seedlings are young, however, seedlings can be destroyed (Jackson 1920). The risk of adult feeding damage to pea or faba bean crops in the spring is highest when PLW are at high densities or when adults arrive relatively early in the crop phenologies of their reproductive hosts (Nielsen 1990; Williams *et al.* 1995; Vankosky *et al.* 2011a). In the absence of compensatory growth, adult foliar feeding may disrupt the nutrient balance and growth patterns of plants, resulting in a reduction in the number and quality of pods (Nielsen 1990; Vankosky *et al.* 2009). George *et al.* (1962) mechanically damaged pea leaves to mimic adult PLW feeding damage and found that loss of one leaflet at the 4-leaflet stage, which is equivalent to 30 PLW feeding notches, led to an 8% yield loss. This is comparable to the findings of Cárcamo *et al.* (2012), who reported an 11% loss in photosynthetic area of pea seedlings to adult PLW damage. Adult foliar feeding is temperature dependent, with feeding highest between 12°C and 21°C (Landon *et al.* 1995). Adult PLW prefer to feed on younger leaves and stipules (Landon *et al.* 1995); the extent of PLW feeding damage to crops in the spring depends on the time of arrival and on weevil density (Williams *et. al.* 1995). While PLW adult foliar feeding can be damaging, PLW larval feeding is considered much more important (Nielsen 1990; Williams *et al.* 1995; Vankosky *et al.* 2009; Cárcamo *et al.* 2015).

Larval PLW are limited to feeding on the *Rhizobium*-containing root nodules of peas or faba beans (Jackson 1920), both of which are associated with the nitrogen-fixing bacteria *Rhizobium leguminosarum,* specifically the biovar *viciae* (Doyle and Luckow 2003; Vankosky *et al.* 2009). Johnson and O'Keefe (1981) detected *Rhizobium* in the gut of PLW larvae and proposed that PLW larvae feed directly on this bacterium. Larval feeding on *Rhizobium* root nodules damages the current pea crop by reducing root nodulation (Cárcamo *et al.* 2015) and disrupts the nitrogen balance of the plant (El-Dessouki 1971; Hunter 2001; Cárcamo *et al.* 2015);

it also limits availability of fixed nitrogen for future crops in a rotation (Corre-Hellou and Crozat 2005; Cárcamo *et al.* 2015). Younger pea plants at the 2nd node are more susceptible to damage from larval feeding from those at the 5th node (Cárcamo *et al.* 2015)

Larval damage can be prevented by supplying crops with fertilizer, as this prevents root nodulation (Arkhipchenko *et al.* 2005; Vankosky *et al.* 2011b), but this is an unrealistic strategy for pulse producers who grow peas or faba beans as a part of a crop rotation for the purpose or restoring fixed nitrogen levels in the soil. If larval densities are at carrying capacity (17 larvae per plant), 98% of pea root nodules can be destroyed which corresponds to a 27% reduction in pea pods produced, along with a 17% loss in pod nitrogen and 22% loss in pea nitrogen (El-Dessouki 1971). In faba bean, larval and adult feeding damage together can result in yield losses of 28% of pods (Nielsen 1990). Because larval damage is subterranean, it is difficult to monitor.

In the Prairie Provinces, pulse producers attempt to combat potentially damaging PLW populations by sowing seeds treated with thiamethoxam (Cruiser), which was registered on an emergency basis in response to the PLW invasion (Cárcamo and Vankosky 2011; Cárcamo *et al.* 2012). Foliar insecticide sprays do not reliably protect pea yields in Alberta (Cárcamo and Vankosky 2011) and application must be timed to coincide with PLW migration to crops in the spring to prevent egg production and oviposition (King 1981; Bardner *et al.* 1983; Ester and Jeuring 1992; Vankosky *et al.* 2009). The current action threshold for application of foliar insecticide is the presence of adult feeding notches on 30% of clam leaves at the 2 to 3 node stage (Cárcamo and Vankosky 2011). These recommendations are helpful, but more information on PLW damage, yield losses, and economic thresholds is needed for true integrated pest management of this pest.

Yield losses from PLW are difficult to predict as they are likely affected by a number of factors, including first the level of soil nitrogen (Cárcamo *et al.* 2015), the severity of adult and larval feeding damage and the intensity of intraspecific competition (George 1962; Nielsen 1990; Lohaus and Vidal 2010; Vankosky *et al.* 2011a). Some studies have revealed relationships between the number of adult feeding notches and larval damage in peas (Cantot 1980; Dore and Meynard 1995), but others have not (Nielsen 1990). Adult feeding intensity is related to adult population density but larval populations were not related to adult populations in a cage study conducted in Alberta (Vankosky *et al.* 2011a). Studies conducted in other pea growing regions show adult feeding intensity is highly variable and dependant on climate conditions (Landon *et al.* 1995; Lohaus and Vidal 2010).

The current method of estimating PLW populations in the Prairie Provinces is to survey adult feeding damage in pea crops. Adult feeding damage is surveyed annually by Alberta Agriculture and Forestry (2017) and by the Saskatchewan Ministry of Agriculture (2016) to create forecast maps for PLW populations in the upcoming growing season. Pea producers use these forecast maps to decide whether or not to plant Cruiser-treated seeds. Pea producers will also survey adult feeding damage within pea fields at the start of the growing season to make decisions on foliar insecticide applications (Alberta Agriculture and Forestry 2014). Pea leaf weevil behaviour is tied to olfaction, making semiochemical-baited traps a possible tactic to monitor adult PLW populations (Vankosky *et al.* 2009; Evenden *et al.* 2016). A male-produced aggregation pheromone, 4-methyl-3,5-heptandione (Blight *et al.* 1984) attracts both male and female PLW adults in the spring (Blight *et al.* 1984; Nielsen and Jensen 1993; Quinn *et al.* 1999; Evenden *et al.* 2016) and fall (Evenden *et al.* 2016). Pea leaf weevil adults also use host plant volatiles to locate suitable host plants (Landon *et al.* 1995; Landon *et al.* 1997) and are responsive to these cues during both periods of adult activity (Landon *et al.* 1997). Exploitation of these semiochemicals as artificial lures in insect traps could be a useful tool for monitoring the PLW range expansion in the Prairie Provinces (Vankosky *et al.* 2009; Evenden *et al.* 2016).

Semiochemical lures targeting PLW have been tested in a number of studies. Cone traps baited with male PLW feeding on faba bean plants attract PLW in faba bean fields in the spring in the United Kingdom (Blight and Wadhams 1987). These attractive semiochemicals were identified as the male-produced aggregation pheromone, 4-methyl-3,5-heptanedione, and the attractive volatiles released from faba beans; (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, and linalool (Blight *et al.* 1984). Synthetic copies of these semiochemicals attract PLW to traps positioned in faba beans in the spring (Blight *et al.* 1984). Landon *et al.* (1995) proposed that (*Z*)-3-hexenyl acetate, which makes up a large percentage of the volatile profile of field peas and faba beans, compared to other legumes, may be an especially important volatile for host-plant detection by PLW. Semiochemical-baited cone traps were successfully deployed to monitor PLW during the spring migration in faba bean crops in Denmark (Nielsen and Jensen 1993). Pea leaf weevil adults respond to pheromone-baited cone traps positioned in North American pea fields during the spring migration in Washington State (Quinn *et al.* 1999). Evenden *et al.* (2016) were the first to show that PLW respond to semiochemical-baited traps in both the spring and fall activity periods in studies conducted in the Canadian Prairie Provinces. Studies of PLW semiochemical traps performed in Europe found cone traps to be successful at capturing and retaining adults (Blight *et al.* 1984; Blight and Wadhams 1987; Nielsen and Jensen 1993). Evenden *et al*. (2016) found that these cone traps were unsuccessful in Alberta and instead used pitfall traps constructed from Solo cups and Choloplast lids. Besides the semiochemical lure, the type, colour, and placement of an insect trap contribute to the attractiveness of a semiochemical trap to a target insect (Reddy *et al.* 2011).

The objective of this research is to continue to develop a semiochemical trap to monitor established and expanding populations of PLW in the Prairie Provinces. An optimal semiochemical trap will reliably capture PLW, be cost-effective, and PLW captures in traps will be correlated with PLW activity in pea fields. In this experiment, semiochemical lures similar to those used by Blight *et al.* (1984) and Evenden *et al.* (2016) are tested, at the same and at higher release rates, and in various combinations, to determine an optimal semiochemical lure for PLW. Various insect traps are also tested in an attempt to find a user-friendly trap. Differences in PLW response between seasons, sexes, and with age are also studied to better understand how PLW olfaction is related to physiology throughout the PLW adult stage.

2.2 Methods

2.2.1 Semiochemical blend, dose and lure type

In order to establish an optimal combination of semiochemicals to use as a lure for PLW in pea fields, a series of field experiments were performed in 2013, 2014, and 2015 testing the attraction of PLW adults to various semiochemical lures that consisted of the PLW aggregation pheromone with or without various host plant volatiles (Tables 1, 3 and 4). The PLW aggregation pheromone, 4-methyl-3,5-heptanedione, was synthesised by collaborators at Scotts Canada, Delta BC (Evenden *et al.* 2016) and dispensed into various release devices depending on the experiment. The bean volatiles tested were those identified previously as released from faba bean shoots: (*Z*)-3-hexen-1-yl acetate; (*Z*)-3-hexen-1-ol; and linalool (Blight *et al.* 1984).

Commercial sources (Scotts Canada) of the bean volatiles ((*Z*)-3-hexen-1-yl acetate; (*Z*)-3-hexen-1-ol; and linalool) were used to formulate the lures (Tables 1 to 4). Lures were shipped in refrigerated containers to the University of Alberta and stored at 4°C for 1-3 days before transport to the field in refrigerated containers and at -20°C for longer-term storage between trapping experiments. At each field site (n = 9-14) in southern Alberta (Appendix A), lures were positioned in pitfall traps (Appendix B, Figure B-1). Pitfall traps consisted of 473 ml plastic cups (Solo, Lakeforest, Illinois, United States of America) positioned in the ground so that the top of the cup was flush with the surface. Captured insects were preserved in propylene glycol antifreeze (Prestone, USA) that was refreshed at each trap check. Lures were hung from a 15 x 15 cm piece of white plastic Coroplast that was positioned above each trap and secured into the ground with 15 cm nails. The white Coroplast also served as a canopy to protect the trap from rain. Baited pitfall traps were positioned 25 m apart in random order along a linear transect 1 m from the edge of pea fields at each site in southern Alberta. In 2013 and 2014, experiments were performed during the spring and fall periods of adult activity. In 2015, semiochemical trapping was conducted continuously throughout the growing season. Semiochemical lures were replaced at the beginning of each activity period in 2013 and 2014 and at 6 week intervals in 2015. Traps were checked weekly, weevils were removed from the propylene glycol, and the number of PLW in each trap was recorded. Captured PLW were separated by sex based on the terminal segments of the abdomen following Jackson (1920). The condition of captured weevils in 2014 and 2015 was used to estimate age category of the individuals as newly eclosed (scales present; flexible elytra) or overwintered (loss of scales; rigid elytra).

2.2.1.1 Trapping Experiment 2013

In 2013, the attractiveness of pitfall traps baited with one of two doses of pheromone (21 mg and 42 mg of 4-methyl-3,5-heptanedione) presented in one of two release devices (250 µl and 400 µl Eppendorf tubes), with and without host plant volatiles were tested and compared to unbaited control traps) (Table 1) at ten sites in southern Alberta (Appendix A, Table A-1, Figure A-1). Release rates for lures are given in Table 2. Testing two different pheromone release devices allowed us to test two different release rates of pheromone (0.18 mg/day or 0.5 mg/day at 30°C for 250 µl and 400 µl Eppendorf tubes, respectively). Pheromone dose determined the longevity of the lure, with higher doses of pheromone having greater longevity. The pheromone lure with the shortest longevity is 21 mg of pheromone in a 400 µl tube which is expected to last for 6 weeks at a constant 30°C.

Semiochemical lures were tested in pitfall traps (Appendix B, Figure B-1) placed 25 m apart along a linear transect at the edge of each field in mid-May. Traps were placed in the field within 5-days post-seeding and were checked every 5-8 days until late June, 2013 (Spring 2013 trapping period). Traps were rebaited with fresh lures and trap order was re-randomized at the same sites in early-August, 2013. Traps were checked weekly until mid-September (Fall 2013 trapping period).

Table 1. Pheromone lures of different doses released from different release devices with and without host plant volatiles tested for attractiveness to pea leaf weevil (*Sitona lineatus*) in spring and fall 2013.

Pheromone dose and release device tested		Host plant volatiles tested		
Pheromone[1] Dose (mg)	Pheromone Release Device	(*Z*)-3-hexenyl acetate Dose (mg) in 250 µl Eppendorf	(*Z*)-3-hexenol Dose (mg) in 250 µl Eppendorf	Linalool Dose (mg) in 250 µl Eppendorf
21	250 µl Eppendorf	0	0	0
21	250 µl Eppendorf	21	34	3x50
21	400 µl Eppendorf	0	0	0
21	400 µl Eppendorf	21	34	3x50
42	250 µl Eppendorf	0	0	0
42	250 µl Eppendorf	21	34	3x50
42	400 µl Eppendorf	0	0	0
42	400 µl Eppendorf	21	34	3x50
0	None	0	0	0

[1] Pea leaf weevil pheromone: 4-methyl-3,5-heptanedione

Table 2. Release rate data from semiochemical lures tested in pea fields in 2013, 2014 and 2015. Release rates were determined gravimetrically by our semiochemical lure supplier, Scott's Canada.

Semiochemical	Dose	Release device	Release rate (mg/day) @ 20°C	Release rate (mg/day) @ 30°C
4-methyl-3,5-heptanedione	21 mg	250 µl Eppendorf	0.03	0.18
	42 mg	250 µl Eppendorf	0.03	0.18
	21 mg	400 µl Eppendorf	0.2	0.5
	42 mg	400 µl Eppendorf	0.2	0.5
(*Z*)-3-hexenyl acetate	21 mg	250 µl Eppendorf	0.35	1.6
	700 µl	Bubble cap	13	N/A
(*Z*)-3-hexenol	34 mg	250 µl Eppendorf	0.15	0.28
	700 µl	Bubble cap	3.7	17
linalool	50 mg	250 µl Eppendorf	Not detected	0.04
	200 µl	Bubble cap	4	N/A

In the spring 2013 trapping period, the Eppendorf tubes were heat sealed prior to shipment to the University of Alberta. Probably due to air pressure changes during air transport, some of the seals leaked and pheromone was released to the outside of the tube on some lures (21 mg of pheromone in 400 µl tubes, 42 mg of pheromone in 250 µl tubes, and 42 mg of pheromone in the 400 µl). To correct for this, a metal crimp cap was used in addition to heat sealing the Eppendorf tubes for lures used in fall 2013 and subsequent trapping periods. To account for the different Eppendorf tubes (capped versus uncapped) used in the spring and fall 2013 trapping periods, the spring and fall trap captures were analyzed separately and not directly compared.

2.2.1.2 Trapping Experiment 2014 The two most attractive pheromone lures determined from the 2013 experiment (21 mg of 4-methyl-3,5-heptanedione in 250 µl or in 400 µl Eppendorf tubes) were tested again in 2014 (Table 3). The host plant volatile lures tested in 2014 included those tested in 2013 and higher release rate lures that are commercially available (Scotts Canada, Delta BC) (Table 3). These lures were tested in pitfall traps placed 25 m apart in a linear transect 1 m from the edge of nine pea fields in southern Alberta (Appendix A, Table A-2, Figure A-2). Lures were placed in the field in mid-May, within 5 days post-seeding and checked weekly for 4 to 6 weeks (Spring 2014 trapping period). Traps were rebaited with fresh lures and trap order was re-randomized at the same sites in mid-August, 2014. Traps were checked weekly from mid-August to early September and a final time after two weeks, in mid-September (Fall 2014 trapping period).

In 2014, non-target Curculionidae captured in semiochemical traps were also collected and identified to genus (*Otiorhynchus, Ceutorhynchus, Perapion, Hypera,* and *Sitona).* The number of non-PLW *Sitona* compared to *S. lineatus* that were captured in traps in 2014 was determined.

Table 3. Semiochemical lures of different doses released from different release devices tested for attractiveness to pea leaf weevil *(Sitona lineatus)* adults in spring and fall 2014.

Pheromone dose and release device tested	Host plant volatiles tested		
Pheromone[1] dose (mg) in 250 µl Eppendorf	(*Z*)-3-hexenyl acetate dose and release device	(*Z*)-3-hexenol dose and release device	Linalool dose and release device
0	21 mg, 250 µl Eppendorf	34 mg, 250 µl Eppendorf	3x50 mg, 250 µl Eppendorf
21	21 mg, 250 µl Eppendorf	34 mg, 250 µl Eppendorf	3x50 mg, 250 µl Eppendorf
42	21 mg, 250 µl Eppendorf	34 mg, 250 µl Eppendorf	3x50 mg, 250 µl Eppendorf
0	700 µl, bubble cap	700 µl, bubble cap	200 µl, bubble cap
21	700 µl, bubble cap	700 µl, bubble cap	200 µl, bubble cap
42	700 µl, bubble cap	700 µl, bubble cap	200 µl, bubble cap
21	0	0	0
42	0	0	0
0	0	0	0

[1] Pea leaf weevil pheromone: 4-methyl-3,5-heptanedione

2.2.1.3 Trapping Experiment 2015

The optimal lures identified in trapping experiments in 2013 and 2014 were tested in 2015: the low pheromone dose with and without the low dose of host plant volatiles (Table 4). Lures were randomly placed in two pitfall traps separated by 50 m at the edge of 14 pea fields in southern Alberta (Appendix A, Table A-3, Figure A-3). Traps were placed during the last week of April within 3 days following seeding and were checked weekly until the first week of September. Semiochemical lures were replaced with fresh lures on week 7 and 13 of trap check, resulting in three trapping periods (Spring, Summer, Fall 2015 trapping periods). Trap catch was monitored weekly throughout the season.

Table 4. Semiochemical lures tested for attractiveness to pea leaf weevil *(Sitona lineatus)* adults in spring, summer and fall 2015.

Pheromone dose and release device tested	Host plant volatiles tested		
Pheromone[1] dose (mg) in 250 µl Eppendorf	(*Z*)-3-hexenyl acetate dose and release device	(*Z*)-3-hexenol dose and release device	Linalool dose and release device
21	0	0	0
21	21 mg, 250 µl Eppendorf	34 mg, 250 µl Eppendorf	3x 50 mg, 250 µl Eppendorf

[1] Pea leaf weevil pheromone: 4-methyl-3,5-heptanedione

In 2015, the number of PLW captured in baited traps was compared with other measurements of weevil density, including adult feeding damage and larval density. Adult feeding damage was assessed when pea plants were at the 5th or 6th-node stage which is the time when adult feeding damage correlates with adult population density (Vankosky *et al.* 2011a). The characteristic adult feeding notches were counted on 10 pea plants within 1m of each semiochemical trap between the 6th and 7th trap checks (11-13 June). Larval feeding damage was surveyed during pea flowering, when larval activity peaks (Dore and Meynard 1995) between the 7th and 8th trap checks (19-21 June). Three plants were chosen within 1 m of each trap and a 15 cm x 5 cm soil core sample was taken around each plant. In addition, a soil sample around another three plants within 1m of each other was taken equidistantly from each of the traps, 10 m into the field. Soil from core samples was processed through a #30 mesh sieve and roots were washed and dissected to count all larvae in each core sample (Nielsen 1990).

2.2.1.4 Statistical Analyses

To determine which semiochemical lures were most effective, PLW captures were analyzed using generalized linear mixed models with negative binomial error distributions created using the function *glmer.nb* in the lme4 package (version 1.1-10) of the statistical program R (version 3.1.3). Models were selected based on best fit by comparing AIC values, –log likelihood values, and the distribution of residuals. For the statistical analyses, the total number of PLWs captured per trap in each season (ie. the sum of PLW captured each week) was used as a response variable, with (1|Site) as a blocking term. Repeated measures models with a (Week|Site) random term were also built, but season total models with (1|Site) were used based on better fit. Model fits were optimized using the methods described in the *Troubleshooting* section of the lme4 package documentation (R version 3.1.3). Models were first constructed with all possible interaction terms included and non-significant interaction terms were subsequently removed in a step-wise manner. Relevant p-values were calculated using the *Anova* function in the package car (version 2.0-25) (R version 3.1.3). Post-hoc Tukey's multiple comparisons tests were performed on factors with more than one level using the function *ghlt* in the package multcomp (version 1.4-4) (R version 3.1.3). Similar models were attempted to determine if the captures of each non-target weevil genera was affected by pheromone or host plant volatile lures, but the counts of non-target weevils were too low for this analysis. The effect of seasonality on PLW trap capture was tested only in 2014 (Spring, Fall) and 2015 (Spring, Summer, Fall). In both years, negative binomial mixed effects models that incorporated a season factor were used to compare trap capture across season. A summary of the statistical models used in this analyses are included in Table 5.

Table 5. Statistical models for 2013, 2014 and 2015 semiochemical trapping experiments.

Year	Model Structure	Purpose
2013	glmer.nb(Total PLW per season in 2013 ~ Volatiles[1] present in lure + (1|Site))	Compares PLW captures in unbaited (blank) traps to traps baited with pheromone and pheromone or host plant volatiles. Site is included as a block.
	glmer.nb(Total PLW per season in 2013 ~ Pheromone dose[2] + Pheromone release device[3] + host plant volatiles[4] + (1|Site))	Compares PLW captures in baited semiochemical traps based on pheromone dose, pheromone release device and the presence or absence of host plant volatiles. Site is included as a block.
	glmer.nb(Total PLW per season in 2013 ~ Lure[5] + (1|Site))	Compares all baited semiochemical traps for spring 2013 and for fall 2013 to each other and to an unbaited (blank) trap. Site is included as a block.
2014	glmer.nb(Total PLW in 2014 ~ Season[8] * Pheromone lure[6] * host plant volatile lure[7] + (1|Site))	Compares PLW captures in traps for 2014 based on the season (spring or fall), the pheromone lure present (none, 21 mg pheromone in 250 µl Eppendorf or 42 mg pheromone in 250 µl Eppendorf) and based on the host plant volatile lure (none, low release rates from Eppendorf tubes or high release rates from bubble caps) present. Site is included as a block.
	glmer.nb(Total PLW per season in 2014 ~ Pheromone lure[6] + host plant volatile lure[7] + (1|Site))	Compares PLW captures in traps for spring 2014 or for fall 2014 based on the pheromone lure present (none, 21 mg pheromone in 250 µl Eppendorf or 42 mg pheromone in 250 µl Eppendorf) and based on the host plant volatile lure (none, low release rates from Eppendorf tubes or high release rates from bubble caps) present. Site is included as a block.
	glmer.nb(Total PLW per season in 2014 ~ Lure[9] + (1|Site))	Compares PLW captures in all semiochemical traps and in unbaited (blank) traps for spring 2014 or for fall 2014. Site is included as a block.
2015	glmer.nb(Total PLW in 2015 ~ Lure[10] + Season[11] + (1|Site))	Compares PLW captures in 2015 based on season (spring, summer or fall) and on the semiochemical lure present (pheromone or pheromone plus host plant volatiles). Site is included as a block.
	glmer.nb(Total PLW per season in 2015 ~ Lure[10] + (1|Site))	Compares PLW captures in spring 2015, summer 2015 or fall 2015 based on the semiochemical lure present (pheromone or pheromone + host plant volaitles). Site is included as a block.

To determine if traps were differentially attractive to male and female PLW, total trap capture in each baited trap in 2014 and 2015 was subjected to a Two-Sided Exact Binomial Test which determined the proportion of males to females within each semiochemical trap. To compare the sex ratio between semiochemical traps with a given season, chi-square contingency table analyses were performed (2 x 6 for spring 2014; 2 x 8 for fall 2014; 2 x 2 for spring 2015; 2 x 2 for fall 2015). For the analysis of sex ratio in spring 2014, the following treatments were excluded due to low PLW captures: blank traps (n = 3), traps baited with low host plant volatiles and without pheromone (n = 2), and traps baited with the high host plant volatile lure and without pheromone (n = 3). For fall 2014 sex ratio analysis, blank traps were excluded from analyses due to low PLW captures (n = 1) in these traps.

In 2014 and 2015, age classes of captured weevils were separated based on scale wear, sclerotization, and elytra rigidity. All weevils captured in spring 2014 and spring 2015 had overwintered; most PLW captured in fall 2014 and in fall 2015 were newly eclosed but some had overwintered and persisted through the summer. Weevils captured in fall 2014 or fall 2015 that had overwintered had loss of scales, heavily sclerotized exoskeletons, rigid eltytra – likely due to the absorption of flight muscles for oogenesis (Schotzko and O'Keeffe 1986) – and some had evidence of mating: females with damaged pygidiums or males with everted aedeagi. Adult PLW have previously been reported to survive for 11 months in the field in Idaho (Schotzko and O'Keeffe 1988). For fall 2014 and fall 2015, the proportion of newly eclosed and overwintered PLW were compared between males and females captured within each semiochemical trap using Two-Way Tests of Equal Proportions. The number of newly eclosed and overwintered male and female PLW were compared between semiochemical traps with a chi-square analysis. This analysis was done separately for fall 2014 and fall 2015, and for fall 2014 analysis, PLW captures were again excluded from blank (unbaited) traps or in traps baited with either host plant volatile lure and without pheromone due to low PLW captures in these traps.

To determine if PLW trap capture in semiochemical traps tested in 2015 is correlated with adult feeding damage or with larval density, a series of Pearson correlations were performed using (R version 3.1.3). P-values were adjusted using the Holm method to limit the Type I (family) error in multiple comparisons. For this analysis, Pearson correlations were performed between adult feeding damage or larval density and the PLW captured in each semiochemical trap in each week of 2015, the cumulative PLW captured in each semiochemical trap by week, and the total PLW captured in each season (spring, summer, or fall). Measures of adult feeding damage included: the number of feeding notches on 10 pea plants near that semiochemical trap and the total number of feeding notches per site. Measures of larval density included: the number of larvae found in soil samples collected near each semiochemical trap, the number of larvae found in soil samples collected between semiochemical traps, and the total number of larvae found at each site.

2.2.2 Trap type

Previous studies employed modified Legget cone traps baited with semiochemical lures to attract (Blight *et al.* 1984; Blight and Wadhams 1987) and monitor (Nielsen and Jensen 1993) PLW adults in Europe. Modified Legget traps did not retain PLW adults when tested in pea fields in southern Alberta (Evenden *et al.* 2016). Pitfall traps, as described above, are currently the most effective trap known to capture PLW in the Prairie Provinces. These traps, however, use propylene glycol as a killing agent, are messy to handle and retain considerable non-target bycatch. To determine if a more suitable trap design could be developed to attract and retain PLW in Alberta, I compared different trap types (Appendix A, Table A-4) in 2013, 2014 and 2015. In each of these experiments, the various types of traps tested (Table 6) were baited with a low dose (21 mg) of pheromone released from a 250 µl Eppendorf tube and positioned in pea fields in August of each year, after the pea crop was harvested at each site. Details on trap construction and photographs of traps are given in Appendix B.

2.2.2.1 Trap Type Experiment 2013

In 2013, the trap capture of PLW in nine different insect trap types was compared (Table 6; Appendix B). Trap types tested included: 1) the Solo cup pitfall trap (Evenden *et al.* 2016), described above; 2) a yellow bucket trap, designed to emulate traps used to monitor palm weevils (Vacas *et al.* 2012); 3) yellow cone traps similar to those used to capture PLW in Europe, positioned on the ground to capture walking PLWs (Blight *et al.*1984; Blight and Wadhams 1987; Nielsen and Jensen 1993); 4) Yellow cone traps positioned 0.75 m above the ground on yellow pyramids to capture walking and/or flying PLWs. Cone traps on pyramids resembled the traps used to monitor plum curculio *Conotrachelus nenuphar* (Coleoptera: Curcluionidae)

(Clement *et al.* 2010) and pecan weevil *Curculio caryae* (Coleoptera: Curculionidae) (Mulder *et al.* 2003). The methods described in Gardosik and Lehman (2005) for pyramid trap construction were slightly modified; 5) Yellow cone traps positioned 1 m above the ground on rebar stakes to target flying PLWs; 6) Green non-saturating Unitraps (Scotts Canada) (17 cm diameter; 23 cm height) positioned 1 m above the ground; 7) Yellow non-saturating Unitraps (17 cm diameter; 23 cm height) positioned 1 m above the ground; 8) Multi-coloured non-saturating Unitraps (green lid, yellow funnel, white bucket; 17 cm diameter, 23 cm height) positioned 1 m above the ground; and 9) Yellow sticky cards (18 cm x 14 cm) (Scotts Canada) attached to a rebar stake at 1 m. Each trap was placed in random order along a linear transect with 25m between traps at the edge of the pea fields. All traps were tested at a total of eight pea fields in southern Alberta (Appendix A, Table A-4, Figure A-4). Traps were placed in the field in early August and checked every 5-7 days for a total of five trap collections. Trap capture was transported to the laboratory where PLW were identified and counted.

2.2.2.2 Trap Type Experiment 2014

In 2014, the trap capture of PLW in eight different insect trap types was compared (Table 6; Appendix B). Trap types tested included: 1) the Solo cup pitfall trap used in previous experiments; 2) the Solo cup pitfall trap with wire mesh (6 mm x 6 mm) positioned on the mouth of the cup at ground level to exclude large bycatch; 3) a pitfall trap constructed from PVC piping, with 2 mm diameter entry holes at the soil surface and propylene glycol as the killing agent; 4) a pitfall trap constructed from PVC piping with 20 mm x 10 mm ovals and with propylene glycol as the killing agent;. 5) a yellow pan trap (6.5 cm x 20 cm x 27 cm), buried into the soil so that the top of the pan trap was level with the soil surface, and half-filled with propylene glycol as a trapping mediuml; 6) green boll weevil cone traps positioned on the ground, which were previously ineffective for retaining PLW in Alberta by Evenden *et al.* (2016); 7) green boll weevil cone traps positioned on the ground with entrance holes larger than 2 mm sealed to prevent PLW escape; and 8) a cylinder of yellow sticky cards (50 cm circumference, 14 cm length; constructed by overlapping 1-2 cm of the 14 cm ends of three 14 cm x 18 cm yellow sticky cards) placed just above the crop stubble on a wooden stake, similar to the omnidirectional sticky trap tested by Fisher and O'Keeffe (1979). Yellow cone traps were constructed from 946 mL yellow plastic drinking cups (Jean's Plastics Party Supplies Gift, Ebay), 100 mm plastic funnels, and a 30 mL snap cap vial. Construction methods are described in detail in Appendix B.

The effect of trap position within the field was also tested in 2014. Two transects each containing the 8 tested trap types were positioned 25m apart at each pea field. One transect was erected along the edge of each field and another 25m into the field, so that the traps formed an 8 x 2 grid. These traps were placed in five pea fields (Appendix A, Table A-4, Figure A-4) in mid-August (post-harvest) and trap capture was checked twice, once in the last week of August and again in the second week of September. Trap capture was transported to the laboratory where PLW were identified and counted.

2.2.2.3 Trap Type Experiment 2015

In 2015, the trap capture of PLW in three different insect trap types was compared (Table 6; Appendix B). Traps types tested included: 1) the Solo cup pitfall trap with propylene glycol, which was consistently the best trap in previous experiments; 2) a commercially available box trap developed for trapping wireworms. This trap is lined with yellow sticky cards to capture beetles that walk up a small ramp into the box of the trap where the semiochemical lure is located (Vernon 2004); and 3) a commercially produced pitfall-type trap designed to capture wireworms (Vernon and Herk 2016). These pitfall traps have three parts: a brown lower cup (400 ml) with lip, which is set into the ground; a clear lining cup (200 ml), and a brown plastic top which snaps onto the brown bottom and excludes large insects and small animals with small plastic pegs. In order to secure the semiochemical lures onto these wireworm traps, two 1 mm holes were drilled into each trap and steel wire was used to secure the lures. Two of each type of trap were randomly placed 25 m apart on a linear transect at the edge of each pea field (for a total of 6 traps per transect). Traps were placed in three pea fields (Appendix A, Table A-4, Figure A-4) in mid-August and were serviced three times, until mid-September. Trap capture was transported to the laboratory where PLW were identified and counted.

2.2.2.4 Statistical Analyses

Mixed effects models were used to analyze the effect of trap type on total PLW capture in all three years of experimentation. In 2013, the number of PLW captured in each trap was compared using the following mixed effects model: *Total PLW ~ trap type + (1|site).* In 2014, the effect of trap position was incorporated into the model as: *Total PLW ~ trap type + trap position + (collection date| site).* Trap capture in the three trap types assessed in 2015 was analyzed by the model: *Total PLW ~ trap type + (1|site).*

Table 6. Trap types tested in 2013, 2014 and 2015

Year	Trap type[1,2]	Placement	Trap origin
2013	Pitall trap constructed from Solo cups, with Coroplast lid and propylene glycol as a killing agent	Pitfall	Hand constructed
	Yellow unitrap suspended 1 m above the ground on rebar stake	Above crop	Commercially available
	Green unitrap suspended 1 m above the ground on rebar stake	Above crop	Commercially available
	Multicoloured unitrap suspended 1 m above the ground on rebar stake with green lid, yellow funnel & white base	Above crop	Commercially available
	Yellow sticky card (18 cm x 14 cm) placed 1 m above the ground on a rebar stake	Above crop	Commercially available
	Yellow cone trap placed 1 m above the ground on a rebar stake	Above crop	Hand constructed
	Yellow cone trap placed 0.75 m above the ground on a yellow Coroplast pyramid	At top of crop	Hand constructed
	Yellow cone trap secured directly on the ground with tent pegs	At ground	Hand constructed
	Yellow bucket trap with ½ of a Vapona insecticide strip	Pitfall	Hand constructed
2014	Pitall trap constructed from Solo cups, with Coroplast lid and propylene glycol as a killing agent	Pitfall	Hand constructed
	Pitall trap constructed from Solo cups, with Coroplast lid and propylene glycol as a killing agent, and with a ring of 6 mm x 6 mm chicken wire mesh to exclude large bycatch	Pitfall	Hand constructed
	Yellow pan trap	Pitfall	Commercially available
	Unmodified boll weevil (Legget) trap	At ground	Commercially available
	Boll weevil (Legget) trap modified with all holes >2 mm sealed with hot glue	At ground	Modified from commercially available
	PVC pitfall trap with small holes (twenty 2 mm x 2mm holes)	Pitfall	Hand constructed
	PVC pitfall trap with large holes (six 20 mm x 10 mm holes)	Pitfall	Hand constructed
	Cylinder of yellow sticky cards placed just above the crop stubble on a wooden stake (“omnidirectional” sticky trap)	At top of crop	Modified from commercially available
2015	Pitall trap constructed from Solo cups, with Coroplast lid and propylene glycol as a killing agent	Pitfall	Hand constructed
	Vernon ramp trap for wireworms, lined with yellow sticky cards	Pitfall	Commercially available
	Vernon pitfall trap for wireworms, with propylene glycol as a killing agent	At ground	Commercially available

[1] All tested trap types were baited with 21mg of pea leaf weevil aggregation pheromone in a 250 µl Eppendorf tube

[2] Trap photos and design details are included in Appendix B.

2.2.3 Seasonal Plasticity to Semiochemical Cues in the Laboratory

2.2.3.1 Insect Collection

To determine if PLW response to semiochemical cues is plastic and depends on the physiological state of the weevils, an olfactometer bioassay was developed. Pea leaf weevils were collected from field pea or faba bean fields and transported to the Lethbridge Research Centre where they were kept at 24°C under 16L:8D light conditions until use in the bioassay. Weevils used in olfactometer experiments were collected at three times when weevils are in different physiological states: 1) in the spring after overwintering, but prior to feeding on reproductive hosts (4 May and 5 May, 2015); 2) in spring after aggregation on reproductive hosts (9 June – 11 June, 2015); and 3) in summer when newly emerged weevils are reproductively immature (28 July 2015). Collected weevils were housed communally in cages (20 cm wide x 20 cm deep x 30 cm tall) and fed either alfalfa (May and July collections) or alfalfa and pea plants (June collections). Alfalfa plants were collected and rinsed before feeding to PLW. Pea plants were grown in the greenhouse at the Lethbridge Research Centre. Water was provided to PLW in 50 mL and 100 mL vials via a paper towel wick. Water was also misted over cages every 2-3 days.

2.2.3.2 Olfactometer Bioassays

A four-choice olfactometer (Analytical Research Systems, Inc.) (Figure 1) was used to compare weevil response to natural sources of semiochemicals at various points in the season reflecting different physiological states of the weevils. Air was moved through the olfactometer with a 4-choice arena olfactometer air delivery system with vacuum (Analytical Research Systems, Inc.) which vacuumed air out of the bottom of the olfactometer arena, via the insect inlet adaptor, at a rate of 3.0L/min. For each of the four arms entering the olfactometer, charcoal-filtered air moved at a rate of 1.0L/min from the olfactometer air delivery system through bubblers containing distilled water, then through a plexiglass chamber containing the odour source and into the olfactometer. Suspended above this system was a 60W incandescent bulb by a ring clamp to provide 57 to 62 lux light intensity across all trials. A webcam was suspended above the arena to record all olfactometer trials.

Approximately 23h before starting an olfactometer bioassay on weevils in each physiological state, natural sources of semiochemicals were amassed. Sources of natural semiochemicals consisted of: 1) Five PLW males on 4 pea plants; 2) Five PLW males with a pot of soil; 3) Four pea plants alone; and 4) a pot of soil as a control. Ten male PLW were selected from holding cages and sex was confirmed by viewing weevils under a dissecting microscope. For treatment 1, five males were placed in a 1.67 L pot containing four pea plants between the 4th and 6th node growth stage and placed in a plexiglass tube (80 cm height x 20 cm diameter) sealed with mesh at either end. For treatment 2, five additional males were placed in a separate cage and supplied with pea plants. The following day, the males in the cage were transferred to a similar plexiglass tube containing a pot of soil and sealed with mesh at either end. For treatment 3, an uninfested pea plant at the 4th to 6th node growth stage was placed in a similar plexiglass container. For treatment 4, a similar 1.67 L plant pot containing only soil was placed in a similar plexiglass container. The four plexiglass containers were each placed into the four plant odour source chambers of the four-way olfactometer. Charcoal-filtered, humidified air entered each odour source chamber at 3.0L/min and then entered the olfactometer arena.

Olfactometer bioassays were conducted under ambient temperature conditions (22-24°C) and between 8:30 am and 4:30 pm, when PLW are expected to be active (Landon *et al.*1995; Schoztko and O'Keeffe 1986). Between each olfactometer trial, the olfactometer arena was opened, cleaned with 95% EtOH then resealed, and the olfactometer was run for at least 5 minutes without odour sources before commencing the next trial. Weevils (male or female) were collected from holding cages five minutes before the start of the trial and positioned individually in a petri dish. The individual test weevil was placed in the olfactometer via the insect inlet adapter on the lower surface of the arena, at which point the video camera was switched on, and the weevil's movement was recorded for 30 minutes. At a later date, videos were visually observed and the movement of each PLW through the olfactometer quadrants was recorded for the 30 minute trial period. A PLW was considered to have responded in the olfactometer if it exited the insect inlet adaptor into one of the four quadrants of the olfactometer arena during the 30 minute trial period. For each of the three physiological states (overwintered, mating, or newly-eclosed) and for both sexes of weevils, the proportion of PLW that responded was calculated. The first quadrant entered by a weevil was considered its "first volatile choice". The amount of time spent by each PLW in each quadrant of the olfactometer (or in the insect inlet adaptor, which represented no choice) was recorded by observing each video. The quadrant of the olfactometer in which each PLW spent the most time was recorded as the “preferred volatile choice”. Additional analysis was attempted using the object-tracking program Lolitrack, but was abandoned due to time constraints. Detailed methods for Lolitrack analysis are provided in Appendix C.

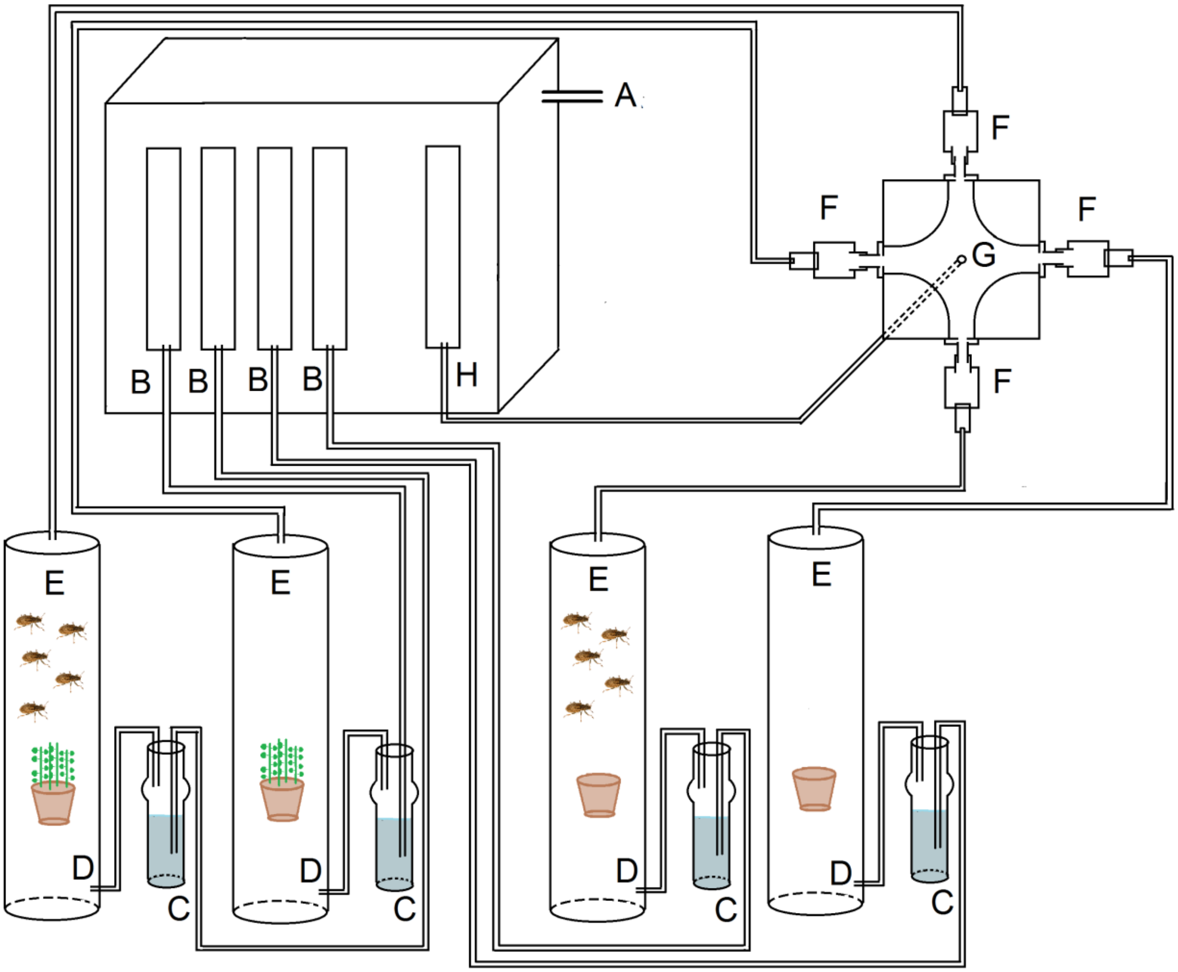

Figure 1. Diagram of olfactometer bioassay setup (not to scale). At point **A**, a lab air source was connected to the 4-Choice Arena Olfactometer Air Delivery System with Vacuum (Analytical Research Systems, Inc.). The 4-Choice Arena Olfactometer Air Delivery System with Vacuum is designed to filter dirt, particulates, condensed water or oil droplets and hydrocarbon vapors from the air steam. The air then exits through each of the four flow rate meters at **B** at a rate of 1.0L/min. Next, the clean air passes through distilled water held in bubblers at **C**. Humidified, clean air enters each of the four odour source chambers at **D**. Each odour source chamber held one of: 1) Five male PLW on four pea plants; 2) Four pea plants alone; 3) Five male PLW and a pot of soil; 4) A pot of soil as a control. Odour-impregnated air then exited through the top of the odour source chambers at **E** and entered each respective arm of the olfactometer at **F**. Air was vacuumed out of the bottom centre of the olfactometer area at **G**. Point **G** was also the insect inlet adaptor, where PLW test subjects were added to the olfactometer. The vacuum flow meter at **H** maintained the flow of air being vacuumed out of the olfactometer at 3.0L/min. Not shown in this diagram are the incandescent bulb and webcam which were suspended above the olfactometer arena.

2.2.3.3 Statistical Analyses

The frequency of responding PLW versus non-responding PLW was compared between weevils of different physiological states and sex using a Chi-square test. For further analysis, only the PLW that responded in olfactometer bioassays were considered. Chi-square tests were used to compare the first volatile choice amongst responding PLW of various physiological states and sexes. For each physiological state and sex, the distribution of first choices was compared to an even distribution with a Chi-square test. Similarly, the preferred volatile choice (corresponding to the quadrant of the olfactometer in which each PLW spent the most time) was compared between responding weevils of different physiologies and sexes using a Chi-square test. For each physiological state and sex of weevils, the preferred volatile choices were compared to an even distribution using a Chisquare-test.

2.3 Results

2.3.1 Semiochemical blend, dose and lure type

2.3.1.1 Trapping Experiment 2013

In the spring 2013 trapping period, the combination of volatiles present in the semiochemical trap lure significantly impacted the season-long trap capture of PLW ($\chi^2 = 131.15$, df = 2, $p < 0.001$; Figure 2). Traps baited with aggregation pheromone (Z-value = -10.34, $p < 0.001$) or with pheromone plus host plant volatiles (Z-value = -11.45, $p < 0.001$) captured significantly more PLW than unbaited control traps. Pheromone + host plant volatile traps also captured significantly more PLW than traps baited with pheromone alone (Z-value = 2.52, p = 0.03). Similarly in fall 2013, there was a significant effect of the combination of volatiles present in the semiochemical lure on the season-long catch of PLW ($\chi^2= 18.84$, df = 2, $p < 0.001$; Figure 2). Traps baited with pheromone or pheromone plus host plant volatile captured significantly more PLW in fall 2013 than unbaited control traps (Z-value = -2.12, p = 0.08 for pheromone—blank; Z-value = -3.97, $p < 0.001$ for pheromone plus host plant volatile —blank). Traps baited with pheromone plus host plant volatile also captured significantly more PLW in fall 2013 than traps baited with pheromone alone (Z-value = 2.94, p = 0.009).

Additional analyses of the 2013 data excluded trap capture in the unbaited control traps and compared the efficacy of the semiochemical-baited traps based on pheromone dose and pheromone release device while considering the presence or absence of host plant volatiles. In the spring of 2013, pheromone dose did not affect PLW captures ($\chi^2 = 1.69$, df = 1, p = 1.9; Figure 3) nor did pheromone release device size ($\chi^2 = 0.40$, df = 1, p = 0.4; Figure 4). In fall 2013, pheromone dose did not significantly affect PLW captures, but there was a trend towards increased PLW captures in traps baited with 21 mg of pheromone over traps baited with 42 mg pheromone (Z-value = 1.87, p = 0.06; Figure 3). The type of pheromone release device did not significantly affect PLW captures ($\chi^2 = 0.242$, df = 1, p = 0.62; Figure 4). No significant interactions were found between the pheromone dose, pheromone release device, or addition of host plant volatiles for either the spring or fall model. In fall 2013, all four combinations of pheromone dose and release device tested captured PLW on 11 September, which was 6 weeks after trap placement in the field, indicating that pheromone lures have a longevity of at least 6 weeks in the field.

A direct comparison of all the trap capture in all traps tested in 2013, including baited traps and the unbaited control traps, showed that the presence of a semiochemical lure significantly affected the number of PLWs trapped in the spring trapping period ($\chi^2 = 148.19$, df = 8, $p < 0.001$; Figure 5). The post-hoc Tukey comparison test determined that the unbatied traps captured significantly fewer PLW than any of the baited traps (all with $p < 0.001$) and that all baited traps captured a similar number of PLW in the spring 2013 ($p < 0.05$). For the fall 2013 trapping period, the semiochemical lure present in the trap also significantly affected the number of PLWs captured ($\chi^2 = 27.99$, df = 8, p = 0.005; Figure 5). In the fall, not all of the baited traps captured more PLW than the unbatied trap. Traps that captured significantly more PLW than the control trap included those with both pheromone and host plant volatiles: 1) the low pheromone dose (21 mg) released from a 250 µl Eppendorf tube with the host plant volatile lures (Z-value = 3.36, p = 0.02); 2) the low pheromone dose (21 mg) released from a 400 µl Eppendorf tube with host plant volatile lures (Z-value = 3.24, p = 0.03); 3) the high pheromone dose (42 mg) released from a 400 µl Eppendorf tube with host plant volatile lures (Z-value = 3.21, p = 0.04); and the high pheromone dose (42 mg).

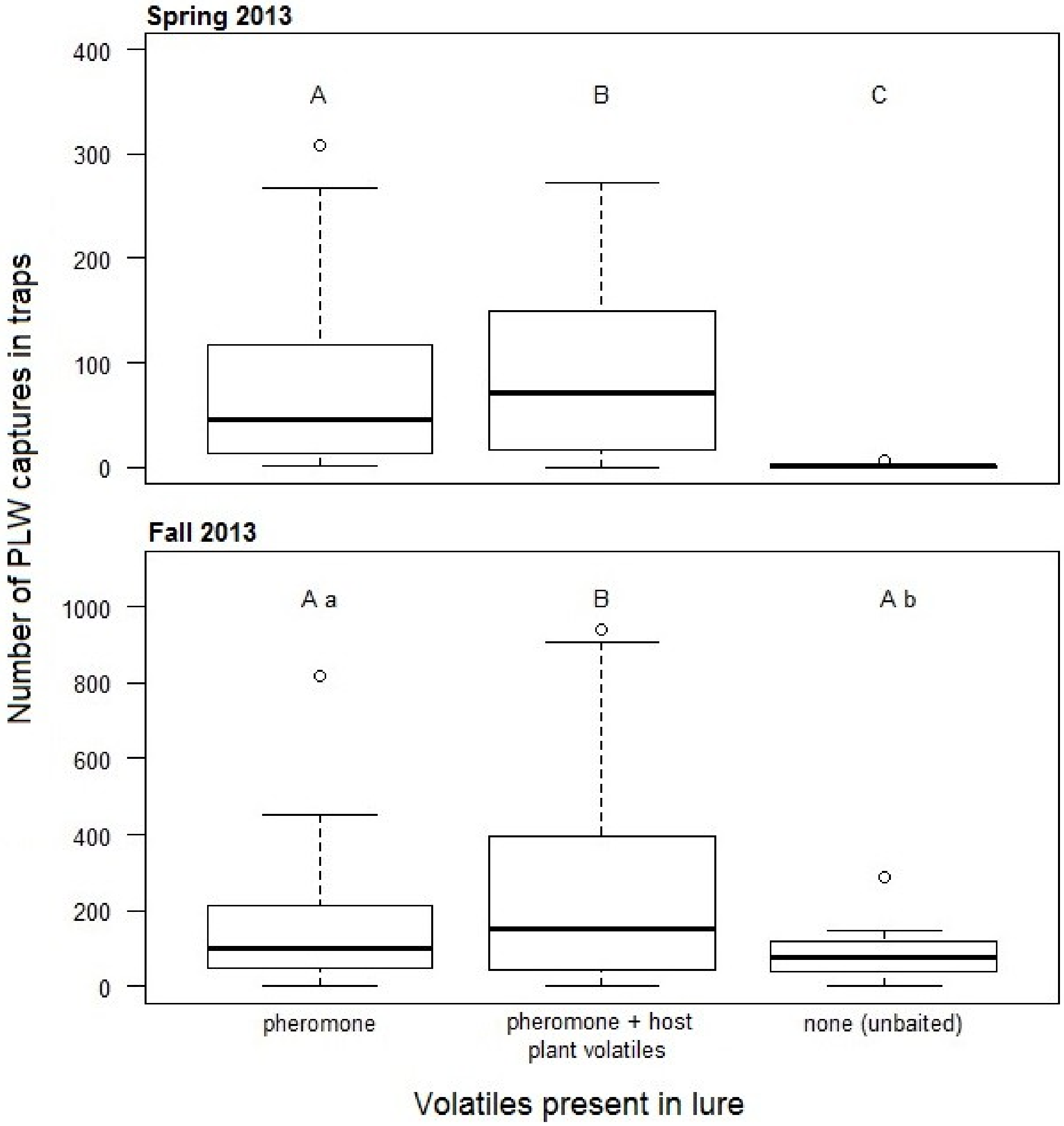

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Figure 2. Box plot of season-long capture of pea leaf weevils (*Sitona lineatus)* in semiochemical-baited traps with different volatile blends tested in spring and fall of 2013. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. Captures of weevils in response to variously baited traps were compared separately for each season, using the model glmer.nb(*Total PLW per season ~ Semiochemical blend + (1|Site))*. Note that the y-axis scales differ for the spring and fall due to relatively higher PLW captures in the fall. Captures of weevils were pooled across pheromone dose and device size. For each plot, volatiles with significantly different seasonal PLW captures have different capital letters for α = 0.05 and have different lowercase letters for α = 0.10.

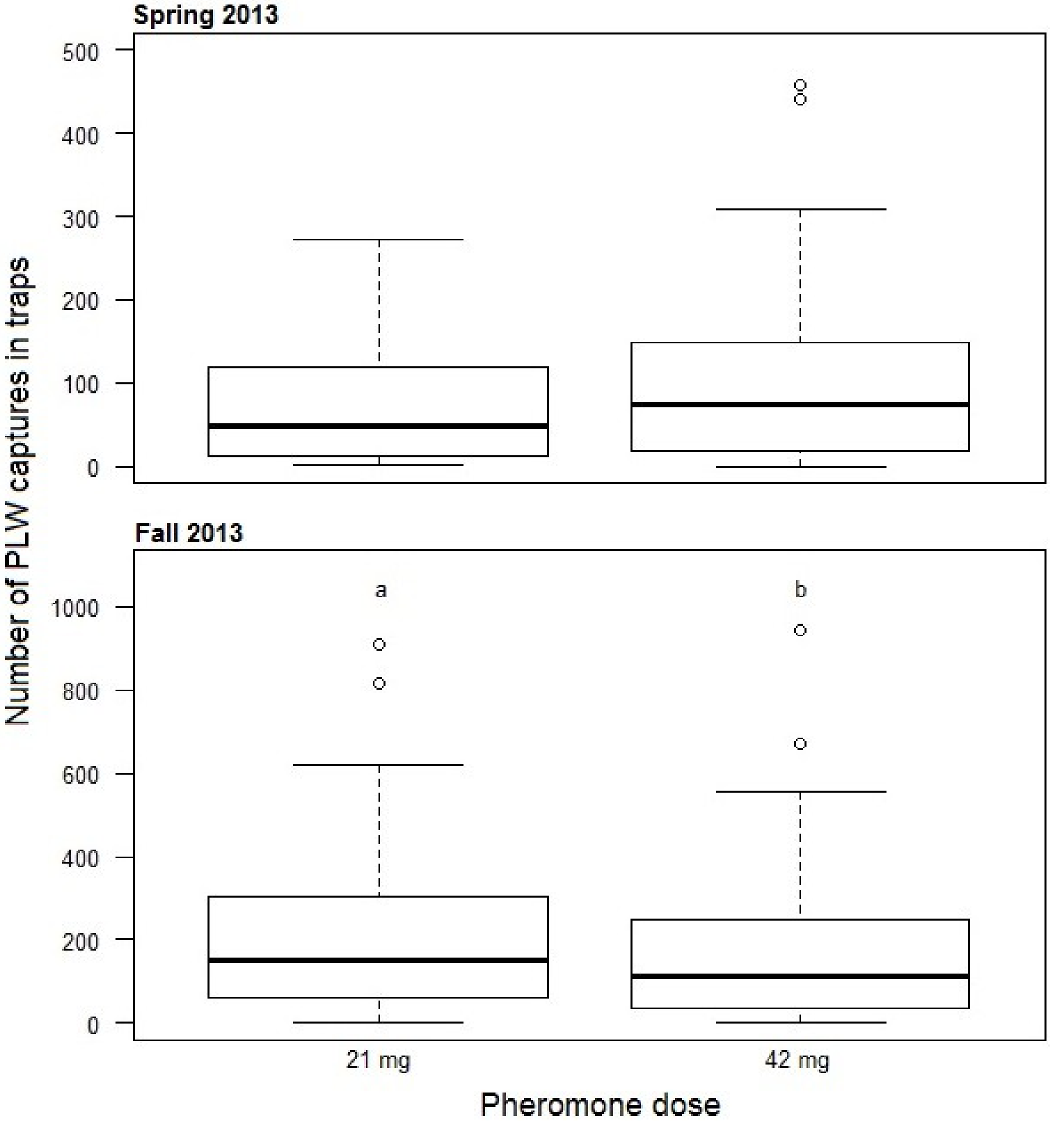

Figure 3. Box plot of season-long capture of pea leaf weevils (*Sitona lineatus)* in baited traps with different doses of pheromone tested in spring and fall of 2013. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. Comparisons between doses were made in each season separately using the model glmer.nb(*Total PLW per season ~ pheromone dose + pheromone device size + host plant volatile + (1|Site))*. Unbaited (blank) traps were excluded for this analysis. Note that the y-axis scales differ for the spring and fall due to relatively higher PLW captures in the fall. Pheromone doses with significantly different seasonal PLW captures have different lowercase letters for α = 0.10. No significant differences were found at α = 0.05.

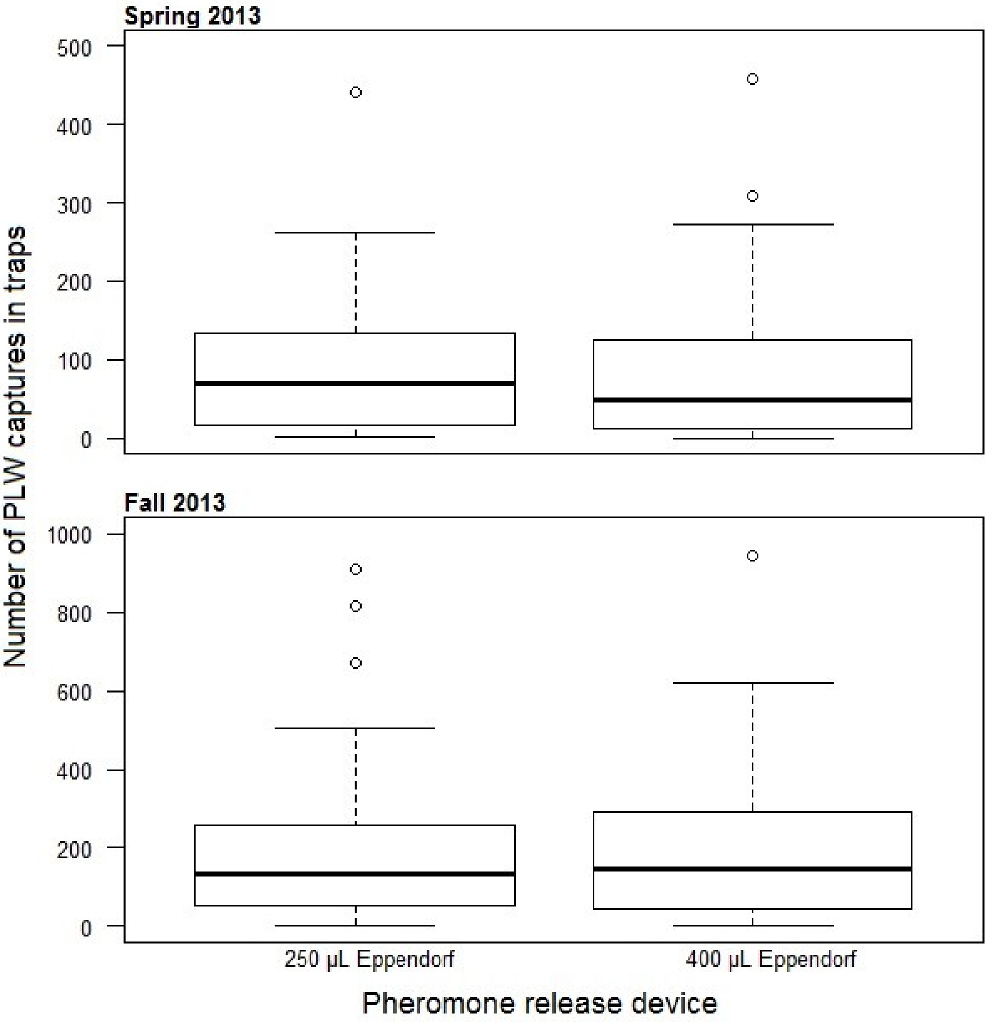

Figure 4. Box plot of season-long capture of pea leaf weevils (*Sitona lineatus)* in baited traps with different pheromone release devices tested in spring and fall of 2013. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. Comparisons between release devices were made in each season separately using the model glmer.nb(*Total PLW per season ~ pheromone dose + pheromone device size + host plant volatiles + (1|Site))*. Unbaited (blank) traps were excluded for this analysis. Note that the y-axis scales differ for the spring and fall due to relatively higher PLW captures in the fall. There were no significant differences (α = 0.05) in season long trap capture between traps baited with the two pheromone release devices tested.

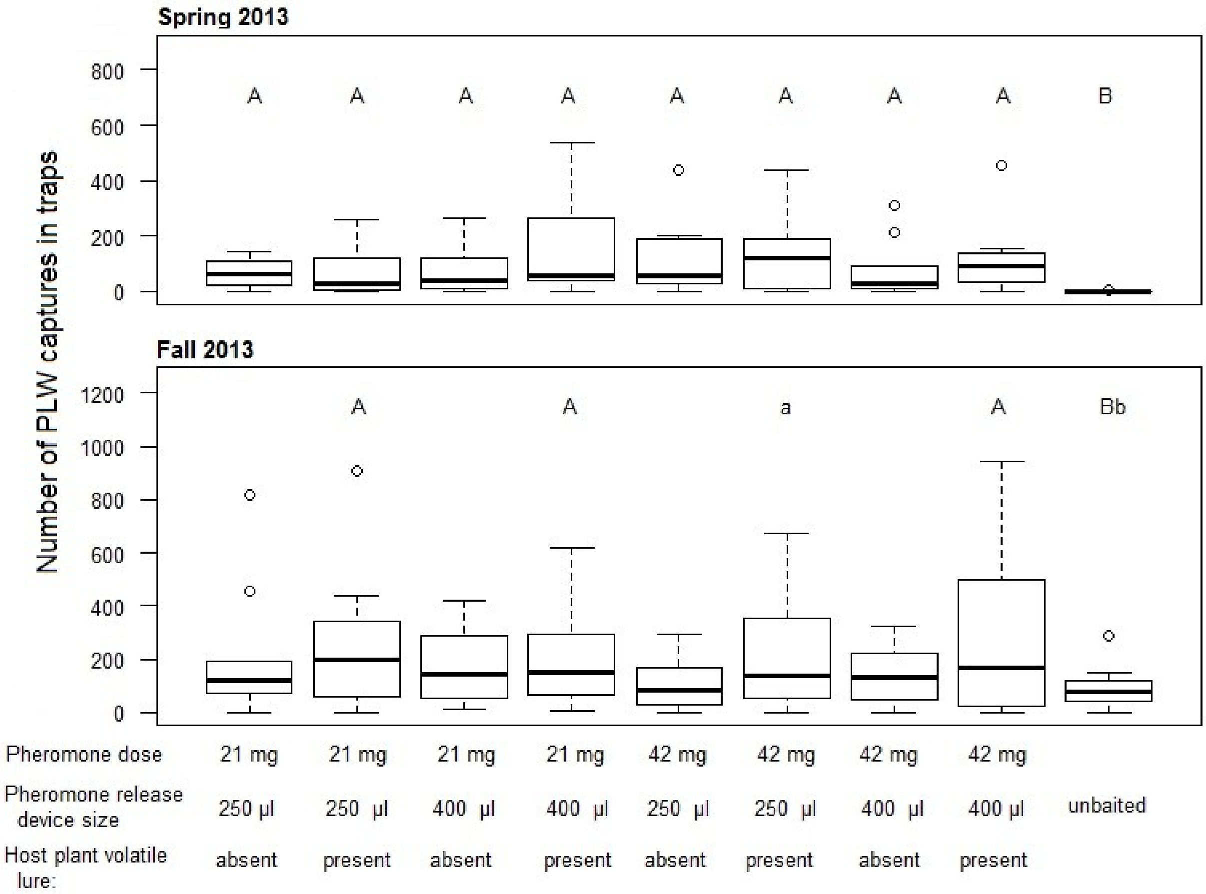

Figure 5. Box plot of season-long capture of pea leaf weevils (*Sitona lineatus)* in blank traps and in all baited traps tested in spring and fall of 2013. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. Comparisons between treatments were made in each season separately. All eight semiochemical lures tested in 2013 were compared to each other and the blank trap using the model glmer.nb(*Total PLW per season in 2013 ~ Lure + (1|Site))*. Note that the y-axis scales differ for the spring and fall due to relatively higher PLW captures in the fall. Semiochemical traps with significantly different seasonal PLW captures have different uppercase letters for α = 0.05 and have different lowercase letters for α = 0.10.

2.3.1.2 Trapping Experiment 2014

Pea leaf weevil captures in semiochemical traps peaked in spring 2014 in the second and third weeks of May, which corresponded to 1-2 weeks after seeding. PLW captures in semiochemical traps dropped off in the last week of May and were negligible in June. In fall 2014, PLW activity peaked in the week ending on 19 August, which occurred approximately two weeks after harvest. In 2014, 998 *Sitona* were captured in semiochemical traps and 98.8% of these were identified as pea leaf weevil *(S. lineatus).* Only 12 individuals were identified as *S.cylindricollis*, the sweetclover weevil.

When the data from the 2014 trapping experiment was analyzed using the model glmer.nb(*Total PLW in 2014 ~ Season * Pheromone lure * Host plant volatile lure + (1|Site)),* season did not significantly affect PLW captures on its own. The pheromone lure, however, had a significant affect on PLW captures ($\chi2 = 146.85$, df = 2, $p < 0.001$), and there was a significant interaction between season and the pheromone lure component ($\chi2 = 6.19$, df = 2, $p = 0.05$). The interaction between season and pheromone lure is largely driven by the relative success of the low pheromone dose compared to the high pheromone dose at two sites in the spring. This difference is less pronounced in the fall. In the spring, the low dose of pheromone tended to capture more PLW but in the fall, the high dose of pheromone captured numerically more PLW. The host plant volatile lure significantly affected PLW captures ($\chi2 = 9.44$, df = 2, $p = 0.009$) as well as a significant interaction between season and the host plant volatile lure component ($\chi2 = 8.14$, df = 2, $p = 0.02$). The addition of host plant volatiles to the pheromone lure increased PLW captured in the fall but not in the spring.

Thus, each season was then modeled separately: glmer.nb(*Total PLW per season in 2014 ~ Pheromone lure * Host plant volatile lure + (1|Site)).* For the spring 2014 trapping period, PLW captures were significantly affected by the pheromone dose ($\chi2 = 107.94$, df = 2, $p < 0.001$; Figure 6), and the post-hoc Tukey comparison showed that traps baited with either the low (Z-value = -10.35, $p < 0.001$) or high (Z-value = -9.57, $p < 0.001$) pheromone doses captured significantly more PLW than traps without pheromone. Traps baited with either type of pheromone lure captured a similar number of PLW (Z-value = 1.28, $p = 0.39$). In the 2014 spring trapping period, the host plant volatile lures did not significantly affect the number of PLW captured ($\chi2 = 0.46$, df = 2, $p = 0.79$; Figure 7). Different results were obtained in the fall of

2014, when the pheromone lure (Figure 6) and host plant volatiles (Figure 7) both significantly affected PLW captures (pheromone lure: $\chi2 = 69.26$, df = 2, $p <0.001$; host plant volatile lure: $\chi2 = 18.27$, df = 2, $p < 0.001$). Post-hoc Tukey comparisons showed that traps baited with one of the two pheromone release devices tested (250 µl and 400 µl, both with 21 mg of pheromone) both captured significantly more PLW than traps without a pheromone lure (Z-value = -7.64, $p < 0.001$ for low pheromone – no pheromone; Z-value = -7.73, $p < 0.001$). Trap capture in traps baited with the two pheromone lures did not differ from each other (Z-value = -0.057, p = 0.998). Traps baited with semiochemical lures that included host plant volatiles captured more PLW than those without host plant volatile lures (low host plant volatile —no host plant volatile: Z-value = -3.73, p = 0.005, high host plant volatile —no host plant volatile: Z-value = -3.74, p = 0.0005). There was no effect of host plant volatile dose on trap capture as traps baited with host plant volatiles released from Eppendorf tubes captured a similar number of PLW as traps baited with host plant volatiles released from bubble cap lures (Z-value = -0.13, p = 0.991).

A direct comparison of trap capture of in all baited traps in both seasons in 2014 showed that PLW captures were significantly affected by the semiochemical lure in both the spring ($\chi2 = 107.77$, df = 8, $p < 0.001$; Figure 8) and the fall ($\chi2 = 68.52$, df = 8, $p < 0.001$; Figure 8). Post-hoc Tukey tests for both the spring and fall models found that the six traps that included PLW pheromone in the lure captured significantly more PLW than unbaited (blank) traps ($p<0.001$ for all spring comparisons; $p<0.01$ for all fall comparisons). The six traps that had PLW pheromone included in the lure also captured significantly more PLW than the two traps baited with just host plant volatile lures in the spring ($p < 0.001$ for both). In the fall, the four traps that were baited with a combination of PLW pheromone and host plant volatile lures captured significantly more PLW than the two traps baited with host plant volatile lures alone (all $p < 0.01$), but the number of PLW trapped did not significantly differ between traps baited with pheromone alone and traps baited with host plant volatile lures alone (high host plant volatile – high pheromone p = 0.120, high host plant volatile – low pheromone p = 0.105, low host plant volatile – high pheromone p = 0.345, low host plant volatile – low pheromone p = 0.330). In both seasons, traps baited with the low or high release rate of host plant volatile lures but without pheromone did not significantly differ from each other (p = 1.000 for spring; p = 0.105 for fall) or from the blank trap (p = 0.999 for spring; p = 0.146 and p = 0.268 respectively for fall). The traps baited with a pheromone lure also did not differ from each other (p ranges from 0.859 to 1.000 in these comparisons for spring and from 0.350 to 1.000 for fall).

For spring 2014, the proportion of male to female PLW did not differ between semiochemical traps ($\chi2 = 7.70$, df = 4, $p = 0.17$; Figure 9). However, binomial tests performed to determine the ratio of male to female PLWs captured within each trap found that the sex ratio sometimes differed from 1:1. For spring of 2014, trap capture was male biased in traps baited with the low pheromone and low host plant volatile doses (63% male, $p < 0.001$, $n = 184$), the low pheromone and high host plant volatile doses (65% male, $p < 0.001$, $n = 221$), the high pheromone dose (61% male, $p = 0.05$, $n = 92$), the high pheromone and low host plant volatile doses (58% male, $p = 0.04$, $n = 154$), or the high pheromone and high host plant volatile doses (66% male, $p < 0.001$, $n = 119$). For the unbaited control trap and traps baited with either the low or high host plant volatile lures alone (ie. those traps lacking a pheromone lure component), the total number of PLWs captured in spring 2014 was low ($n = 3$, $n = 1$, $n = 3$, respectively) and these traps had no significant bias towards either sex. Traps baited with the low pheromone dose but no host plant volatile lure captured 209 PLWs in spring 2014, but trap catch was not significantly male- or female-biased (54% male, $p = 0.27$, $n = 209$).

The proportion of male to female PLW also did not significantly differ between semiochemical traps in fall 2014 ($\chi2 = 7.12$, df = 7, $p = 0.42$; Figure 9). However, binomial tests performed on PLW captured within each trap found that sex ratios were not always 1:1. In the fall 2014, trap capture in traps baited with the high dose of host plant volatile without pheromone was significantly female-biased (24% male, $p = 0.05$, $n = 17$). In the fall, no traps had trap capture that was significantly male-biased at $\alpha = 0.05$, but captures in the low pheromone plus high host plant volatile traps were almost significantly male-biased (57% male, $p = 0.058$, $n = 204$). All other traps were not significantly male- nor female-biased in the fall 2014 trapping period. Blank traps were not included in the analysis for fall 2014 as PLW captures were low ($n = 1$).

In the fall of 2014, 15.8% of PLW captured in traps were suspected to have overwintered and belong to the parental generation, based on their heavy sclerotization and their lack of cuticular scales. Exact proportion tests found that the proportion of newly eclosed or overwintered PLW sometimes differed between male and female PLW captured in a given semiochemical trap (Figure 10). For PLW captured in traps baited with a low dose of pheromone, a greater proportion of male (29%) than female (7%) PLW belonged to the parental generation (χ2 = 5.47, df = 1, p-value = 0.02). The same is true for PLW captured in other traps: 1) baited with a low dose of pheromone and a low dose of host plant volatile (χ2 = 9.16, df = 1, p = 0.002; 30% of males and 13% of females had overwintered); 2) baited with a low dose of pheromone and a high dose of host plant volatile (χ2 = 6.27, df = 1, p = 0.01; 15% of males and 3% of females had overwintered); 3) baited with a high pheromone dose and a low host plant volatile dose (χ2 = 17.58, df = 1, p < 0.0001; 32% of males and 8% of females had overwintered); and 4) baited with a high pheromone dose and a high host plant volatile dose (χ2 = 12.36, df = 1, p < 0.001; 20% of male and 6% of female PLWs had overwintered). Traps baited with a high pheromone dose also followed this trend but this was only significant at α = 0.10 (χ2 = 3.5, df = 1, p = 0.06; 25% of males and 10% of females belonged to the parental generation). In unbaited control traps or traps baited with only host plant volatile lures, the proportion of parental *versus* newly eclosed PLW did not differ between males and females. PLW captures in these traps, however, were low overall. When the proportion of newly eclosed to overwintered male and female PLW was compared between semiochemical traps tested in 2014 using a chi-square analysis, there was almost a significant difference between semiochemical traps at α = 0.05 (χ2 = 24.14, df = 15, p = 0.06). A post-hoc chi-square analysis determined that this result was driven by the difference between traps baited with a low dose of pheromone and a high dose of host plant volatiles and traps baited with a low dose of pheromone and a low dose of host plant volatiles (p = 0.03); there was also a difference in the proportion of newly eclosed or overwintered males and females between traps baited with a low dose of pheromone and a high dose of host plant volatiles and traps baited with a high dose of pheromone and a low dose of host plant volatiles (p = 0.04).

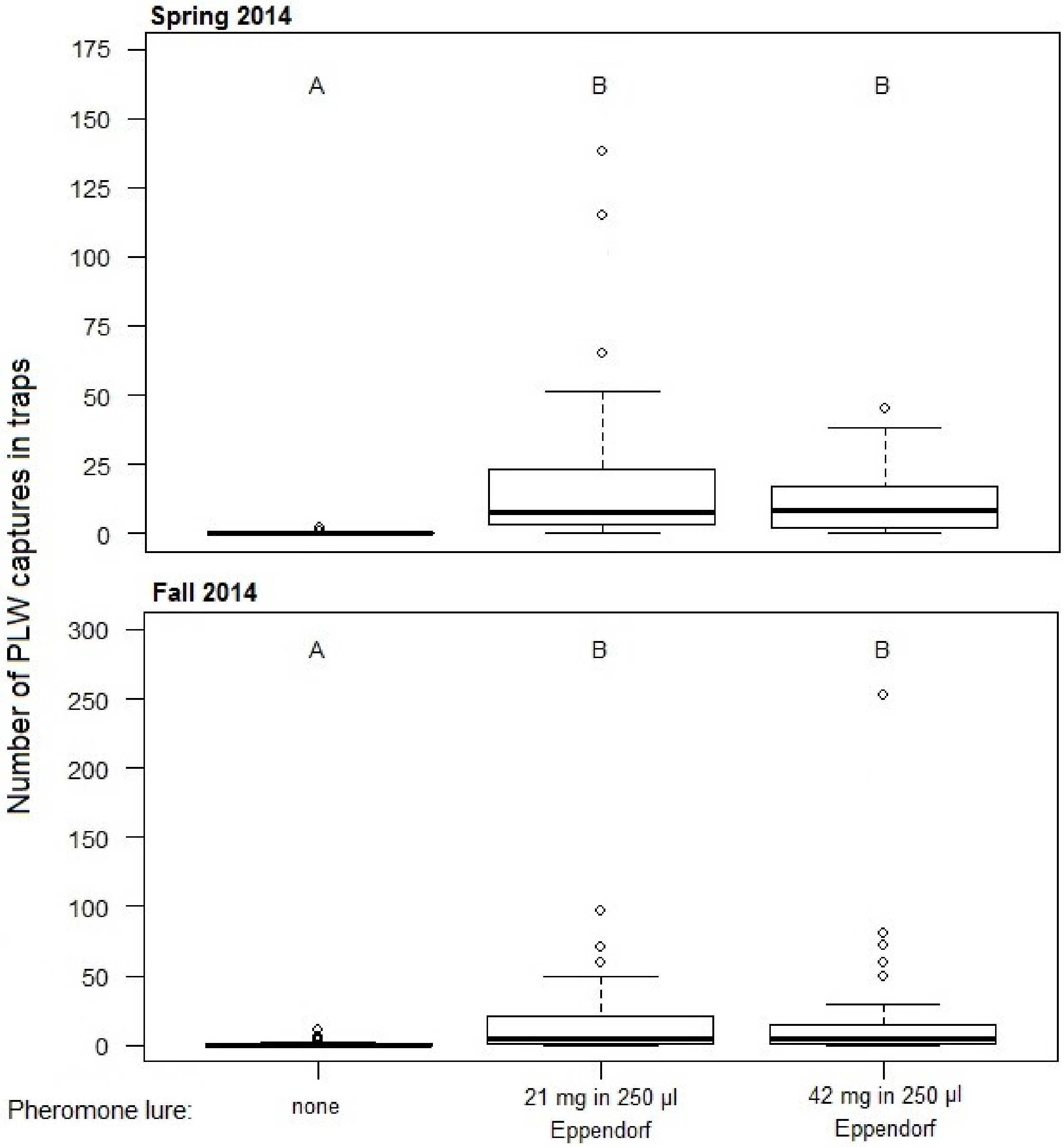

Figure 6. Box plot of the effect of the presence of pheromone in baited traps on season-long capture of pea leaf weevils (*Sitona lineatus)* in traps tested in spring and fall of 2014. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. The total number of PLW captured was analyzed separately for the spring and fall using the model glmer.nb(*Total PLW per season ~ Pheromone lure + host plant volatile lure + (1|Site))*. Note that the y-axis scales differ for the spring and fall due to relatively higher PLW captures in the fall. Pheromone doses with significantly different PLW captures have different letters for α = 0.05.

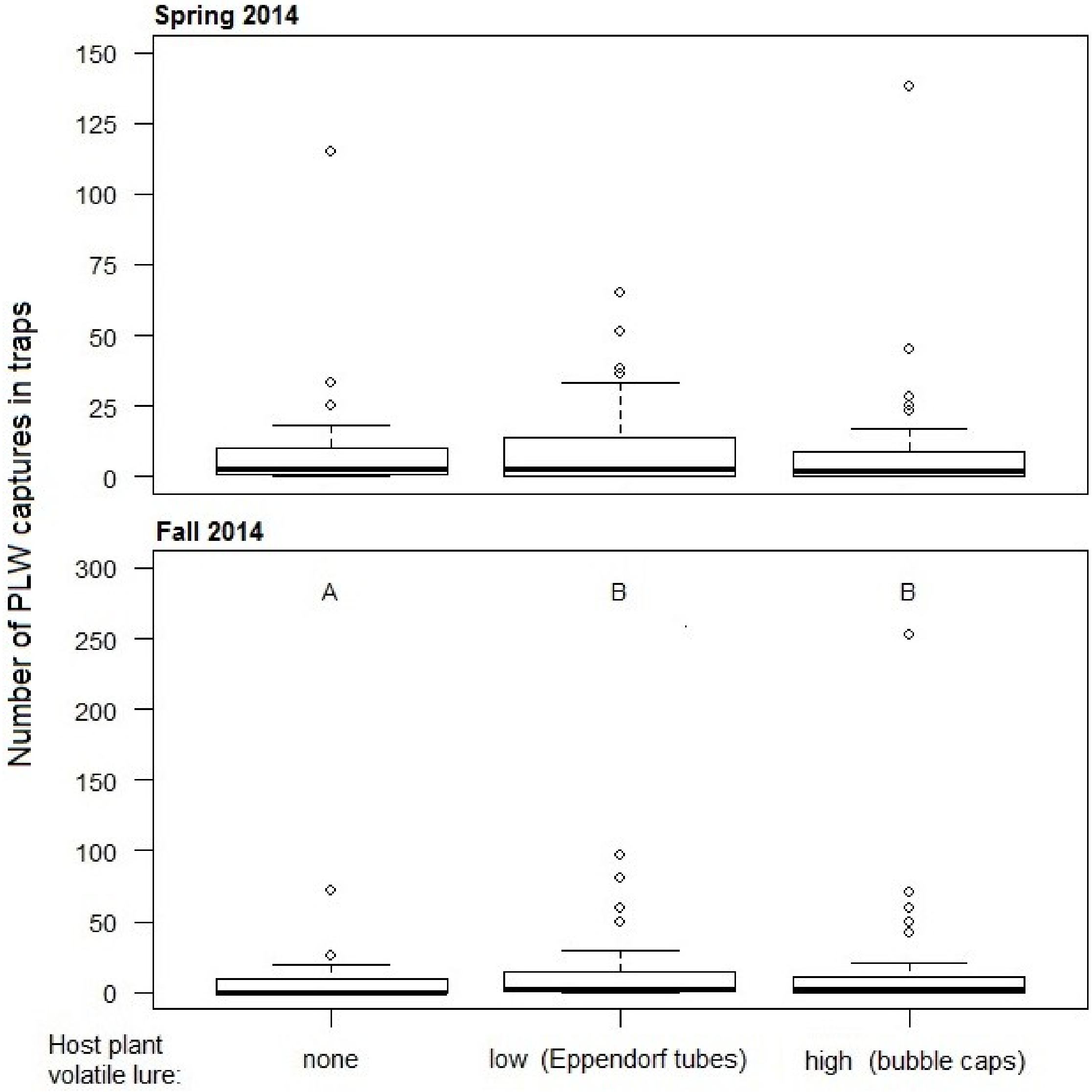

Figure 7. Box plot of the effect of the presence of different release rates of host plant volatiles in baited traps on season-long capture of pea leaf weevils (*Sitona lineatus)* in traps tested in spring and fall of 2014. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. The total number of PLW captured was analyzed separately for the spring and fall using the model glmer.nb(*Total PLW per season ~ Pheromone lure + host plant volatile lure + (1|Site))*. Note that the y-axis scales differ for the spring and fall due to relatively higher PLW captures in the fall. “Low” host plant volatile lures were released from Eppendorf tubes (Table 3) and “high” host plant volatile lures were released from bubble caps (Table 3). Host plant volatile lures with significantly different seasonal PLW captures have different letters for α = 0.05.

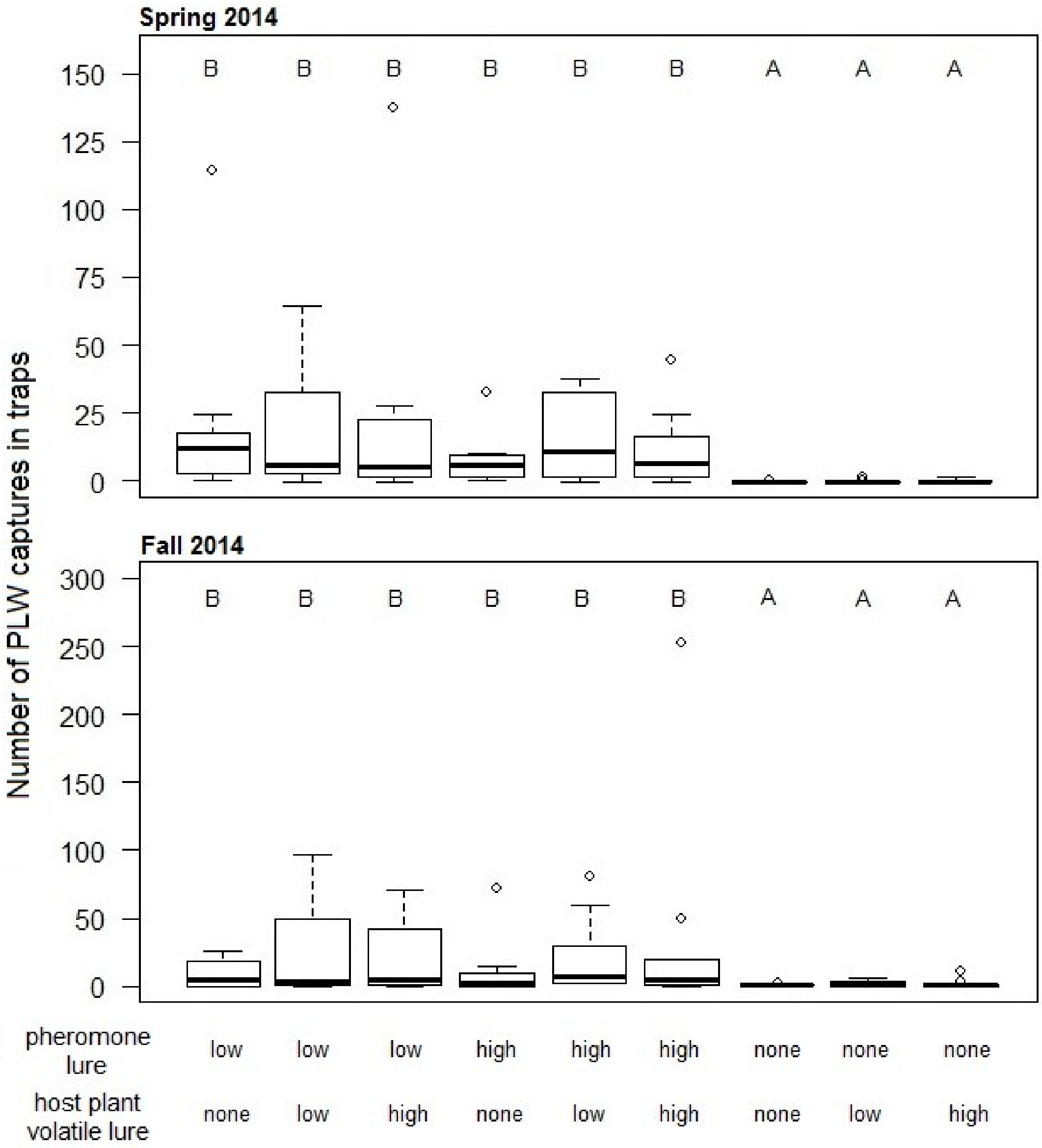

Figure 8. Box plot of season-long capture of pea leaf weevils (*Sitona lineatus)* in all baited traps tested in spring and fall of 2014. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. Comparisons between treatments were made in each season separately. All nine treatments tested in 2014 were compared to each other and the blank trap using the model glmer.nb(*Total PLW per season ~ Lure + (1|Site))*. Note that the y-axis scales differ for the spring and fall due to relatively higher PLW captures in the fall. Semiochemical lures are described in Table 3. Semiochemical lures with significantly different seasonal PLW captures have different letters for α = 0.05.

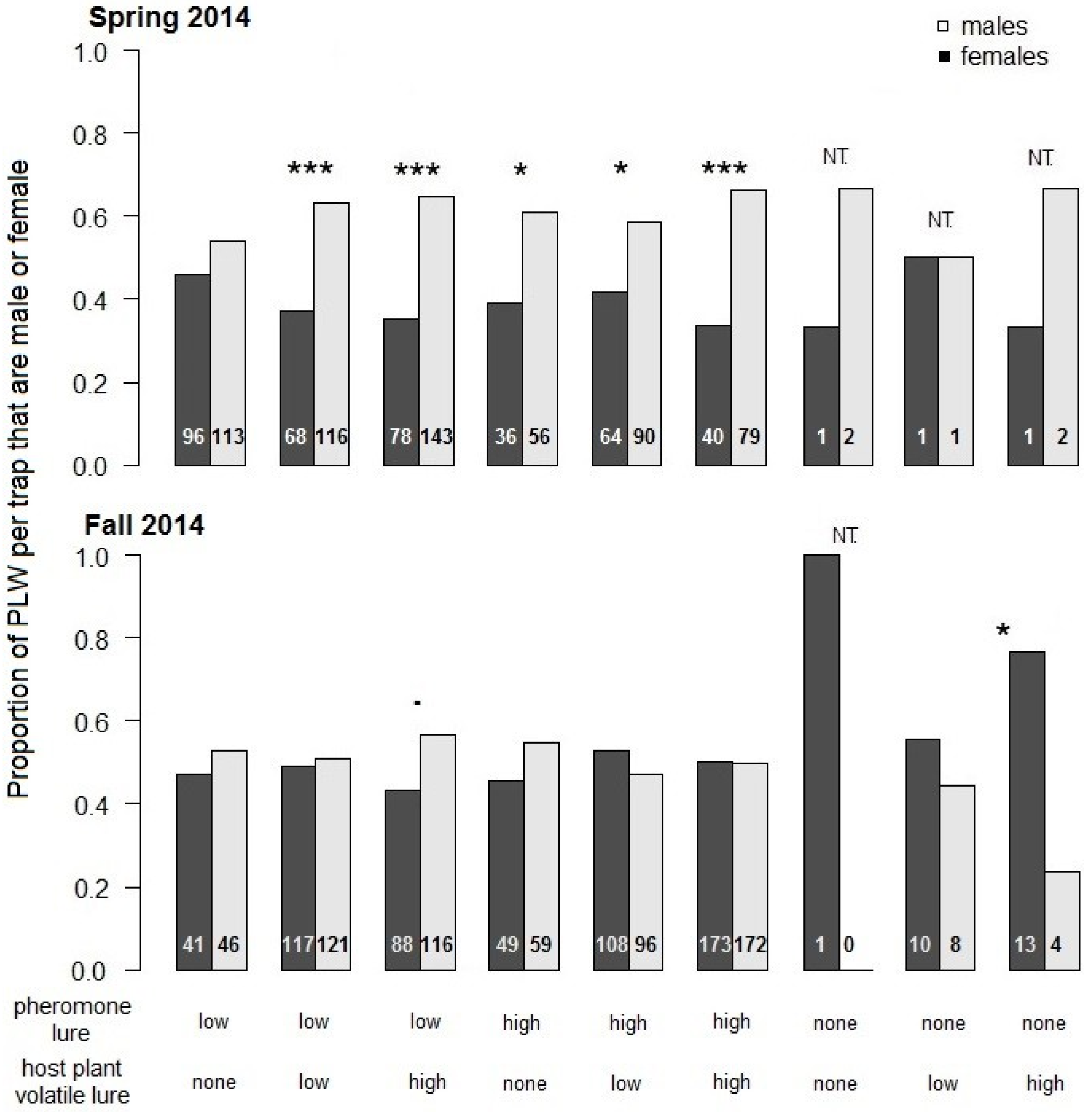

Figure 9. Proportion of male and female pea leaf weevils (*Sitona lineatus)* captured in semiochemical traps in 2014. Semiochemical lures are described in Table 3. Trap capture was separated by sex in each semiochemical trap and the proportion of males and females captured was compared with a two-sided binomial test within trap type. Significant differences between the proportion of male and female PLW captured within a semiochemical trap are denoted with "." for α = 0.10, " * " for α = 0.05, and " *** " for α = 0.001. When PLW captures in a given semiochemical trap were sufficiently low, a binomial test was not performed; this is indicated on the plot with "NT". Numbers within bars on the graph denote the total number of male or female PLW captured in a given trap. The number of male and female PLW was compared between semiochemical traps using a 6 x 2 Chi-square contingency table for spring 2014 and a 8 x 2 contingency table for fall 2014. Sex ratios did not significantly differ between semiochemical traps for spring 2014 ($\chi2 = 7.70$, df = 5, p = 0.17) or for fall 2014 ($\chi2 = 7.13$, df = 7, p = 0.42).

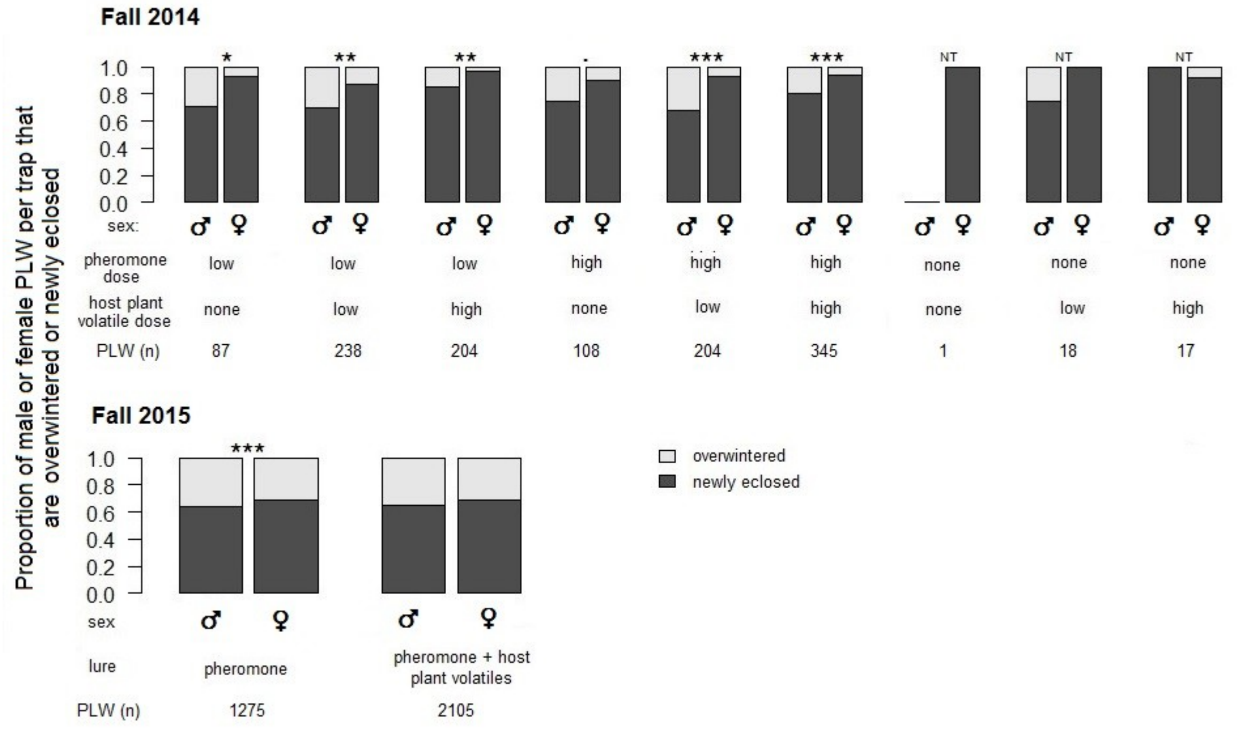

Figure 10. The proportion of newly eclosed and overwintered male and female pea leaf weevil (*Sitona lineatus)* captured in each semiochemical trap type tested in fall 2014 and fall 2015. For each semiochemical trap in each season, a Two-Way Test of Equal Proportions was used to determine if the proportion of overwintered and newly eclosed PLW was similar between males and females. Significant differences between the proportion of newly eclosed male and female PLWs are denoted with "." for α = 0.10, " * " for α = 0.05, " ** " for α = 0.01, and " *** " for α = 0.001. "NT" indicates that PLW captures in a given semiochemical trap were too few to perform this statistical analysis. A 6 x 4 chi-square contingency table was used for fall 2014 and a 2 x 4 chi-square contingency table was used for fall 2015 to compare the number of newly eclosed and overwintered male and female PLW between different semiochemical traps. Traps lacking a pheromone lure were not included in the chi-square comparison (indicated with "NT" on the plot) due to minimal captures of PLW in these traps. The number of newly eclosed or overwintered male and female PLW did not significantly differ between traps for fall 2014 ($\chi2 = 24.14$, df = 15, p = 0.06) or for fall 2015 ($\chi2 = 1.19$, df = 3, p = 0.75).

2.3.1.3 Trapping Experiment 2015

In 2015, PLW captures significantly differed between the spring, summer and fall ($\chi2$ = 267, df = 2, $p < 0.0001$; Figure 11). Significantly more PLW were captured in either the spring or the fall than in the summer (spring – summer $p < 0.05$, fall – summer $p < 0.05$). A similar number of PLW were trapped in the spring and fall, although the fall captured numerically more PLW (fall—spring p = 0.986). In the spring, the average PLW captured per week per trap was 7.63 for pheromone-baited traps and 6.13 for pheromone plus host plant volatile-baited traps. In the summer, trap catch was much lower: 0.27 for pheromone-baited traps and 0.23 for pheromone plus host plant volatile-baited traps. In the fall, PLW captures were much higher than in the spring or summer and the average PLW captured per week per trap was 13.01 for pheromone-baited traps and 21.48 for pheromone plus host plant volatile-baited traps. This difference in PLW captures between pheromone and pheromone plus host plant volatile-baited traps is significant for fall 2015 ($\chi2$ = 273, df = 1, $p <0.0001$; Figure 11).

In spring 2015 (Figure 12), PLW captures in semiochemical traps were highest in the week ending on 6 May, which was the first week of trapping and occurred 1 week post-seeding. A warm, dry spring in 2015 compared to 2014 could explain the difference in PLW activity seen between these years. In fall 2015 (Figure 12), the period of PLW activity, when PLW were captured in traps, was longer than the period of PLW activity in spring 2015. Pea harvest occurred at 2015 sites in the first week of August.

In spring 2015, PLW captures were significantly male-biased for low dose pheromone traps (61% male, $p < 0.0001$, n = 748) and the low dose pheromone plus low dose host plant volatile traps (63% male, $p < 0.0001$, n = 602) traps (Figure 13). When compared with a chi-square analysis, these sex ratios did not significantly differ between traps ($\chi2$ = 0.926, df = 1, p = 0.34). The small total number of PLWs trapped in the summer of 2015 in pheromone (55% male, p = 0.21, n = 11) and in pheromone plus host plant volatile (50% male, p = 0.39, n = 8) traps was not significantly male or female biased (Figure 13). For the fall 2015 trapping period, the number of PLWs captured was significantly male-biased at $\alpha = 0.05$ in traps baited with pheromone (54% male, p = 0.009, n = 1297) and at $\alpha = 0.10$ in traps baited with pheromone plus host plant volatile (52% male, p = 0.059, n = 1102) (Figure 13). A chi-square analysis determined that the ratio of males to females also did not differ between semiochemical traps tested in fall 2015 ($\chi 2 = 0.75$, df = 1, p = 0.39).

In the fall of 2015, 34% of PLW captured in traps appeared to have overwintered. For PLW captured in the pheromone traps tested in fall 2015, a greater proportion of male than female PLW were suspected to belong to the parental generation ($\chi 2 = 4.01$, df = 1, p = 0.045; 37% of males and 32% of females trapped had overwintered; Figure 10). For PLW captured in the pheromone plus host plant volatile traps in fall 2015, the proportion of parental versus newly eclosed PLW did not differ between males and females ($\chi 2 = 2.62$, df = 1,p = 0.11; 35% of males and 32% of females had overwintered). The proportion of newly eclosed to overwintered male and female PLW did not significantly differ between semiochemical traps tested in fall 2015 ($\chi 2 = 1.19$, df = 3, p = 0.75).

Adult feeding damage was not correlated with PLW captures in pheromone-baited traps, but was sometimes correlated with PLW captures in traps baited with both pheromone plus host plant volatile lures. Trap capture in pheromone plus host plant volatile lure-baited traps was significantly correlated to the number of adult feeding notches observed on 10 pea plants closest trap (r = 0.84, p = 0.02, Figure 14) only in the second week of trapping in 2015. Pea leaf weevil capture in pheromone plus host plant volatile lure- baited traps in each of the remaining 19 weeks was not correlated with adult feeding damage directly around the trap. Adult feeding damage near the pheromone plus host plant volatile traps was, however, correlated with the cumulative number of PLW captured in these traps to the 18th week (collected on September 2; r = 0.82, p = 0.05), 19th week (collected on September 9; r = 0.85, p = 0.02) and 20th week (collected on September 16; r = 0.85, p = 0.02). The total PLW captured in each season in the pheromone plus host plant volatile-baited traps were significantly correlated with adult feeding notches near that trap in both the summer (r = 0.77, p = 0.03) and fall (r = 0.83, p = 0.01) but not for the spring (r = 0.24, p = 0.99) (Figure 15). Interestingly, the PLW captured in semiochemical traps in summer and fall 2015 were not from the same generation of PLWs whose feeding damage was assessed, but were from the next generation. Adult feeding damage was not significantly correlated with season-long PLW captures in traps baited with pheromone alone in the spring (r = -0.14, p = 0.99), summer (r = - 0.20, p = 0.99), or fall (r = 0.14, p = 0.99) of 2015 (Figure 15).

In contrast to the correlations between trap catch and adult feeding damage, PLW larval density was only weakly correlated with trap catch in traps baited with pheromone alone ($r = 0.72$, $p = 0.10$; Figure 16). Larval density was correlated with PLW captures in pheromone traps in summer 2015, when PLW captures overall were low ($n = 23$ PLW captured in pheromone traps). Larval density was not correlated with PLW captures in traps baited with both pheromone and host plant volatiles.

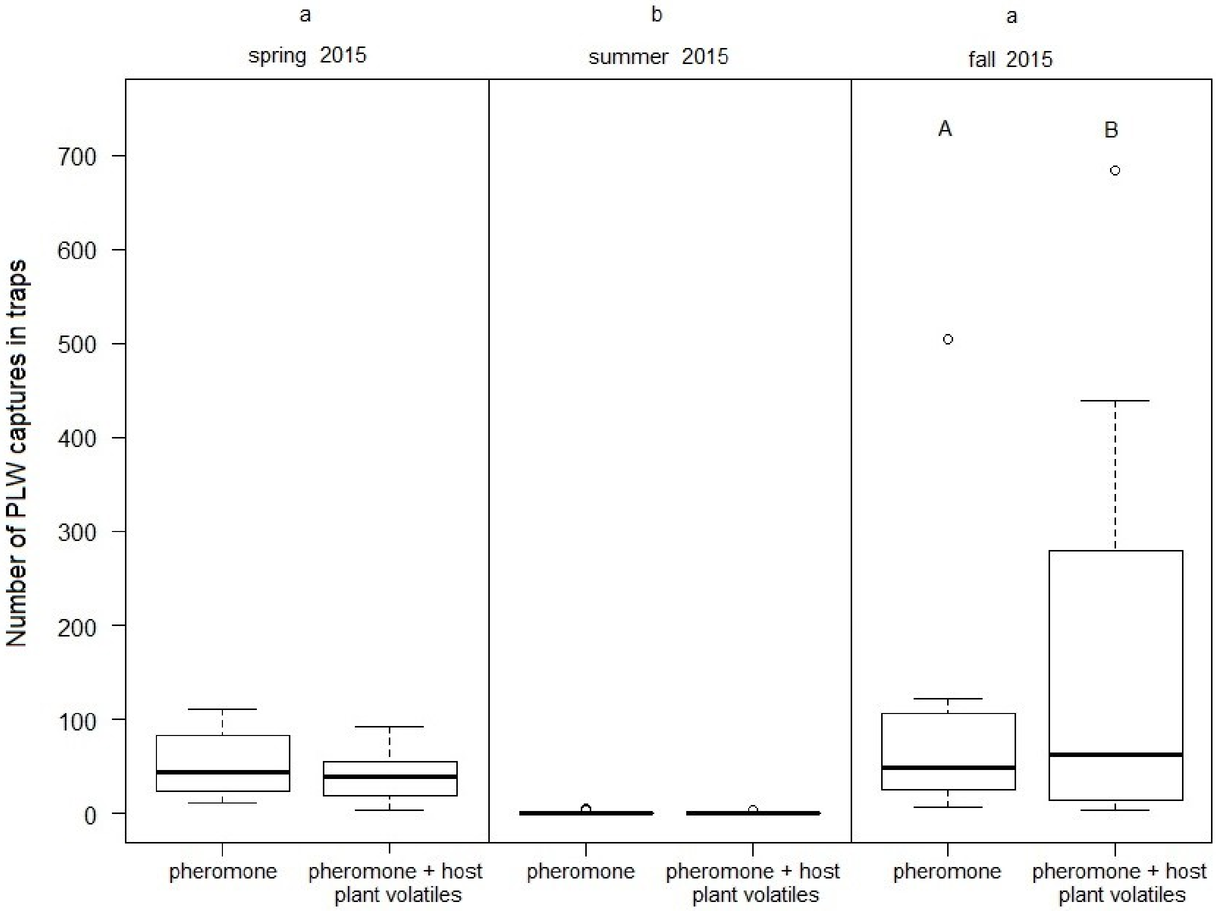

Figure 11. Box plot of season-long capture of pea leaf weevils (*Sitona lineatus)* in baited traps tested in spring, summer and fall of 2015. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. The total number of PLW captured in each season was compared using the model glmer.nb(*Total PLW in 2015 ~ Lure + Season + (1|Site))*. Significant differences in the total PLW captured between each season are denoted at the top of the plot with lowercase letters for α = 0.05. PLW captures within each season were compared between semiochemical traps using three separate models (glmer.nb(*Total PLW per season ~ Lure + (1|Site))* which were run separately for each season. Significant differences in PLW captures between two semiochemical traps of a given season are denoted with capital letters for α = 0.05.

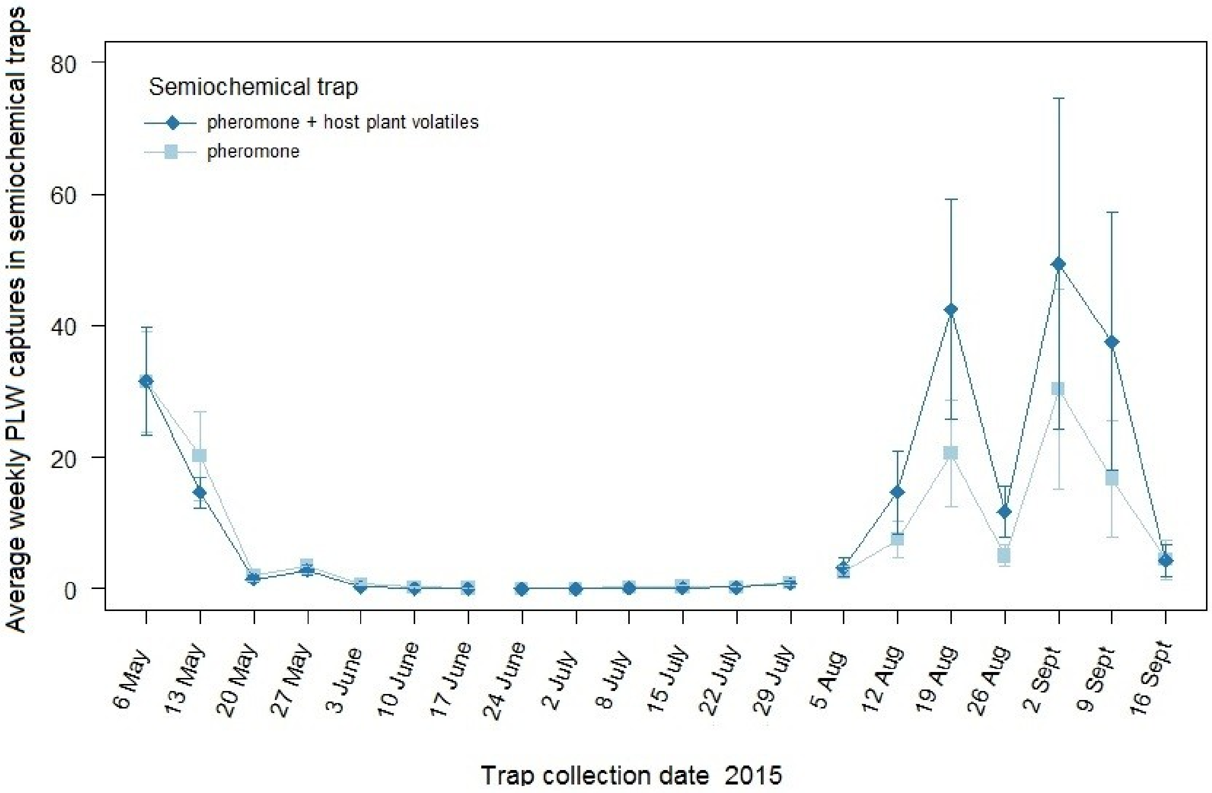

Figure 12. Average weekly captures of pea leaf weevils (*Sitona lineatus)* in semiochemical traps tested in 2015. Collection dates from 6 May-7 June , 24 June to 29 July, and 5 August to 16 September constitute the spring, summer and fall trapping periods, respectively. Traps were checked weekly and semiochemical lures were replaced on 17 June 29 July. Pea fields used in this experiment were seeded in the last week of April, 2015. By 20 May, after peak springtime captures of PLW, pea crops had 2 to 4 nodes of growth. Adult feeding damage was assessed on 11 to 13 June, when peas had 5 or 6 nodes of growth. Larval populations were assessed on 19 to 21 June, when peas were flowering. Pea fields were harvested in the first week of August.

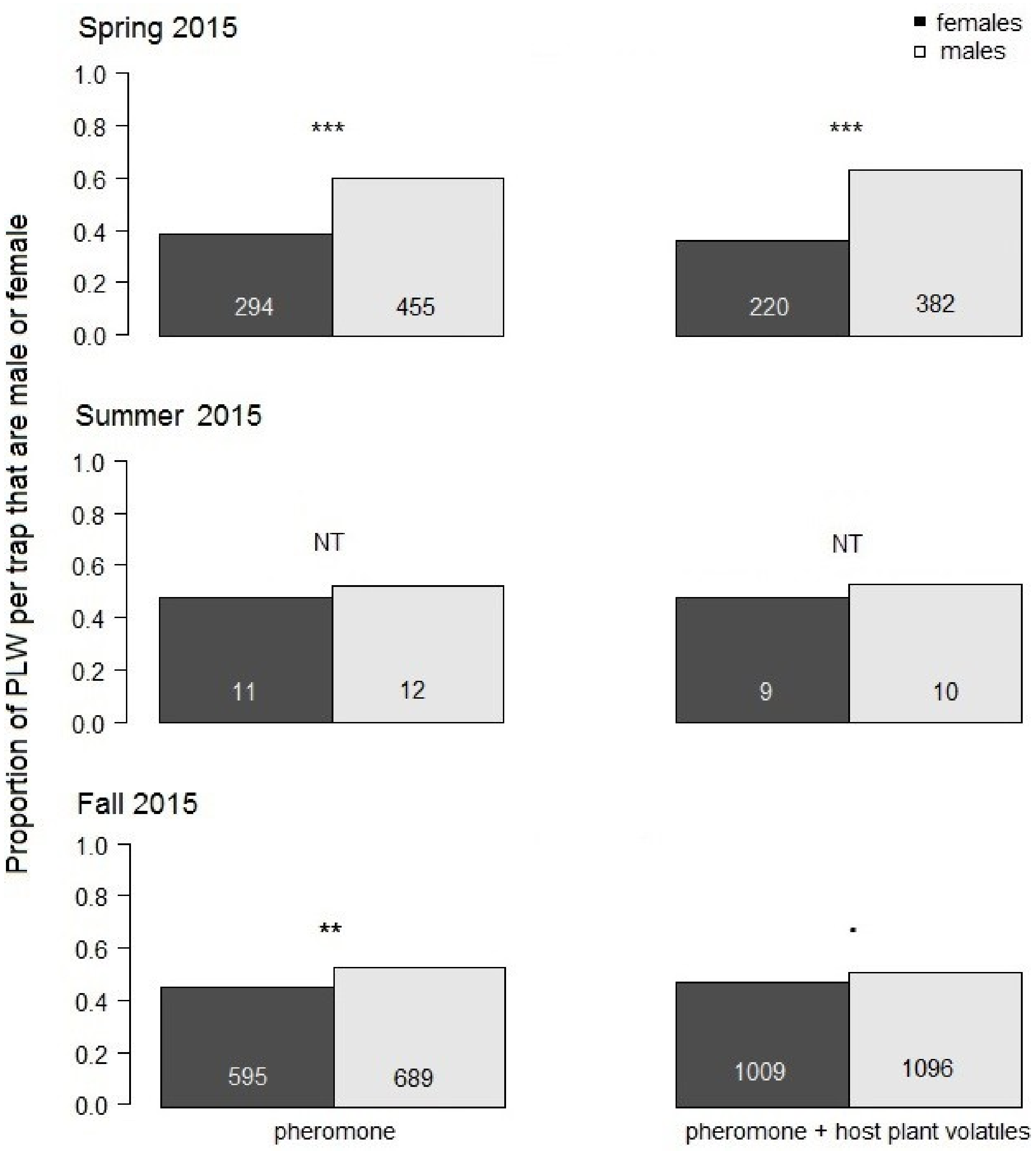

Figure 13. Proportion of male and female pea leaf weevils (*Sitona lineatus)* captured in semiochemical traps in 2015. In each season, trap capture was separated by sex in each semiochemical trap and the proportion of males and females captured was compared with a two-sided binomial test within trap type. Significant differences between the proportion of male and female PLW captured within a semiochemical trap are denoted with "." for α = 0.10, " * " for α = 0.05, " ** " for α = 0.01, and " *** " for α = 0.001. When trap captures were sufficiently low, a binomial test was not performed; this is indicated with "NT".

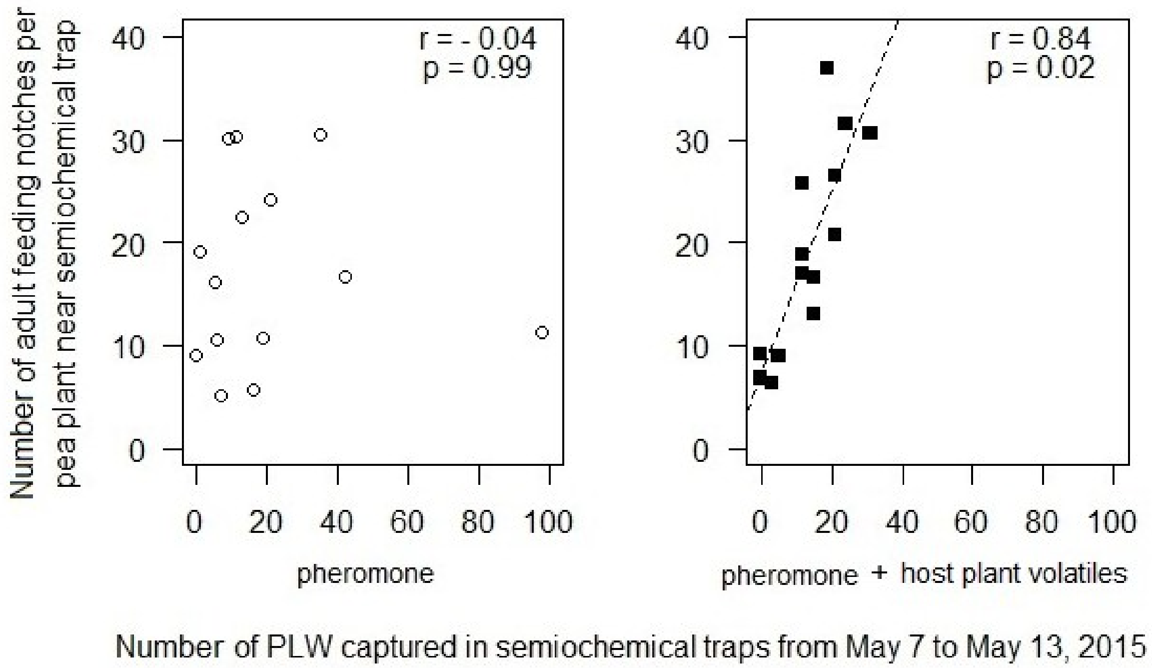

Figure 14. Pearson correlation between adult pea leaf weevil (*Sitona lineatus)* feeding damage near semiochemical traps and the number of PLW collected in that trap during the second week of trap collection from 7 May to 13 May, 2015 when peas were in the seedling stage. Adult feeding damage is measured as the average number of adult feeding notches per plant on ten pea plants closest to that semiochemical trap. Pearson correlations were adjusted using the Holm method.

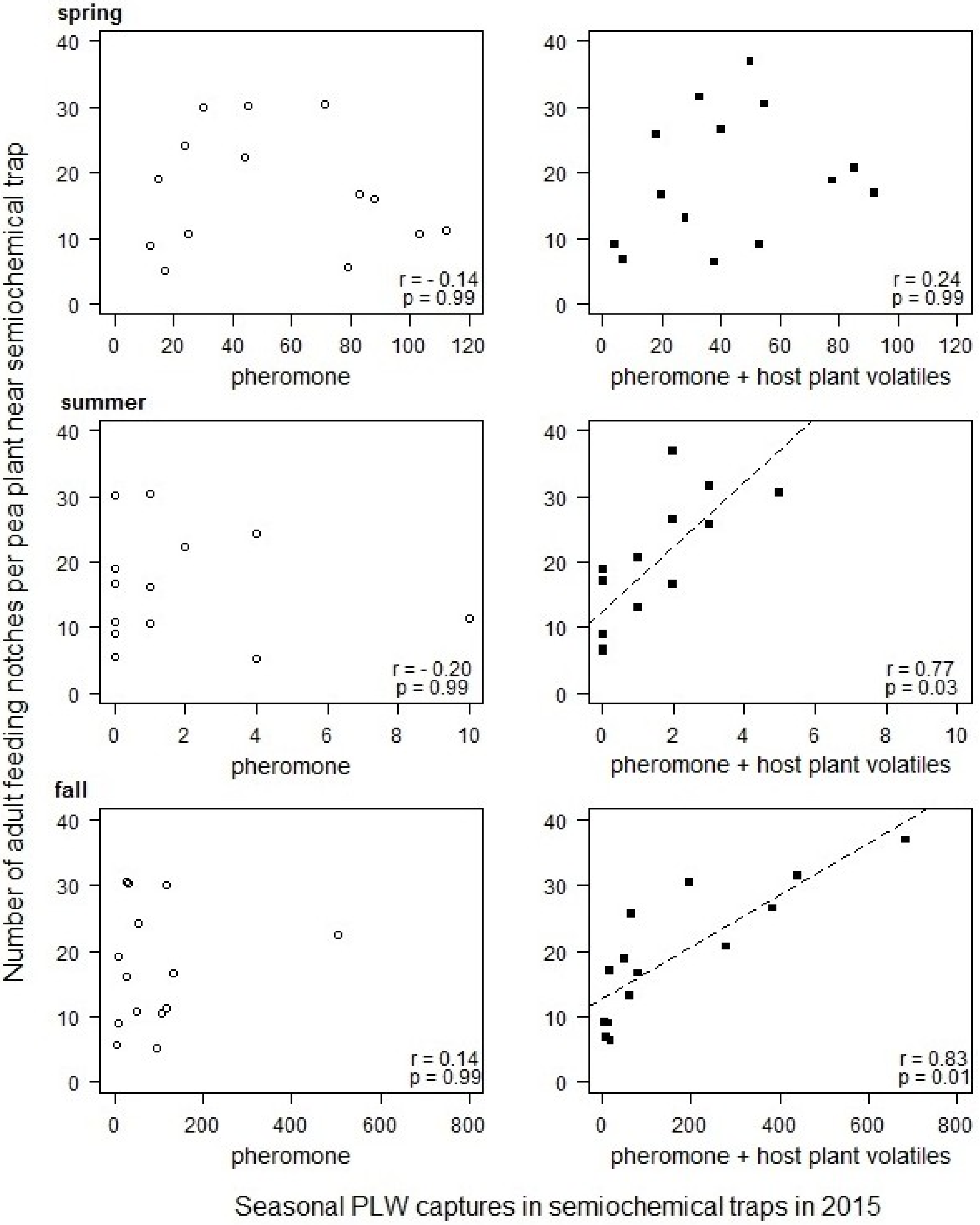

Figure 15. Pearson correlation between adult pea leaf weevil (*Sitona lineatus)* feeding damage near semiochemical traps and the number of pea leaf weevils collected in that trap during each season in 2015. Adult feeding damage is measured as the average number of adult feeding notches per plant on ten pea plants closest to that semiochemical trap. Pearson correlations were adjusted using the Holm method.

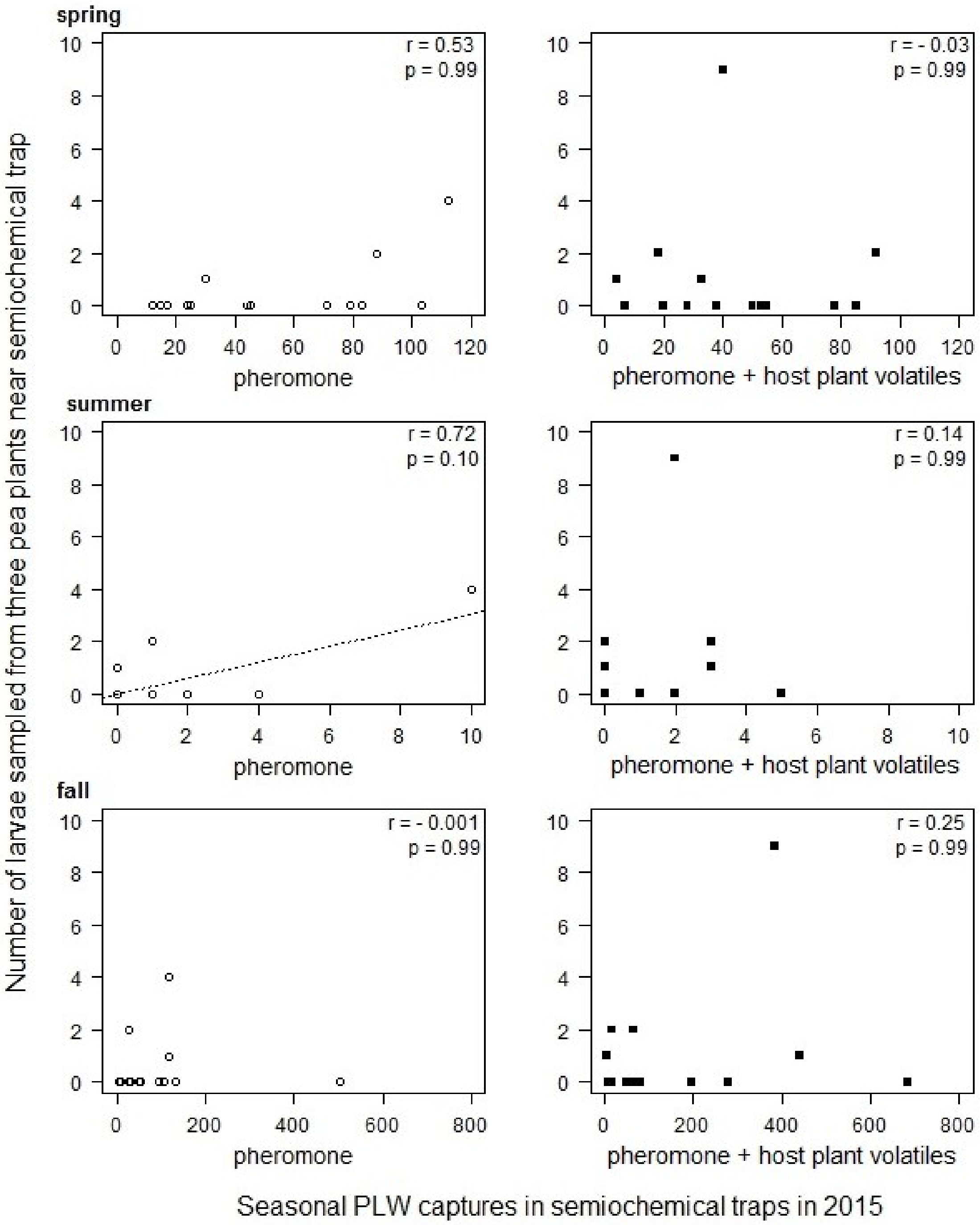

Figure 16. Pearson correlations between larvae sampled near semiochemical traps and the total number of pea leaf weevils (*Sitona lineatus)* captured in that semiochemical trap each season. Larvae were sampled from soil core samples of the roots of three pea plants near each semiochemical trap. Pearson correlations were adjusted using the Holm method.

2.3.2 Trap Type

2.3.2.1 Trap Type Experiment 2013

There was a significant difference between the number of PLWs captured and retained in the different trap types tested in 2013 ($\chi^2 = 99.31$, df = 8, $p < 0.0001$; Figure 17). The pitfall traps constructed from Solo cups captured significantly more PLW than any of the other traps tested in 2013 ($p < 0.0001$). The remaining trap types captured and retained a similar (negligible) number of PLW. In addition, Unitraps captured a high level of Hymenopteran bycatch and are not recommended for monitoring PLW. Yellow sticky cards were difficult to handle and did not effectively capture PLWs perhaps because they only faced one direction and became easily dust covered and less sticky. Cone traps suspended in the air did not capture many insects at all compared to cone traps placed directly on the ground. Cone traps placed on the ground and on pyramid traps made excellent spider homes and this may have reduced successful PLW captures in these traps.

2.3.2.2 Trap Type Experiment 2014

In the 2014 trap type experiment, there was again a significant difference between the number of PLW successfully captured and retained in the different trap types tested ($\chi^2 = 125.84$, df = 7, $p < 0.001$). The unmodified Solo pitfall cup, the Solo pitfall cup modified with chicken wire to exclude large animals, and the yellow pan trap were all successful at capturing and retaining PLW (Figure 18). The remaining traps captured a similar (negligible) number of PLW. There was no effect of trap position on the capture of PLW as traps placed on the edge of the field captured a similar number of PLW as those placed on a parallel transect 25m into the field ($\chi^2 = 0.063$, df = 1, $p = 0.802$).

2.3.2.3 Trap Type Experiment 2015

A low number of PLW were captured in the trap type experiment in 2015 and there was no significant difference between the number of PLW captured in these three trap types tested ($\chi^2 = 2.42$, df = 2, $p = 0.298$).

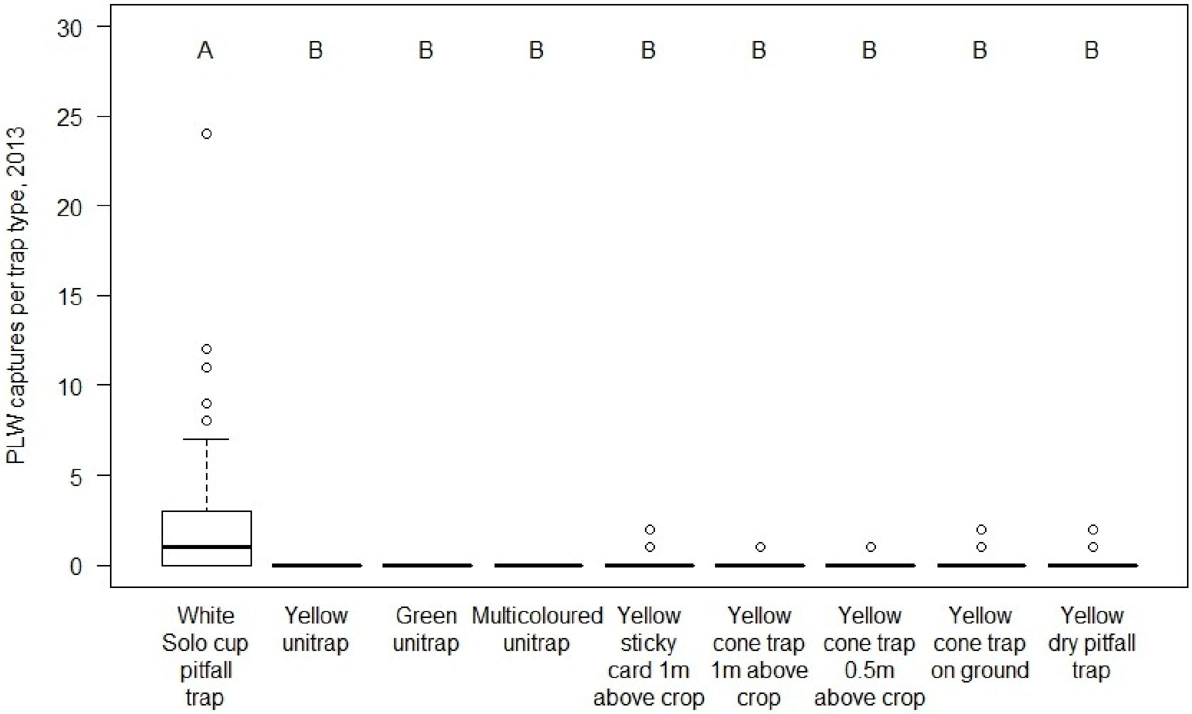

Figure 17. Box plot of pea leaf weevils (*Sitona lineatus)* captured in different trap types baited with 21 mg of PLW aggregation pheromone released from a 250 µl Eppendorf tube from 8 August – 6 September 2013. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. Trap types were compared to each other using the model glmer.nb(*Total PLW ~ Trap type + (1|Site).* Traps with significantly different PLW captures have different letters ($p<0.05$). Trap capture of PLW in the Solo cup pitfall trap was greater than in all other trap types tested.

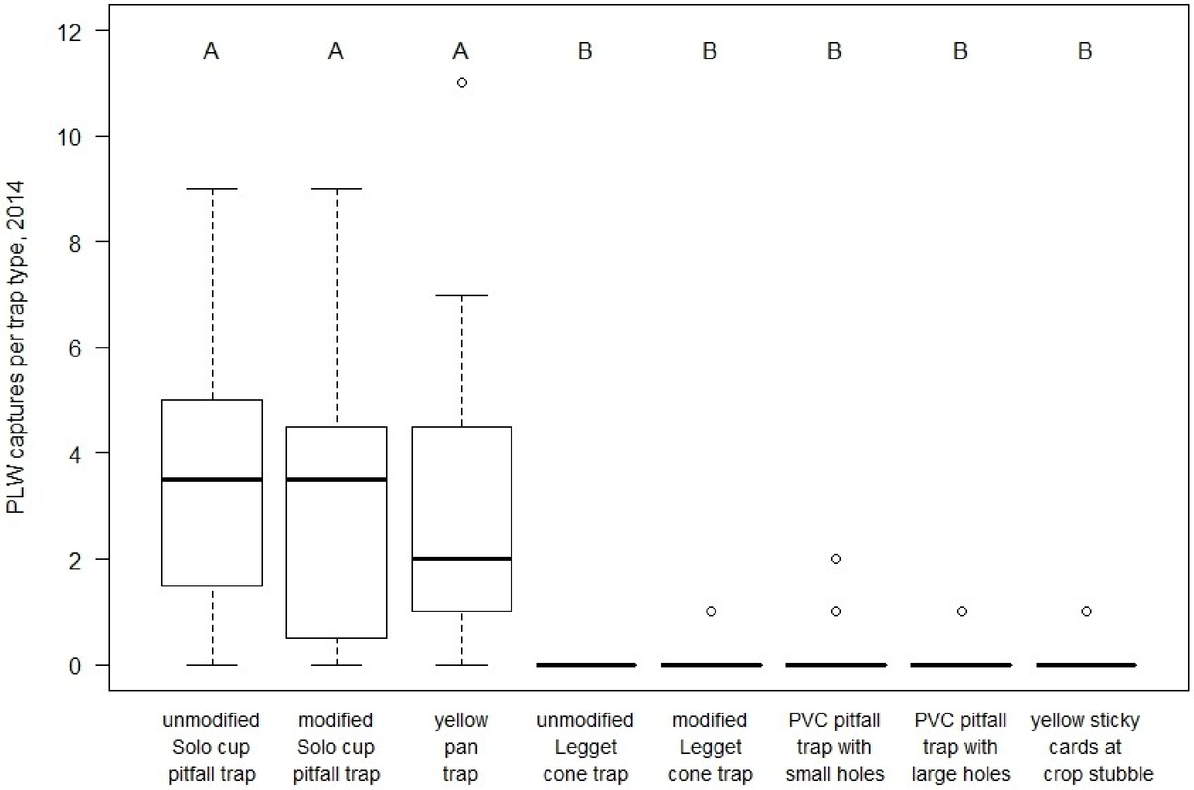

Figure 18. Box plot of pea leaf weevils (*Sitona lineatus)* captured in different trap types baited with 21 mg of PLW aggregation pheromone released from a 250 µl Eppendorf tube from 7 August – 15 September, 2014. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. Trap types were compared to each other using the model glmer.nb(*Total PLW ~ Trap type + Trap placement (1|Site)*. Traps with significantly different PLW captures have different letters ($p < 0.05$).

2.2.3 Seasonal Plasticity to Semiochemical Cues in the Laboratory

2.2.3.1 Olfactometer Bioassays

The proportion of PLW that responded in olfactometer bioassays did not differ significantly among treatment groups comprised of male or female weevils in various physiological states (newly overwintered, reproductively active, or newly eclosed) and sex (χ^2 = 5.41, df = 5, p = 0.368; Figure 19). For each of the six treatment groups, 51.9% to 73.5% of PLWs responded. When analyzed separately, sex did not influence the proportion of PLW that responded in the olfactometer (χ^2 = 2.07, df = 1, p = 0.15), although males (68.6% response) tended to respond more frequently than females (58.7%). Physiologial state alone did not influence the proportion of responding PLW (χ^2 = 2.44, df = 2, p = 0.295), although overwintered PLW (70.6% response) tended to respond more frequently than reproductively active (59.14% response) or newly eclosed PLW (60.76% response).

The first choice of odour source did not differ between treatment groups (χ^2 = 16.53, df = 15, p = 0.348; Figure 20). Weevil sex (χ^2 = 1.06, df = 3, p = 0.786) and physiological state (χ^2 = 8.08, df = 6, p = 0.233) did not significantly influence the first choice of odour source in the olfactometer. For each combination of sex and physiological state tested, the first odour choice varied in some instances from an expected even distribution (Figure 20). For all groups of PLW the most frequent first choice was either the area with volatiles from 5 male PLW alone or the area with volatiles from 5 male PLW on pea.

For recently overwintered females (χ^2 = 9.52, df = 3, p = 0.023), the distribution of first choice of odour source was significantly different from an equal distribution, as recently overwintered females responded more frequently than the expected 25% to the quadrant supplied with volatiles from male PLW and peas (47.82%) and to the quadrant supplied with just male PLW volatiles (30.34%). The frequency of response of recently overwintered females that responded to the quadrant supplied with pea volatiles (4.35%) or to the control quadrant (17.39%) was lower than the expected 25%. For recently overwintered males, the distribution of first choices was not significantly different from an even distribution (χ^2 = 2.36, df = 3, p = 0.501). For PLW tested during the period of reproductive activity, in July, the first choice of odour source made by both females (χ^2 = 5.74, df = 3, p = 0.125) and males (χ^2 = 8.8571, df = 3,

$p = 0.0313$) did not differ from an even distribution. The first choice of odour source by male PLW during the period of reproductive activity to male PLW volatiles (46.43%) and to male PLW and pea volatiles (28.57%) was higher than expected in an even distribution. The response to pea volatiles alone (10.71%) and to the control (14.29%) was lower than expected. For newly eclosed, pre-overwintered females ($\chi^2 = 3.19$, df = 3, $p = 0.363$) and males ($\chi^2 = 3.46$, df = 3, $p = 0.325$), the distribution of first odour choices did not significantly differ from an even distribution.

The overall preferred odour choice did not differ significantly between PLW in the different treatment groups ($\chi^2 = 16.53$, df = 15, $p = 0.348$; Figure 21). Weevil physiological state ($\chi^2 = 12.38$, df = 6, $p = 0.054$) did not significantly impact the overall odour preference. There was, however, a trend towards a difference in overall odour preference between recently overwintered and mating weevils, with mating PLW exhibiting a stronger preference for volatiles from male PLW, and with recently overwintered PLW responding to volatiles from male PLW and from male PLW on pea. Weevil sex ($\chi^2 = 6.03$, df = 3, $p = 0.11$) did not significantly affect the overall preferred odour source, however, females tended to respond more frequently to volatiles from male PLW than males did; this is especially true for females versus males that are newly eclosed. Male PLW also tended to respond more frequently to volatiles from pea plants alone than did female PLW.

For all treatment groups of weevils tested, except for recently eclosed males, the distribution of preferred odour sources significantly differed from a null distrubition. Both overwintered females ($\chi^2 = 13.76$, df = 3, $p < 0.01$) and overwintered males ($\chi^2 = 8.12$, df = 3, $p = 0.0436$, the distribution of preferred odours differed from the null hypothesis of an even distribution. The response of overwintered females to volatiles from male PLW alone (44.12%) and to volatiles from male PLW and pea plants (35.29%) was higher than expected. Interestingly, overwintered females preferred the control odour source (a pot of soil) more frequently than pea volatiles alone (2.94%). For overwintered males, the preferred odour source was most frequently male PLW alone (44.12%). Overwintered males responded to volatiles from male PLW on pea plants was close to what is expected in an even distribution (26.47%). Overwintered males responded less frequently than expected to volatiles from pea plants alone (17.65%) or to the blank control (11.76%). Males ($\chi^2 = 51.20$, df = 3, $p < 0.0001$) and females (χ^2

= 46.62, df = 3, $p < 0.0001$) tested during the period of reproductive activity also showed unequal preference of odours. Females tested during the period of reproductive activity showed a strong overall preference for volatiles from male PLW alone (65.38%). The response to male PLW and pea volatiles (17.31%), pea volatiles alone (5.77%) and to the control (11.54%) was lower than expected. Similar to females, males tested during the period of reproductive activity showed a strong preference for volatiles from male PLW alone (73.17%). Response to volatiles from male PLW on pea plants (12.20%), pea plants alone (9.76%) and the control (4.88%) was lower than expected. For newly eclosed weevils, females did not prefer each odour equally (χ^2 = 41.69, df = 3, $p < 0.0001$). Again, there was a strong preference for volatiles from male PLW alone (63.46%) and response to volatiles from male PLW and pea plants (13.46%), pea plants alone (7.69%) and the control (15.38%) was less frequent than expected. Newly eclosed male PLW were the only group of weevils tested for which the distribution of preferred volatile choice did not differ from an even distribution (χ^2 = 5.74, df = 3, $p = 0.125$). For each physiological state and sex tested, the most frequently preferred odour source was five male PLW.

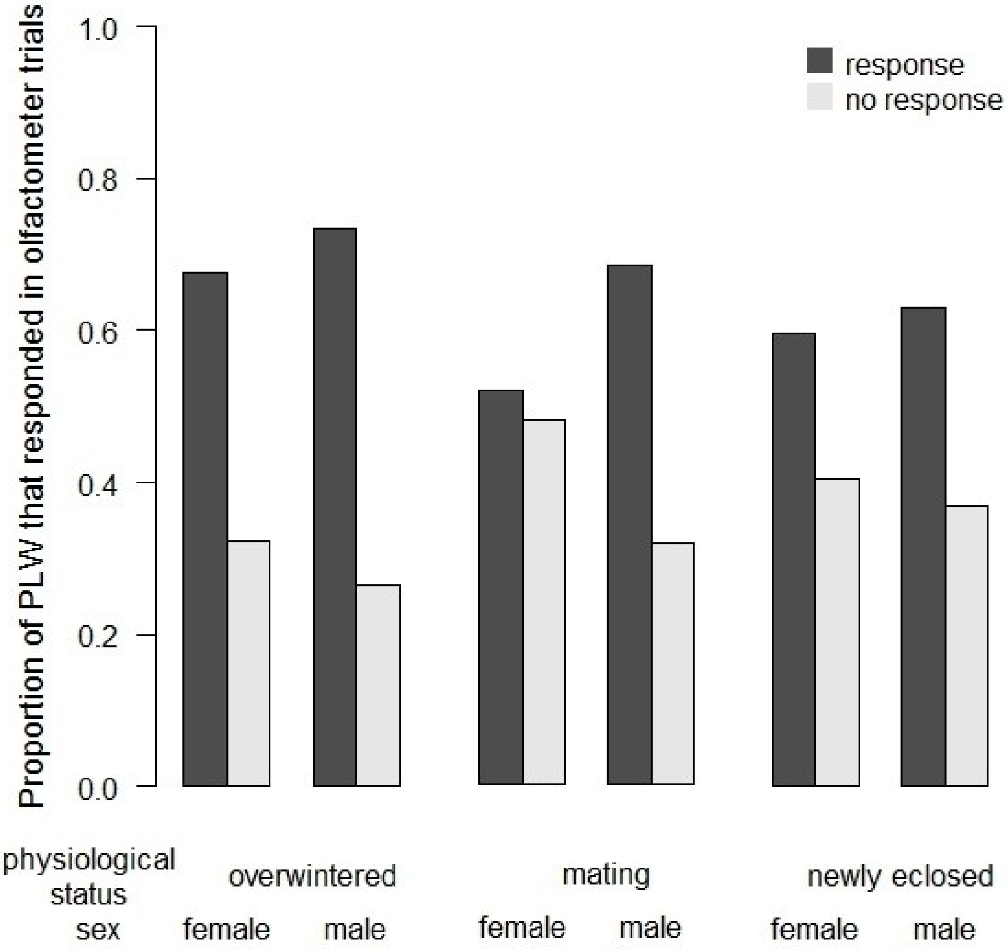

Figure 19. Proportion of male and female pea leaf weevils (*Sitona lineatus)* in different physiological states that responded in olfactometer trials. A 2 x 6 chi-square contingency table was used to determine the proportion of responsive to non-responsive weevils for each combination of physiological state (overwintered, mating, or newly eclosed) and sex of weevil tested in olfactometer bioassays. The proportion of PLW that responded did not significantly differ with physiological state or sex ($\chi^2 = 5.4118$, df = 5, p = 0.3677).

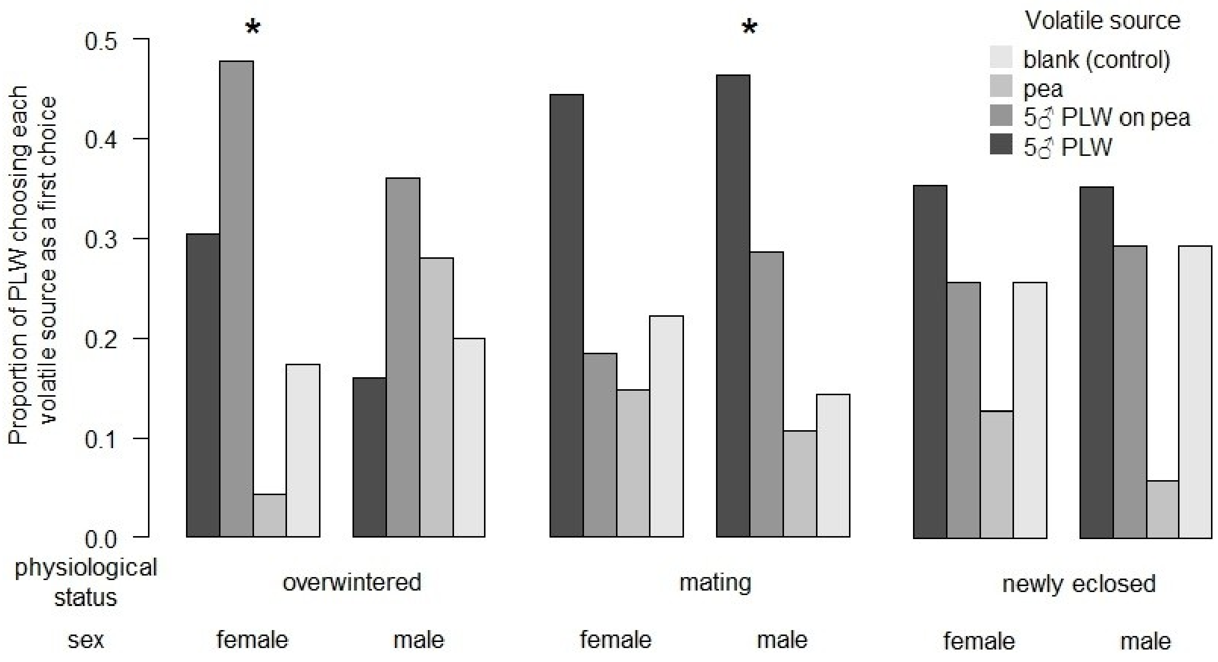

Figure 20. Distribution of the first odour choice in olfactometer trials by overwintered, mating, or newly eclosed, male or female pea leaf weevils (*Sitona lineatus).* Only PLW that responded in olfactometer trials were included in this analysis. First odour choice did not differ by weevil sex or physiological state, based on a chi-square contingency table analysis (χ^2 = 16.53, df = 5, p = 0.348). For each sex and physiological state tested, the distribution of first choices was subsequently compared to an even distribution using chi-square goodness of fit tests. Asterisks indicate that the first odour choice by overwintered females (χ^2 = 9.52, df = 3, p = 0.0231) and by mating males (χ^2 = 8.86, df = 3, p = 0.031) were significantly different from an even distribution.

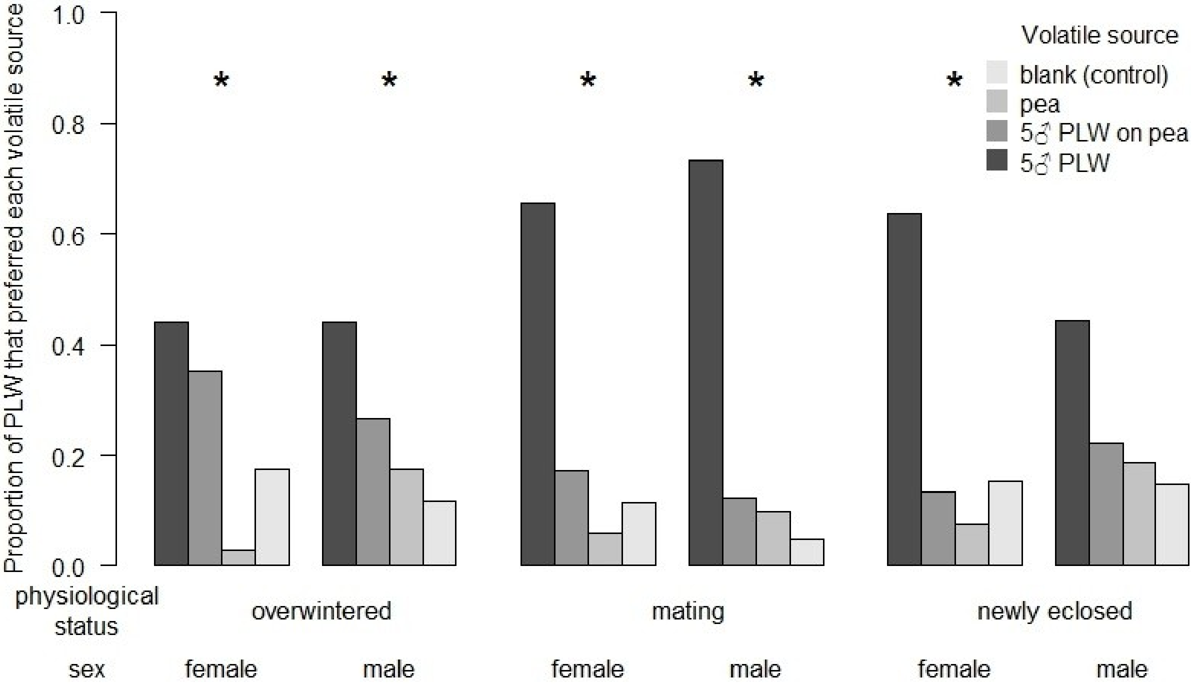

Figure 21.Distribution of preferred odour sources tested in the four-way olfactometer for male and female pea leaf weevils (*Sitona lineatus)* in different physiological states. Only PLW that responded in olfactometer bioassays were included in this analysis. The odour preferences of PLW were compared between weevils by sex and physiological state via a contingency table analysis. Odour preferences did not significantly differ by sex or physiological state at α = 0.05, but this difference was significant at α = 0.10 (χ^2 = 23.06, df = 15, p = 0.083). For each physiological state and sex tested, the distribution of first odour choices was then compared to an even distribution using chi-square goodness of fit tests. Asterisks indicate that the distribution of preferred odour choices of all PLW, except for newly eclosed males, was significantly different from an even distribution at α = 0.05.

2.4 Discussion

2.4.1 Semiochemical Blend, Dose and Lure Type

After comparing various release rates and combinations of PLW pheromone and host plant volatiles, the optimal lure for PLW tested was 21 mg of 4-methyl-3,5-heptanedione in a 250 µl Eppendorf tube, with host volatile lures containing: 21 mg of (*Z*)-3-hexenyl acetate in a 250 µl Eppendorf tube, 34mg of (*Z*)-3-hexenol in a 250 µl Eppendorf tube, and three 250 µl Eppendorf tubes, each with 50mg of linalool. Evaluation of various ground-based and aerial insect traps found that the most successful trap type is a wet pitfall trap, which is easily constructed from Solo cups, Choloplast and nails. Semiochemical-baited traps successfully attracted and retained PLW adults in both the spring and fall, corroborating the findings of Evenden *et al.* (2016). Pea leaf weevil captures in traps baited with the optimal lure were sometimes correlated with feeding damage of the previous generation of adults, indicating that PLW captures in these traps show potential to be representative of true PLW populations in pea fields. Capture of PLW in baited traps in the fall permits the development of assessment of pre-overwintering populations in the fall. Assessment of overwintering population densities could assist producers in planning pest management strategies including whether to plant insecticide-treated pea seed the following spring.

Trap capture in semiochemical-baited traps can increase with semiochemical dose (Byers 2013) because the active space of the pheromone signal is larger (Byers 2008) or the plume is more readily sensed by insects (Dolzer *et al.*2003). Pea leaf weevils, however, did not respond in a dose-dependent manner to semiochemical-baited traps tested in 2013 and 2014. Smart *et al.* (1994) also found that PLW did not respond in a dose-dependent manner to pheromone-baited traps. Perhaps response to a broad range of semiochemical doses relates to the aggregating mating behaviour of PLWs as aggregations may vary from a few to a few thousand male and female PLWs, and therefore PLWs may need to respond to a wide range of PLW pheromone release rates. Similarly, attraction of plum curculio (*Conotrachelus nenuphar)* (Coleoptera: Curculionidae) weevils to trap trees at the perimenter of apple orchards was not enhanced by a 5-fold increase in pheromone dose (Leskey *et al.* 2014). The red palm weevil, *Rhynchophorus ferrugineus* exhibits a dose-dependent response to its male-produced aggregation pheromone at lower release rates, but this relationship breaks down at higher release rates (Vacas *et al.* 2016).

If the quantity of a pheromone signal is increased with weevil aggregation size, enhanced response to high release rates would be advantageous only to a certain point, depending on the optimal aggregation size. This may be similar to the behaviour of granary weevils, *Sitophilus granarius* (Coleoptera: Curculionidae) who are attracted to low concentrations of their aggregation pheromone but cease movement when detecting sufficiently high concentrations of pheromone (Plarre 1994). Future studies which test a broader range of PLW pheromone release rates should be conducted to determine if PLW behavioural response is dose-dependent at lower release rates or if high doses repel or arrest PLW. It is also expected that a dose-dependent response to PLW pheromone would be found in electroantennogram analyses, similar to the lucerne weevil (*Sitona lineellus)* (Unelius *et al.* 2013).

In this experiment, PLW response to pheromone was enhanced by the presence of host plant volatiles in spring 2013, fall 2013, fall 2014 and fall 2015. In fall 2014, the host plant volatile lures were tested on their own, and in this experiment, the pheromone lure was synergized by the host plant volatile lures. Blight *et al.* (1984) and Evenden *et al.* (2016) also found that PLW response to pheromone was sometimes synergized by the presence of host plant volatiles. Although PLW captures in pheromone traps were sometimes enhanced with the addition of a host plant volatile lure, the two host plant volatile lures tested here were not attractive on their own. This is in contrast to laboratory studies that showed PLWs orienting to plant volatile compounds alone in an olfactometer assay (Landon *et al.* 1997). Similarly, the strawberry blossom weevil, *Anthonomus rubi* (Coleoptera: Curcculionidae), is consistently attracted to traps baited with its pheromone, exhibits a synergistic response to traps baited with its pheromone and host plant volatiles, but does not respond to traps baited with host plant volatiles alone (Wibe *et al.* 2014).The cotton boll weevil, *Anthonomus grandis* (Coleoptera: Curculionidae), also displays an increased response to a combination of its pheromone and host plant volatiles over pheromone alone or host plant volatiles alone (Dickens 1989). A synergistic effect of host plant volatiles when combined with pheromone signal would be expected to occur if calling or mating bevhaiour is tied to the host plant (Landolt 1997).

Pea leaf weevils consistently respond to pheromone in the spring and fall, but PLW may be more likely to respond to host plant volatiles in the fall because the plume eminating from the trap is more apparent at that time of year due to a scarcity of host plants. In the fall there would be fewer background host plant volatiles competing with semiochemical lures and PLW need to find plants in the fall to feed before overwintering (Jackson 1920; Landon *et al.*1997). The olfactory response of other insects is influenced by background volatiles. For example, diamondback moth (*Plutella xylostella* (Lepidoptera: Plutellidae)) captures in pheromone-baited traps are enhanced by the addition of host plant volatiles in semiochemical traps placed in cabbage crops but not in canola crops (Reddy and Guerrero 2000; Miluch *et al.* 2014). During the spring trapping period, the pea crop is green and edible to PLW (Jackson 1920; Landon *et al.* 1995). By the fall trapping period, however, most pea crops have desiccated, are at or near the harvesting stage and are not as attractive to PLW as green, secondary hosts (Landon *et al.* 1995). As pea plants enter the flowering or bud states, relative emission of (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate decreases compared to other volatiles (Thoming *et al.* 2014). There was an exception to the general findings in fall 2012 (Evenden *et al.* 2016) and spring 2013 in the current study. In fall 2012, the addition of host plant volatile lures to pheromone-baited traps did not enhance PLW captures as they did when tested in other years. This same cohort of weevils was trapped in greater numbers in traps baited with pheromone and host plant volatiles than in traps baited with pheromone alone the following spring (2013). The difference in response to host plant volatile by this cohort likely indicates cohort-specific conditions experienced by these PLWs, such as an abundance of food in fall 2012 or a scarcity of food in spring 2013.

In the current study, the sex ratio of weevils captured in semiochemical-baited traps varies with semiochemical lure and season of trapping. Pea leaf weevil captures in spring 2014, spring 2015 and fall 2015 were male-biased. In fall 2014, traps captured even sex ratios except for traps baited with a high release rate of host plant volatiles and without pheromone, which was significantly female-baised. This may be a result of the strong need for female PLW to feed before overwintering in order to maximize their egg production (Schotzko and O'Keeffe 1986). Blight *et al.* (1984) also reported male-biased captures of PLW in semiochemical traps in the spring. Interestingly, Evenden *et al.* (2016) reported female-biased PLW captures in semiochemical traps in the spring and an even number of males and females in the fall. Both male-biased and female-biased PLW captures in semiochemical traps were reported by Nielsen and Jensen (1993).

Besides investigating sex-specific or physiology-specific differences in PLW response to odours further in olfactometer bioassays, future studies should investigate the peripheral and central olfactory system of PLW. It is possible that PLWs exhibit sexual dimorphism in the expression of olfactory receptor neurons, similar to the clover root weevil (*Sitona lepidus*) (Park *et al.* 2013) and the lucerne weevil (*Sitona lineellus*) (Unelius *et al.* 2013). Male clover root weevils have three types of olfactory sensillae containing olfactory receptor neurons specialized for their male-produced pheromone and four types specialized for host plant volatiles (Park *et al.* 2013). Females, on the other hand, have only two sensillae containing olfactory receptor neurons specialized for pheromone but have five specialized for host plant volatiles. The lucerne weevil (*Sitona lineellus*) also exhibites some sexual dimorphism in EAG response: males showed a stronger EAG response to the (4*S*,5*S*)-isomer of 5-hydroxy-4-methyl-3-heptanone and females showed a similar EAG response to all four isomers (Unelius *et al.* 2013). The pepper weevil, *Anthonomus eugenii* (Coleoptera: Curculionidae) also exhibits sexual dimorphism in response to semiochemicals as male response to pheromone is synergized with the addition of host plant volatiles, but female response to pheromone is not (Muniz-Merino *et al.* 2014). Ju *et al.* (2017) found that the response of the dark black chafer *Holotrichia parallela* (Coleoptera: Scarabaeidae) to its female-produced sex pheromone is synergized by host plant volatiles but that this synergistic response varies between males and females.

Variation in the sex ratio of PLW that respond to semiochemical traps may occur in different cropping systems. Pea leaf weevil captures on pheromone-baited sticky traps during the spring dispersal period in Denmark were female-biased in faba bean fields but had even sex ratios in clover and alfalfa In the same study, pheromone-baited cone traps captured significantly more males than females in clover and wheat but significantly more females than males in alfalfa (Nielsen and Jensen 1993). Blight *et al.* (1984) and Blight and Wadhams (1987) placed traps in fallow fields in the spring that contained bean crops the previous year and recorded male-biased PLW captures in traps baited with synthetic pheromone or pheromone and host plant volatiles but no sex ratio bias in traps baited with natural semiochemicals. In experiments reported here, males and females responded similarly to natural semiochemicals in the olfactometer. This could possibly be explained by a component of the male PLW aggregation that has yet to be identified. Other *Sitona* species also respond to 4-methyl-3,5-heptanedione and a multi-component pheromone blend may enhance discrimination between different *Sitona* species (Toth *et al.* 1998;

Park *et al.* 2013; Unelius *et al.* 2013). Other *Sitona* species also show sex-specific differences in response to pheromone components (Park *et al.* 2013; Unelius *et al.* 2013), and a missing pheromone component may explain some differences in sex ratios of PLW captured in different experiments. Future experiments should investigate if there are unidentified components of the male PLW pheromone.

It is also possible that PLW sex ratios in semiochemical traps differ among experiments because the sex ratio of the overall PLW population differs by location and year. Some PLW populations may be infected with a reproductive parasite that skews sex ratios of their arthropod hosts, such as *Wolbachia* (Werren *et al.* 2008) or *Rickettsia* (Lawson *et al.* 2001). *Wolbachia* and *Rickettsia* have been isolated from *Sitona oboletus* in New Zealand (White *et al.* 2015); however, it is unknown if *Wolbachia* or *Rickettsia* affect *Sitona* sex ratios. It is also unknown if *Rickettsia* infect *Sitona* species. Floate *et al.* (2006) did not find *Wolbachia* in the PLW individual that they investigated; however Floate *et al.* (2006) note that their results likely underestimate the incidence of infected arthropods. It is also possible that PLW sex ratios are tied to PLW density, and that larger or smaller aggregations tend to be female- or male-biased, if sex specific costs and benefits change at different population densities, as occurs in other insects such as the sandfly (*Lutzomyia longipalpis)* (Diptera: Phlebotominae) (Jones and Quinnell 2001). Laboratory feeding experiments by Schotzko and O'Keeffe (1988) found that female PLW have greater longevity than male PLW when maintained in single reproductive pairs but that male and female PLW have similar longevity when maintained in groups of 13 reproductive pairs, indicating that PLW density may affect PLW mortality in a sex-specific way. This in contrast, however, to the sex ratios of PLW captured in fall 2014 and fall 2015 in our experiments, which suggest that males may be longer-lived than females. Future studies should determine if PLW sex ratios in pea fields are density dependent and if PLW sex ratios in semiochemical traps mirror PLW sex ratios in surrounding crops. Electrophysiological bioassays should be performed to determine if PLW exhibit sexual dimorphism in their expression of olfactory receptor neurons.

During the process of separating male and female PLWs in trap catch, weevils could be separated by condition. Weevils captured early in the spring were heavily sclerotized whereas by the end of the spring trapping period, PLW trapped often had rigid elytra that were difficult to open. The rigid elytra may indicate that the flight muscles of these weevils had atrophied in order to allocate more resources to the reproductive system, a condition known as 'oogenesis flight syndrome' (Johnson 1963). In 2015, when semiochemical trapping experiments were conducted continuously throughout the growing season, the majority of PLW captured in semiochemical traps in the summer were heavily sclerotized and had lost many scales. There are other reports of the overwintering generation of PLW surviving the summer (Fisher and O'Keeffe 1979;). Pea leaf weevils that overwintered and survived through the spring and summer into the fall trapping period are unlikely to mate again the following spring and therefore will not contribute to future PLW damage. Jackson (1920) estimated that PLW development from egg laying to pupation takes about 13 weeks. Adult PLW begin to eclose in late July in Alberta (Cárcamo and Vankosky). Overwintered PLW have previously been reported to live through the summer, until August, in Idaho (Schotzko and O'Keeffe 1988). It is therefore likely that the heavily sclerotized PLW captured in the summer in the current study were part of the previous generation that overwintered. The majority of PLW captured in the fall of 2014 and 2015 were suspected to be newly eclosed adults as they were lightly sclerotized and had scales and flexible elytra. A small proportion of the PLW captured in the fall were scale-less, heavily sclerotized and with fused elytra suggesting they survived through the summer into the fall. If the heavily sclerotized, scaleless PLW captured in the fall are from the previous generation, this suggests that the response of overwintered, mated PLW to semiochemicals is plastic as very few weevils are captured in the summer. Unfortunately, our olfactometer bioassays only tested recently overwintered weevils and overwintered mating weevils, but not overwintered weevils in summer (post-mating). Interestingly, the number of PLW from the overwintered generation captured in the fall of 2014 varied from 3% to 13% for female PLW and from 15% to 32% for male PLW. In fall 2015, 31% and 35% or 36% of PLW captures in either semiochemical trap were from the overwintered generation for males and females, respectively. Female PLW may be more sensitive to environmental conditions than males.

Trap capture of PLW in semiochemical-baited traps is related to adult feeding damage early in the spring and during the summer and fall and this relationship was best for traps baited with the combination of pheromone and host plant volatiles. Although there are many examples of enhanced insect trap capture with the addition of host plant volatiles to pheromone-baited traps (Blight et al.1984; Dickens 1989; Reddy and Guerrero 2000; Wibe et al. 2014; Evenden et al. 2016), there are few examples that explicitly relate captures in traps baited with host plant volatiles to insect populations or damage. One example where insect injury level is related to captures in traps baited with host plant volatiles is for the coffee berry borer (*Hypothenemus hampei)* (Fernandes *et al*. 2011). In the current study, adult PLW feeding damage was assessed on pea plants near semiochemical traps, but it is possible that feeding damage is artificially high near semiochemical traps (Smart *et al.*1994). The density of insects and plants in a habitat affect the response of insects to semiochemicals (Anderson *et al.* 2013) and PLW population density is variable from year to year. Pea leaf weevil longevity is related to the availability of food and density of weevils (Schotzko and O'Keefe 1988). Experiments comparing the efficacy of these semiochemical traps in different densities and varieties of pea crops would be helpful when developing a predictive PLW damage model from semiochemical trap captures. Adult feeding density may be artificially high near semiochemical traps if these semiochemicals act as phagostimulants to PLW, which is expected due to the close relationships between host plant location and mate finding (Jackson 1920) and between nutritional state and fecundity (Schotzko and O'Keeffe 1988) in this species. Moujahed et al. (2014) found that PLW herbivory on pea alters the volatile profile emitted by pea, but specific volatiles were not identified. Agelopous et al. (1999) reported that mechanically-damaged faba bean plants increased emission of (*Z*)-3-hexenal, (*Z*)-3-hexenol, and (*E*)-2-hexenal. Pea leaf weevils are responsive to (*Z*)-3-hexenol (Blight et al. 1984) and PLW captures in pheromone-baited traps are enhanced with the addition of a host plant volatile lure that includes (*Z*)-3-hexenol (Blight *et al.* 1984; Evenden *et al.* 2016). Future research should identify if the host plant volatile lures tested here act as feeding stimulants for PLW. Future research should also identify if PLW herbivory induces emission of attractive volatiles from host plants.

Larval density but not adult feeding damage was significantly correlated with PLW captures in traps baited with pheromone alone. The total PLW captured in the summer of 2015 was significantly correlated with the density of larvae near those traps at $\alpha = 0.10$. Pea leaf weevil larval densities 25m from these traps, however, were not significantly correlated with PLW trap captures. Larvae are not highly-mobile and larval density may be artificially high near semiochemical traps compared to the rest of the pea crop if female PLW remain part of the PLW mating aggregation while ovipositing. Some insects that aggregate as adults or juveniles also aggregate during oviposition; this is advantageous when larvae are more successful at high densities, such as when larval feeding is more efficient in groups (Desurmont et al. 2014) or for protection from predation (Arpaia et al. 2009) or dessication (Benoit et al. 2009). Alternatively, ovipositing females may move to areas of lower density to reduce intraspecific competition among larvae (Prokopy 1981). Pea and faba bean plants have a low carrying capacity for PLW larvae (Nielsen 1990) and female PLW are expected to move to areas of lower density while ovipoisting (Schotzko and O'Keeffe 1998). If PLW females indeed prefer to oviposit away from a mating aggregation, it is possible that larval density in our experiment was artificially low near semiochemical traps. Future experiments should therefore compare PLW captures in semiochemical traps to PLW larval and adult densities or damage throughout the entire pea field. To obtain a range of local and comparable weevil densities and damages, each field would have to be divided into sprayed and unsprayed strips. Olfactometer bioassays would also be useful to determine if female PLW exhibit a plastic response to pheromone between periods of mating and oviposition.

The semiochemical traps tested here were highly specific for PLW. However, in 2014, the non-target weevils (Coleoptera: Curculionidae) captured in semiochemical traps were identified as *Hypera, Ceutorhynchus, Otiorhynchus, Perapion* and *Sitona.* The non-*Sitona* weevils are easy to visually separate from PLW and will not be confusing to users of this trap. The non-PLW members of the genus *Sitona* were identified as sweetclover weevil, *S. cylindricollis,* and these captures were very infrequent, making up only 1.2% of *Sitona* captures in 2014. *S. cylindricollis* aggregation pheromone likely overlaps slightly with *S. lineatus* and other *Sitona* species. Toth *et al.* (1998) captured multiple *Sitona* species in traps baited with 4-methyl-3,5-heptanedione in Hungary. Information on the specific aggregation pheromone identity of various *Sitona* species is limited, but *S. lineellus* utilizes a two-component blend: 4-methyl-3,5-heptanedione and (4*S*,5*S*)-5-hydroxy-4-methyl-3-heptanone as an aggregation pheromone (Unelius *et al.* 2013). *S. lepidus* also uses these components in its aggregation pheromone but only males are responsive to 4-methyl-3,5-heptanedione (Park *et al.* 2013). It is expected that *Sitona* species may use multi-component blends to ensure species specificity of the pheromone signal. *Sitona* weevils utilize legumes as host plants (Jackson 1920) and it is likely that there is overlap in attractive host plant volatiles. Besides *S. lineatus,* the *Sitona* species present in the Prairie Provinces are *S. cylindricollis*, *S. flavescens, S. hispidulus, S. lineellus,* and *S. californius* (Bright 1994) and their chemical ecology is virtually unknown. Knowledge on the chemical ecology of the non-*Sitona* weevil bycatch is also limited. These weevils may also have similar pheromones or, more likely, are attracted to the host plant volatile lures included in these traps. For example, *Otiorhynchus sulcatus* is attracted to linalool and (*Z*)-3-hexenol which are emitted by its host plants (Van tol and Visser 1998). Despite some non-PLW weevil bycatch, the overall specificity of these semiochemical-baited traps is high.

2.4.2 Trap Type

The pitfall trap was the most successful trap type tested for attraction and retention of PLW across three field seasons. The pitfall trap constructed from Solo cups consistently captured the most PLW, but it is not the most user-friendly trap. The addition of a ring of wire mesh around the pitfall cup does not hinder PLW captures and may successfully exclude large bycatch, which would be useful in areas abundant in small mammals, amphibians, or large carabid beetles. Vertebrates are not an uncommon bycatch in pitfall traps, and there can be a trade-off between the utilization of pitfall traps for insect monitoring and the loss of vertebrates and arthropod predators, such as carabids to bycatch (Thompson and Thompson 2008). Lemieux and Lindgren (1999) found that the addition of a lid to open-top pitfall traps reduced vertebrate bycatch. By similarly reducing the entrance size, the addition of mesh to covered pitfall traps is expected to also reduce vertebrate bycatch, although this was not statistically tested in this study. The Vernon pitfall trap is expected to be a suitable trap for capturing PLW. Unfortunately, an overall low number of PLW were captured in the 2015 trapping experiment, and we were unable to demonstrate that the Vernon pitfall trap successfully captures PLW in the field. Similar to the pitfall trap modified with mesh, the Vernon pitfall trap includes pegs that limit the trap entrance size with the goal of limiting non-target trap captures. This trap only costs $1 CAD to manufacture (Vernon, personal communication). A possible limitation of the Vernon pitfall trap in the PLW system is that the pitfall cup is slightly smaller than the Solo cups and may fill faster. Vernon pitfall traps may need to be monitored more than once per week in hot weather to ensure that the propylene glycol does not evaporate. Because PLW captures in the 2015 trap type experiment were low overall and because the Vernon pitfall trap is similar to the Solo cup pitfall trap but with some advantages, it is recommend that the Vernon pitfall trap is tested again for PLW in the future. Yellow pan traps also successfully attracted and retained PLW in similar numbers to the two pitfall traps tested. Yellow pan traps are used in the Prairie Provinces to monitor the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Fox and Dosdall 2003; Blake *et* *al.* 2010) and *Delia* spp. (Broatch and Vernon 1997). Yellow pan traps, however, were more susceptible than pitfall traps to evaporation of propylene glycol and capture high quantities of bycatch.

The remaining traps tested for PLW did not successfully retain PLW and are not worthy of further investigation. Unitraps are especially unsuccessful, as not only do they not capture PLW, they capture a high level of hymenopteran bycatch (Mori and Evenden 2013). Yellow sticky cards rarely captured PLWs, even when omnidirectional, and were not worth the mess or effort when placed in the field after harvest. Fisher and O'Keeffe (1979), however, used unbaited yellow sticky cards to successfully monitor peak PLW activity during springtime dispersal. The Legget cone traps previously used for PLW (Blight *et al.*1984; Blight and Wadhams 1987; Nielsen and Jensen 1993; Smart *et al.* 1994) and found to be unsuccessful for PLW by Evenden *et al.* (2016) were not successful in the current study, even when modified to prevent PLW escapes. Although the hand constructed cone traps tested in these experiments were also not successful, a smaller cone trap, designed specifically for PLW or a similarly small insect, may have potential for success. The efficacy of cone traps may also be limited by spiders, who were often found to have built a web in the cone of the trap. Future experiments testing semiochemical trap types for PLW should focus on pitfall traps. The Solo pitfall trap is inexpensive and easy to make, but the Vernon pitfall trap may be just as inexpensive, effective and more user-friendly.

2.4.3 Seasonal Plasticity to Semiochemical Cues in the Lab

Pea leaf weevil adults in the fall are newly eclosed, reproductively immature, and females must forage in order to develop their reproductive tracts (Schotzko and O'Keeffe 1986). Pea leaf weevils do not undergo a true reproductive diapause while overwintering, and continue to feed and develop throughout the winter months when conditions are favourable (Fisher and O'Keeffe 1979). In the spring, PLWs need to secure mates but may be forced to forage if overwintering conditions are poor. It is expected that PLWs respond to aggregation pheromone in the spring when PLWs are actively seeking mates, but it is interesting that PLWs also respond to aggregation pheromone lures in the fall (Evenden *et al.* 2016) when they are not reproductively active. Response to aggregation pheromone in the fall may promote aggregation before overwintering. Aggregation during overwintering occurs in many beetle species (Landolt 1997; Wertheim *et al.* 2005), and is beneficial for proximity to mates in the spring (Landolt 1997;

Bartelt *et al.* 2008; Raak-van den Berg *et al.* 2012), host-plant location, especially when calling is associated with host plants (Landolt 1997; Landolt and Phillips 1997). Insects may also aggregate during overwintering for thermoregulation (Dudeck *et al.* 2015; Howe 1962), or group defense (Wheeler and Carde 2013).

The response of weevils in different physiological states to semiochemical cues tested in the olfactometer was surprisingly similar and is in contrast to our field trapping experiments, where PLW captures were usually male-biased. Weevils responded first to volatile cues produced by male PLW alone or to cues from males and pea plants regardless of the physiological state or sex of the responder. This is in contrast to Landon *et al.* (1997) who found that PLW response to pea volatiles peaked during PLW dispersal periods compared to other times of year. However, Landon *et al.* (1997) found that the lowest response of PLW to pea volatiles occurred in the winter. Overwintering weevils were not tested in the current experiment but their response should be evaluated in the future. In the current study, both sexes of weevil in all physiological states tested spent most of the time during the bioassay in the quadrant of the olfactometer closest to the volatiles released from males alone or males with pea plants. Although PLW sex and physiological state did not statistically affect PLW response in the olfactometer, sex or physiological-state specific trends were seen. Females tended to respond more frequently than males to volatiles from male PLW, especially when newly eclosed. Males tended to respond more frequently than females to volatiles from pea plants alone. Of all the weevils tested, recently overwintered PLW had the strongest response to volatiles from male PLW on pea. Mating PLW, on the other hand, had the strongest preference for volatiles from male PLW alone. Further investigation of the influence of sex and physiological state on PLW response to semiochemicals is needed and should take into consideration a possible interaction between physiological state and sex. It is also still unclear if PLW males produce pheromone when in different physiological states throughout the growing season. Blight *et al.* (1984) demonstrated that male PLW produce the aggregation pheromone 4-methyl-3,5-heptanedione when they are reproductively active in the spring. As PLW respond to this same compound in trapping studies conducted in the fall (Evenden *et al.* 2016), it is assumed that males produce this same compound in the fall. Further experimentation is needed to collect and analyze volatile emissions from males in different physiological states.

Pea leaf weevils used in these olfactometer bioassays may have also been affected by the relatively high temperature and long-daylight conditions that they were held under before being tested in bioassays. Temperature and daylight conditions are important cues for PLW and holding PLW at summer-like conditions may influence their physiology (Hans 1959; Harmon *et al.* 1987; Stein 1972). In future olfactometer bioassays, PLW should be held at conditions that more closely mimic conditions in the field relevant to their physical condition, which may help better elucidate sex-specific or physiology-specific responses of PLW to semiochemicals. Additionally, all male PLW used as volatile sources in the olfactometer had been maintained on pea. If PLW aggregation pheromone is produced when male PLW feed on reproductive host plants, these PLW may have produced pheromone in the lab at a time of year when they would normally not be producing aggregation pheromone.

Weevils did not respond to pea volatiles alone in olfactometer assays. This parallels our field experiment, where PLW did not respond to traps baited with host plant volatiles alone. Pea leaf weevils did respond to pea volatiles in olfactometer bioassays in another study, but this response was not compared to insect-produced cues (Landon *et al.* 1997). Landon *et al.* (1997) also demonstrated that PLWs respond behaviourally to synthetic (*Z*)-3-hexenyl-acetate alone in an olfactometeter assay. Future studies should examine PLW response to a variety of host plants at different times in the season. Future studies should also compare the response of PLW to damaged and undamaged host plants.

2.4.4 Implications of this research

The research presented here clearly shows that PLW are attracted to male PLW aggregation pheromone in both the spring and fall under controlled conditions and in the field. PLW are attracted to various release rates of their aggregation pheromone, but 21 mg of 4-methyl-3,5-heptanedione in a 250 µl Eppendorf tube is a sufficient lure. PLW are not attracted to a lure consisting of PLW host plant volatiles (linalool, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate), on its own. In some trapping periods, PLW response to pheromone-baited traps was enhanced with the addition of the host plant volatile lure. Although PLW feeding damage near traps baited with pheromone and host plant volatile was correlated with PLW captures in these traps, it is unknown if feeding damage in the entire pea crop is correlated with PLW trap captures.

Currently, pea producers use PLW forecast maps based on springtime adult feeding damage (Alberta Agriculture and Forestry 2017; Saskatchewan Ministry of Agriculture 2016) when deciding whether or not to use insecticide treated seeds, which is the best method of estimating PLW damage risk currently available. Monitoring PLW populations in semiochemical traps may be more accurate because: 1) it may be possible to measure the generation of PLWs that have potential to cause damage more directly, and 2) PLW feeding damage varies greatly depending on crop or climate conditions and 3) Semiochemical traps are attractive to PLW in the fall and can be developed to monitor the overwintering generation prior to control in the spring.

The semiochemical traps developed here can also be used to monitor the range expansion of PLW in the Prairie Provinces. Semiochemical traps will be useful tools for monitoring PLW, especially in areas of low PLW density, where PLW populations might not be large enough to justify scouting for PLW feeding damage manually.

2.5 Chapter 2 References

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Chapter 3: General Conclusions and Future Research

3.1 General Conclusions and Future Research

Current pest management practices aim to reduce pest populations in an economically efficient manner while limiting effects on non-target organisms and the environment. This is a science-based approach commonly known as integrated pest management (IPM). In order to be effective, IPM should be multidisciplinary, combining the use of multiple control tactics into a management strategy (Kogan 1998). The development of an IPM program for pea leaf weevil (PLW), *Sitona lineatus*, which is recently invasive in the Canadian Prairie Provinces, is an ongoing effort. Here, I present research on the development of an IPM tactic (Kogan 1998) targeting PLW in the Prairie Provinces: semiochemical monitoring traps. We tested various semiochemical lures and traps during both the spring and fall periods of adult activity to develop a suitable semiochemical trapping system to monitor PLW activity. Along with trapping experiments, olfactometer bioassays measured PLW response to semiochemicals when weevils were in different physiological states throughout the pea growing season.

Pea leaf weevils responded to semiochemical-baited pitfall traps placed at the margins of field pea crops in Alberta in the spring and in the fall. The current study corroborated the findings of Evenden *et al.* (2016): that PLW respond to semiochemical traps during the fall migration. Pea leaf weevils respond similarly to the four pheromone lures tested here, and 21 mg of 4-methyl-3,5-heptanedione in a 250 µl Eppendorf tube is an adequate lure . The host plant volatiles emitted from faba bean (linalool, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate) (Blight *et al.* 1984) are not effective trap lures on their own but enhance PLW captures in pheromone-baited traps in the fall. Two release rates of host plant volatiles were tested in 2014 and were equally effective in the enhancement of attraction of pheromone-baited traps to PLW. Thus, a PLW semiochemical trap based on a pitfall trap baited with either 21 mg of 4-methyl-3,5-heptanedione in a 250 µl Eppendorf tube or with 21 mg of 4-methyl-3,5-heptanedione in a 250 µl Eppendorf tube, 21 mg of (*Z*)-3-hexenyl acetate in a 250 µl Eppendorf tube, 34mg of (*Z*)-3-hexenol in a 250 µl Eppendorf tube, and three 250 µl Eppendorf tubes, each with 50mg of linalool can be used to monitor PLW activity in the spring and fall. These semiochemical traps could be used to monitor PLW range expansion where weevils are at low density (El-Sayed *et al.*2006; El-Sayed *et al.*2009). This trapping system can also monitor the arrival of PLW into a pulse crop in the spring. This could assist pea producers in pest management decisions such as application of foliar insecticide to reduce adult PLW populations and prevent oviposition (Fisher and O'Keeffe 1979; Nielsen and Jensen 1993; Vankosky *et al.*2009).

To further develop this semiochemical monitoring trap, it is necessary to correlate PLW captures in semiochemical traps with larval populations or yield loss (Vankosky *et al.*2009; Bjostad *et al.*1993; Nielsen and Jensen 1993). The experiments here show that PLW captures in semiochemical traps can be indicative of adult feeding damage which is the current measure of PLW activity used in the Prairie Provinces. Future experiments should seek to relate PLW captures in semiochemical traps to subsequent larval density, larval feeding damage, or PLW-induced yield loss. The ideal tool for pea producers would be semiochemical-baited traps positioned in the fall to predict overwintering PLW populations and subsequent damage the following growing season (Vankosky *et al.*2009). This model, however, would require detailed information on the factors that contribute to overwintering mortality. If PLW captures in semiochemical traps can be related to yield loss in peas, such as with the use of grain-yield monitoring systems during harvest, semiochemical-trap based action thresholds may be developed and utilized.

To increase the knowledge of PLW chemical ecology, pheromone production should be investigated further. Pea leaf weevils produce aggregation pheromone during the spring dispersal period and also respond to pheromone during the fall dispersal. It is currently unknown how male pheromone production fluctuates throughout the year but it is likely that PLW males produce pheromone in the fall as well. In Blight et al. (1984) and Blight and Wadhams (1987), male PLW used as odour sources were feeding on reproductive host plants, which females must feed on to attain reproductive maturity (Schotzko and O'Keeffe 1986). Pheromone production could be associated with males feeding on host plants, as is expected for multiple weevil species (Landolt 1997). Future research should determine at what times of year males produce aggregation pheromone and if this requires feeding on reproductive hosts. In the olfactometer bioassays reported here, male PLW used as odour sources had been maintained on pea plants and may have been producing aggregation pheromone at times of year when they may not normally be exposed to reproductive hosts and therefore may not normally produce pheromone. Future research on this pheromone should also investigate if there are any other components involved.

Other *Sitona* species have been captured in traps baited with 4-methyl-3,5-heptanedione (Toth et al. 1998) indicating that there may be overlap in the pheromone of these species. Unelius et al. (2013) identified the male-produced aggregation pheromone of *S. lineellus* as a two-component blend of 4-methyl-3,5-heptaedione and (4*S*,5*S*)-5-hydroxy-4-methyl-3-heptanone. Similarly, Park *et al.* (2013) identified the male-produced aggregation pheromone of *S. lepidus* as 4-methyl-3,5-heptanedione, which attracts males, and (4*S*,5*S*)-5-hydroxy-4-methyl-3-heptanone, which both attracts both males and females. Headspace analyses and electrophysiological studies should be used to investigate male pheromone production. Male PLW pheromone emission may be density-dependent, as some males in an aggregation might be "sneaky", and hijack the pheromone signal of other males while limiting their own pheromone production (Schlyter and Birgersson 1989). It would be interesting to compare the average pheromone emission between individual males and aggregations of various sizes to determine if male PLW pheromone production is density-dependant. This information would also be interesting to compare pheromone emission between PLW aggregations and semiochemical trap lures. Information on the quantity of pheromone released by a male would also be useful for conducting future olfactometer bioassays.

Further research should also investigate the intraspecific differences in PLW response to semiochemicals. In semiochemical trapping experiments, PLW captures have sometimes been female-biased (Evenden *et al.* 2016), male-biased (Blight *et al.* 1984), or even (Blight and Wadhams 1987; Evenden *et al.* 2016). The reason for variability in sex ratios is unknown: perhaps PLW populations have variable sex ratios between season and year, due to sex-specific differences in mortality, or perhaps PLW have sex-specific differences in olfactory response that are apparent in field conditions but not in the olfactometer bioassays. The related *S. lineellus* and *S. lepidus* have sex-specific differences in olfactory receptor neuron expression (Unelius *et al.* 2013; Park *et al.* 2013). It is possible that PLW also has sex-specific differences in olfactory receptor neurons, which might help explain differences in male and female behaviour. Sex-specific differences in response to pheromone-baited traps may be related to sex-specific costs and benefits. For example, at certain densities, it may be more beneficial for one sex to respond to the aggregation pheromone than the other sex, resulting in a population with a biased sex ratio. Male and female PLW may also exhibit differential response to plant volatiles. For example, variable sex ratios in PLW semiochemical trapping experiments may perhaps be explained by a differential influence of background plant volatiles on male or female PLW response. Ju *et al.* (2017) reported that the response of both sexes of the dark beetle chafer *Holotrichia parallela* to its female-produced sex pheromone is synergized by host plant volatiles but that there are sex-specific differences in this synergistic response. It is also possible that response of PLW to pheromone is dependent on mating status. Mated *Tribolium castaneum* (Coleoptera: Tenebrionidae) males and females also exhibited a decreased response to their male-produced aggregation pheromone (Fedina and Lewis 2007). The behavioural response of male *Agrotis ipsilon* (Lepidoptera: Noctuidae) moths to the female *A. ipsilon* sex pheromone is halted after mating until the next scotophase, when males are again attracted to female sex pheromone (Gadenne *et al.* 2001). Interestingly, this behaviour was modulated by the antennal lobe and not by the peripheral olfactory system, allowing for a rapid, transient plasticity in olfactory response. A similar mechanism may operate in female PLW that results in decreased attraction to the aggregation pheromone after mating to promote dispersal and location of an oviposition site further away from the mating aggregation. Based on a limited carrying capacity of individual pea or faba bean plants for PLW larvae (Nielsen 1990), and on the high longevity and fecundity of female PLW, Schotzko and O'Keeffe (1998) predicted that females should migrate to areas of low PLW density during oviposition. To determine the impact of mating status on PLW attraction to semiochemical traps, future studies should compare the reproductive development and mating status of female PLW captured in semiochemical traps to female PLW populations in pea fields. Olfatometer bioassays could also be used to compare the response of females that are umated, recently mated and 1-2 weeks post-mated after female PLW have had time to oviposit.

As presented here, these semiochemical traps are useful for monitoring PLW. The range expansion of PLW in the Prairie Provinces is ongoing, and these traps are particularly useful to monitor PLW in areas where they are suspected to be present but at but in densities too low to justify scouting for adult feeding damage. These semiochemical traps may also be deployed by pea producers in the spring, to monitor the arrival of PLW into pea fields and better time the application of foliar insecticides. As developed here, this semiochemical-baited trap is a useful IPM tool with which to monitor PLW activity. Further development of this trapping system so that it relates trap captures with PLW density in the field will increase the information available to pea producers who seek to make informed management decisions.

The semiochemical lures tested here could also be useful in a trap cropping system. Smart *et al.* (1994) used PLW aggregation pheromone and neem oil, an antifeedant, to create a push-pull trap cropping system in small (36 m x 36 m) plots of faba bean. Compared to untreated subplots, subplots (6 m x 6 m) baited with aggregation pheromone had higher adult and larval feeding damage, and subplots treated with neem oil had less adult feeding damage. Further development of this trap cropping system requires testing larger, more realistic plot sizes. The trap cropping system could also be enhanced with the use of host plant volatiles. Different varieties of pea and faba bean release slightly different chemical profiles, and investigations into which varieties of these crops are the most attractive would be useful for producers. Planting varieties of varying attractiveness could be incorporated into a trap cropping system. Future studies should compare faba bean volatiles, which are more attractive to PLW during oviposition, to pea volatiles, which are more attractive to PLW in early spring, when PLW are emerging from overwintering (Jaworska 1992). Jaworska (1992) proposed that PLW alternate between hosts due to changing nutrient requirements of PLW at different stages in their lifecycle. This is supported by fat extractions performed by Hoefele (unpublished data) on PLW collected from alfalfa, faba bean or pea crops at various times of year. Weevils captured earlier in the season had higher content relative to PLW captured later in the season, and PLW collected on faba bean had lower fat content than PLW collected on peas. Landon *et al*. (1997) also found that PLW response to pea volatiles was plastic and was highest during crop colonization and during PLW eclosion in the fall. In order to be effective, a trap crop system must concentrate reproductive PLW away from the main crop prior to oviposition to prevent larval damage. Future experiments should therefore determine the specific chemical cues from faba bean that are attractive to PLW during or oviposition. Future experiments to test the response of PLW under different nutritional states to semiochemicals may also help us better understand the plasticity of response to semiochemicals across the seasons. Semiochemical tools are useful for manipulating insect behaviour, and besides being useful as monitoring trap lures, the semiochemicals tested in the experiments reported here show potential for use in a trap crop system.

Trap crop systems reduce pesticide use, by limiting the need for their application to only the trap crop. If PLW could be concentrated in an a trap crop prior to oviposition, foliar insecticide sprays could then be targeted on those areas specifically. With timely application, foliar insecticide sprays reduce adult PLW populations, egg production, and the resultant larval populations (Steene *et al*. 1999; Vankosky *et al.* 2009). The efficacy of foliar insecticide sprays is inconsistent (Cárcamo and Vankosky 2011) but would likely be enhanced when combined with semiochemical-based trap cropping systems that concentrate target PLW in an area. Pitfall traps baited with PLW pheromone could be used to lure weevils to the trap crop as well as monitor their arrival in the trap crop, allowing for the timely application of foliar insecticides.

A trap cropping system may also be useful for enhancing the efficacy of natural enemies, such as entomopathogenic fungus or nematodes. Application of *Beauveria bassiana* can reduce larval PLW populations (Poprawski *et al.*1985) and to reduce the number and longevity of eclosing PLW adults (Muller-Kloger and Stein 1970). Infection of PLW with *B. bassiana,* however, requires adequate exposure to effective concentrations of the pathogen (Muller-Kloger and Stein 1970). *Beauvaria bassiana* has been used for control of *S. lineellus* in France (Aeschlimann *et al.*1985). Nematodes, including *Steinernema carpocapsae* (Rhabditida: Steinerematidae), *S. feltiae* (Rhabditida: Steinerematidae), and *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) are also successful biocontrol agents for PLW (Jaworska 1998). *Heterorhabditis bacteriophora* is particularly effective because it can penetrate the insect cuticle and infect adult hosts (Weich and Jaworska 1990; Bedding and Molyneux 1982). Nematode application is generally less cost-effective than pesticide application, but the establishment of nematodes in an area may help manage PLW populations in the long-term (Vankosky *et al.* 2009). A trap cropping system that concentrates PLW in the trap crop area would make it easier to target these insects with entomopathogenic fungus or with nematodes.

A mass-trapping program may also be possible with these semiochemical traps (Blight *et al*. 1984; Blight *et al.* 1987; Nielsen and Jensen 1993; Vankosky *et al.* 2009). Fall would be an ideal time to target PLW for mass-trapping, as they have not yet reproduced, are present in large numbers and highly attracted by semiochemical cues at this time. In the field trapping experiments reported here, a greater number of PLW were trapped in the fall than in the spring. This may be due to overwintering mortality, but may also be due to a difference in competing background volatiles between these two seasons. Regardless, PLW captures in semiochemical traps were highest in the fall in the weeks following harvest. A mass-trapping strategy with semiochemical traps placed near threshing tables may capture a significant portion of the PLW population before they overwinter. Mass-trapping during the spring migration of PLW to reproductive host plants could also significantly reduce PLW populations before they oviposit. In order to be effective, these semiochemical-baited traps must attract and retain PLW in sufficient numbers to reduce their population before larvae cause damage (El-Sayed *et al.* 2006).

The ultimate goal of this research is to develop a semiochemical-based monitoring tool for PLW in the Prairie Provinces. The semiochemical traps developed in this research may help pea producers determine the presence or absence of PLW in a given geographical area, which may be useful in tracking range expansion of this invasive species. These semiochemical traps could also be used by pea producers to monitor the arrival of PLW into a pea crop at the start of the growing season, which is useful for timing the application of foliar insecticides. As they are presented here, these semiochemical traps can be used to reduce the application of pesticide in areas where it is not necessary, which is a main goal of integrated pest management. Further research into IPM strategies that incorporate semiochemical tactics may provide a method to effectively maintain PLW populations at manageable levels in the Prairie Provinces.

3.2 Chapter 3 References

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Appendix A: Locations of pea fields used in trapping experiments

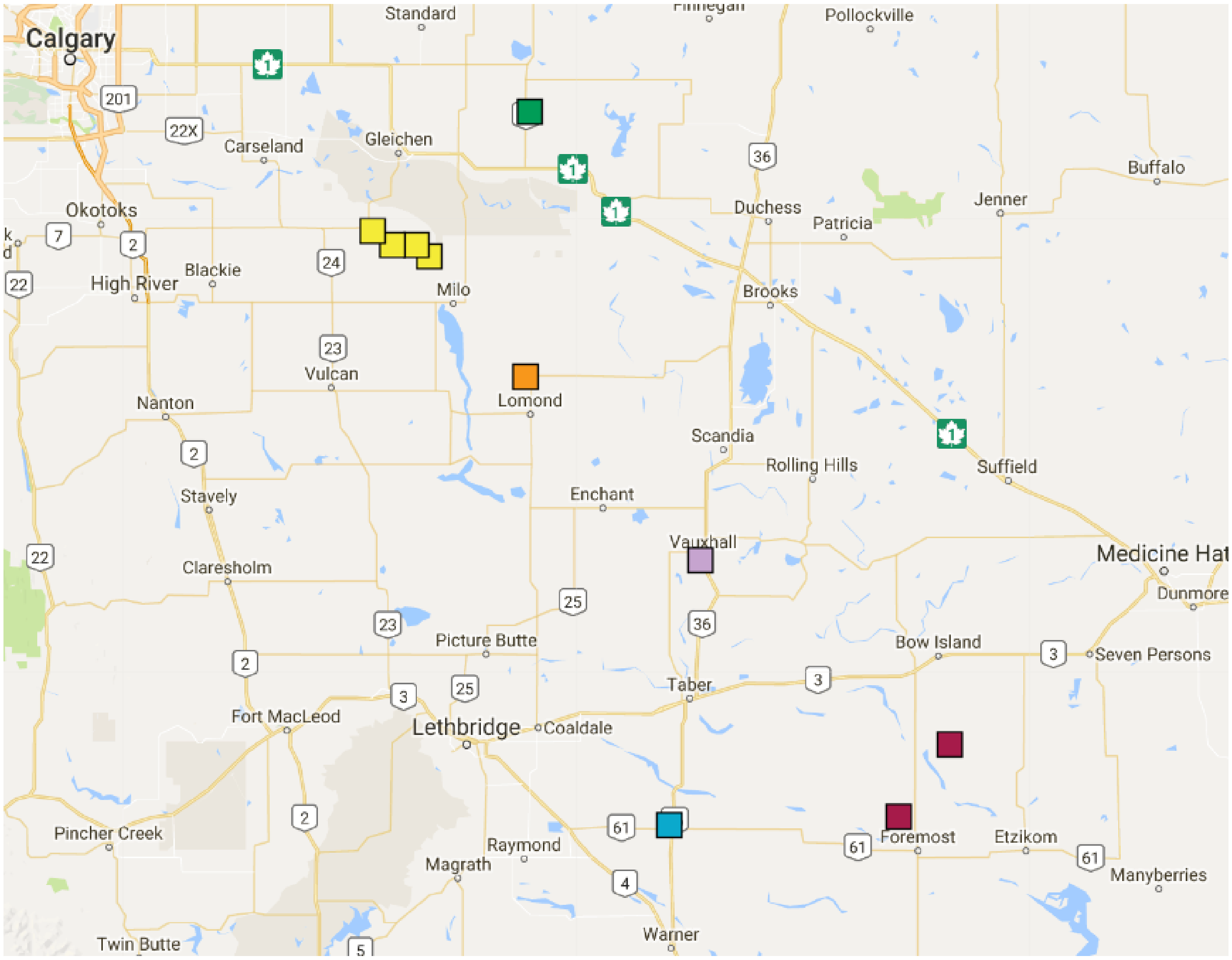

Figure A-1. Locations of pea fields used in 2013 experiments testing semiochemical lures. Colours indicate different counties (yellow = Vulcan County; green = Wheatland County; purple = Municipal District of Taber; red = County of 40 Mile; orange = Newell County; blue = Lethbridge County).

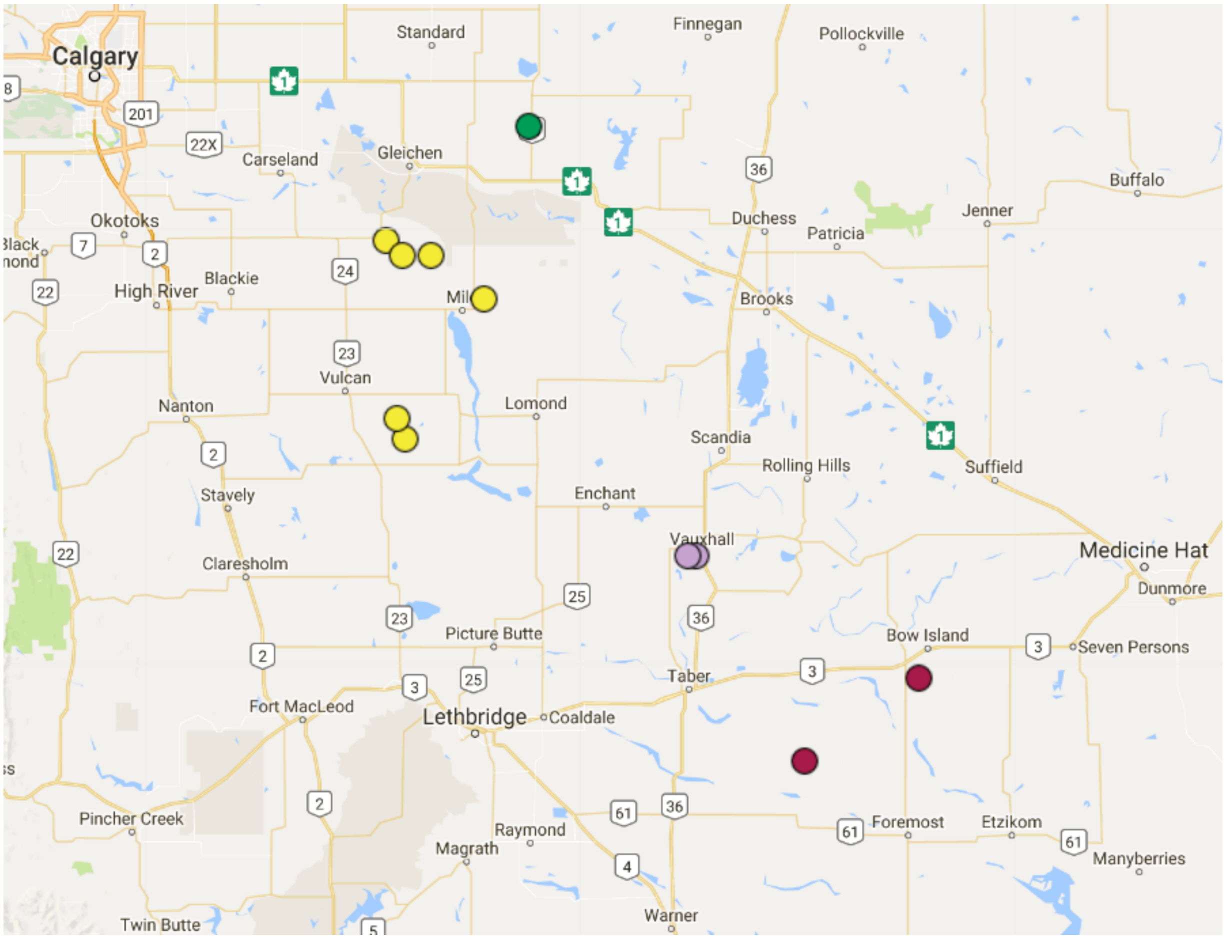

Figure A-2. Locations of pea fields used in 2014 experiments testing semiochemical lures. Colours indicate different counties (yellow = Vulcan County; green = Wheatland County; purple = Municipal District of Taber; red = County of 40 Mile).

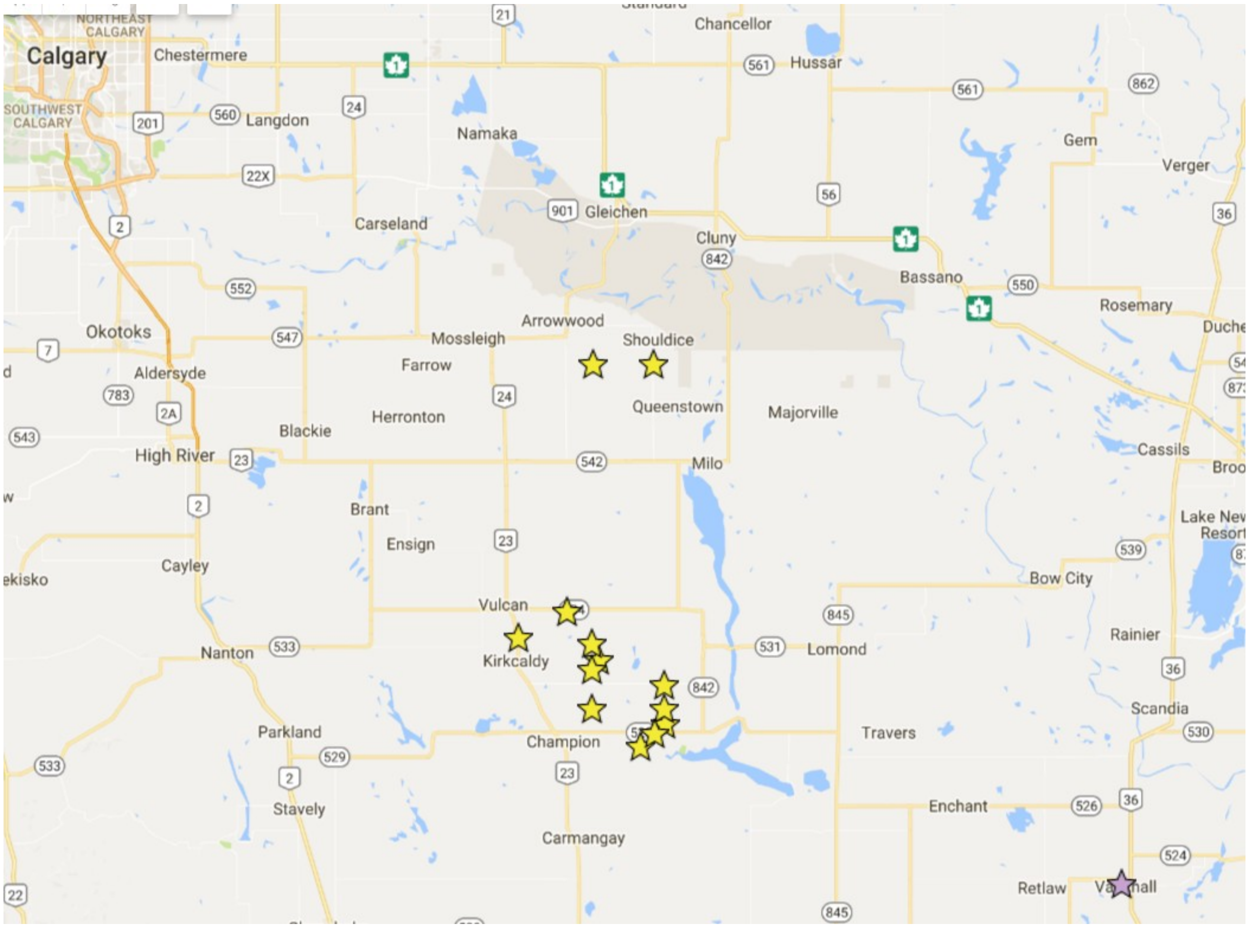

Figure A-3. Locations of pea fields used in 2015 experiments testing semiochemical lures. Colours indicate different counties (yellow = Vulcan County; purple = Municipal District of Taber).

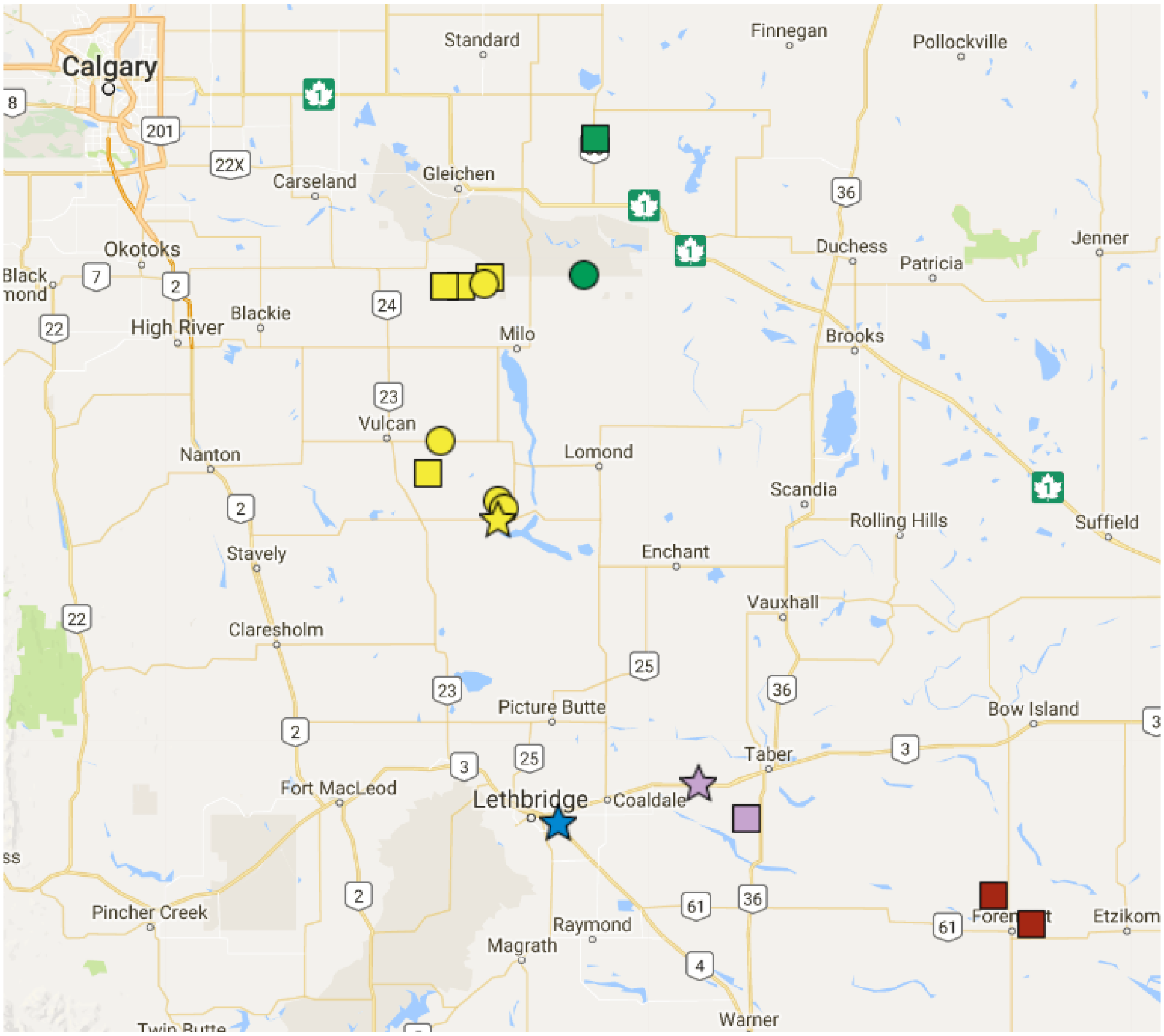

Figure A-4. Locations of pea fields used in experiments evaluating trap type. Colours indicate different counties (yellow = Vulcan County; green = Wheatland County; purple = Municipal District of Taber; red = County of 40 Mile; blue = Lethbridge County). Shapes indicate the year that site was included (square = 2013; circle = 2014; star = 2015).

Table A-1. **Locations of pea fields used in 2013 experiments testing semiochemical lures.**

GPS location	Spring trapping period	Fall trapping period
50.95054, -112.64572	8 May — June 19	31 July — 11 Sept
50.43000, -112.66204	8 May — June 21	2 Aug — 13 Sept
50.6669, -112.95945	9 May — June 19	31 July — 11 Sept
50.68883, -112.99834	9 May — June 19	31 July — 11 Sept
50.68879, -113.07479	9 May — June 19	31 July — 11 Sept
50.71792, -113.1348	9 May — June 19	1 Aug — 13 Sept
49.69867, -111.34797	9 May — June 20	1 Aug — 12 Sept
49.55302, -111.5061	9 May — June 20	1 Aug — 12 Sept
49.53831, -112.21733	10 May — June 21	1 Aug — 12 Sept
50.06847, -112.12007	10 May— June 20	2 Aug — 12 Sept

Table A-2. Locations of pea fields used in 2014 experiments testing semiochemical lures.

GPS location	Spring trapping period	Fall trapping period
50.968016, -112.658763	15 May —19 June	6 August — 15 September
50.309956, -113.069864	1 May — 12 June	7 August — 15 September
50.686135, -112.985319	1 May — 12 June	7 August — 15 September
50.717091, -113.130791	1 May — 12 June	N/A
50.352983, -113.095163	1 May — 12 June	7 August — 15 September
50.717091, -113.130791	N/A	7 August — 15 September
50.352983, -113.095163	22 May — 19 June	6 August — 15 September
49.814041, -111.408598	14 May — 19 June	5 August — 16 September
50.066792, -112.130690	30 April — 11 June	6 August — 16 September
50.067783, -112.153826	30 April — 11 June	6 August — 16 September
49.639738, -111.777606	30 April — 11 June	5 August — 16 September

Table A-3. Locations of pea fields used in 2015 experiments testing semiochemical lures.

GPS location	Dates of lure (re)placement	Trap collection dates		
		spring	summer	fall
50.07312, -112.11507	27 April, 17 June, 29 July	6 May, 13 May, 20 May, 27 May, 3 June, 10 June, 17 June	24 June, 2 July, 8 July , 15 July, 22 July, 29 July	5 August, 12 August, 19 August, 26 August, 2 September, 9 September, 16 September
50.33935, -113.08341	29 April, 17 June, 29 July			
50.32713, -113.09755	29 April, 17 June, 29 July			
50.26321, -112.95983	29 April, 17 June, 29 July			
50.28091, -112.96317	29 April, 17 June, 29 July			
50.30991, -112.96271	29 April, 17 June, 29 July			
50.28108, -113.09755	29 April, 17 June, 29 July			
50.2361, -113.00562	29 April, 17 June, 29 July			
50.25165, -112.97902	29 April, 17 June, 29 July			
50.35799, -113.09755	29 April, 17 June, 29 July			
50.39622, -113.14332	29 April, 17 June, 29 July			
50.36519, -113.23324	29 April, 17 June, 29 July			
50.68872, -113.09447	6 May, 17 June, 29 July			
50.68783, -11298235	6 May, 17 June, 29 July			

Table A-4. Locations of pea fields used in 2013, 2014, and 2015 experiments evaluating trap types.

Year	GPS location	Setup date	Trap collection dates
2013	50.96207, -112.65681	31 July	8 August, 14 August, 21 August, 28 August, Sept 4
	50.68924, -113.04615		
	50.70643, -112.96186		
	50.68872, -113.09447		
	50.34299, -113.14246	1 August	9 August, 15 August, 21 August, 30 August, 6 September
	49.69619, -112.21742		9 August, 15 August, 22 August, 29 August, 5 September
	49.55260, -111.49679		
	49.49863, -111.38539		
2014	50.71011, -112.68769	21 August	27 August, 15 September
	50.69203, -112.97621		
	50.40087, -113.10272		
	50.29134, -112.93829		
	50.27679, -112.92114		27 August, 3 September
2015	49.75944, -112.35360	15 August	19 August, 26 August, 9 September
	49.68423, -112.76275		
	50.26246, -112.93734		20 August, 29 August, 12 September

Appendix B: Detailed description and photographs of trap types

1. *Solo cup pitfall trap.*

   These traps were constructed from two white, 473 mL Solo cups. One cup was trimmed at the 350 mL fill line and placed inside the other, intact, cup. This pair of cups was then dug into the soil so that the soil surface was level with the top of the intact Solo cup. The inner cups were then filled half full with propylene glycol, which is a sufficient killing agent and is less toxic to mammals than other types of antifreeze. When checking these traps, the trimmed inner cup allows for easy removal of insects and propylene glycol without collapsing the pitfall hole. The inner cup is also useful to remove dirt that has spilled into the trap while placing these traps in the ground. A 15 cm x 15 cm white Coroplast square was used as a lid for each Solo pitfall trap, using four 8 or 10 cm galvanized steel nails to hold the trap lid in place. A steel wire was used to attach semiochemical lures to the centre of each Coroplast trap lid. Trap lids were placed 2-3 cm above the surface of the soil to allow semiochemical plumes to emit from traps and to allow PLWs to enter traps. For 400 µl Eppendorf tubes and bubble cap lures, care was taken during lure assembly and trap servicing to ensure that these lures would not be submerged in the propylene glycol. Solo pitfall traps were serviced by removing the trap lid, removing the inner cup to replace the propylene glycol, and replacing the inner cup and lid.

Figure B-1. Solo cup pitfall trap with attached semiochemical lure.

2. *Unitraps*

Three types of unitraps were tested: a multicoloured unitrap, pictured below, with a green lid, yellow cone and a white base. Entirely green and entirely yellow unitraps were also tested. Unitraps were hung above the pea stubble on rebar stakes so that the bottom of the Unitrap was 1 m above the ground.

Figure B-2. Multicoloured unitrap. Image © Pherobank.

3. *Rectangular yellow sticky card*

Yellow sticky cards (18 cm x 14 cm) were suspended on rebar stakes so that the bottom of the yellow sticky card was 1 m above the ground.

4. *Yellow cone trap secured to ground with tent pegs*

This yellow cone trap was made with the Legget cone trap, similar to traps used by Blight (1984), Blight and Wadhams (1987) and by Nielsen and Jensen (1993) for PLW in faba bean fields in Europe. However, the Legget cone trap did not successfully capture and retain PLWs in pea fields in Alberta (Evenden *et al.* 2016) and it was suspected that PLWs, which are smaller-bodied than cotton boll weevils, were escaping from the Legget traps. These yellow cone traps were made to emulate the Legget cone traps but eliminate areas that PLW were suspected to escape from.

Yellow cone traps were made out of 946 mL yellow plastic drinking cups, with a 100mm clear plastic funnel and a 30 mL plastic snap cap vial, similar to the cone part of the yellow pyramid traps described above. To allow PLW entry into these traps, six 2 cm diameter holes were cut into the lip of each cup, which was then placed upside down on the ground. The plastic funnel was glued to the base of the cup, and the snap cap lid secured around the plastic funnel tip. To allow PLW movement from the yellow cup portion into the plastic funnel, five 1 cm diameter holes were drilled into the base of the cup. A cone mesh (1 mm x 1 mm) cylinder was glued into the inside of the funnel tip, leading into the plastic collection vial, to prevent PLWs from moving from the collection vial back into the funnel. Semiochemical lures were secured to the inside of the yellow cup with steel wire. Three small holes were drilled into the edge of the funnel mouth to allow bungee cords to be attached. These bungee cords led to tent pegs, which secured the cone trap to the ground.


Figure B-3. Yellow cone trap secured to the ground with tent pegs.

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5. *Yellow pyramid trap*

These traps were inspired by the Tedders pyramid traps which have successfully captured other weevils, including plum curculio (*Conotrachelus nenuphar;* Clement *et al.* 2010) and pecan weevil (*Curculio caryae*; Mulder *et al.* 2003). These traps were constructed similar to the directions provided in Gardosik and Lehman (2005) who used these traps for white pine weevil, *Pissodes strobe*. The triangular trap base


Figure B-4. Yellow pyramid trap.

was constructed out of two yellow Coroplast triangles (27 cm base x 60 cm height), with 30 cm slits cut to allow these two pieces to interlock. Two 27 cm x 4 cm x 4 mm slats of wood were joined in an X shape and stapled to the bottom edges (measuring 27 cm long) of the pyramid. Two 20 cm nails were used to stake each pyramid trap into the ground. At the top of each pyramid, a yellow cone trap similar to the one described above was attached. These yellow cones only differed from the traps above in that they lacked the six 2 cm diameter holes at the lip of the cup. These holes were not drilled in order to encourage PLWs who moved up the yellow pyramid to move all the way to the collection vial before escaping the trap. Yellow pyramid traps were serviced by removing the insect collection vial from its lid then replacing it.

6. *Yellow cone trap placed 1m above ground on rebar stake*

These traps were similar to the yellow cone traps were placed in the air to attract flying PLWs. These traps only differed from the yellow cone traps described previously in that they lacked the six 2 cm diameter holes at the bottom of the trap, and that they were hung 1 m above the ground on a rebar stake. Traps were serviced by removing the insect collection vial, removing the captured insects, and replacing the vial.

Figure B-5. Yellow cone trap.

7. *Yellow bucket trap*

Yellow bucket traps were constructed from 946 mL yellow plastic drinking cups and a circular yellow Coroplast lid which fit the top of the cup. Six 2 cm diameter holes were drilled into the lip of the cup. Cups were dug into the ground so that the soil was level with the bottom of the cup lip, with the cup lip (and entry holes) above the soil surface. Half of a Vapona insecticide strip was secured to the inside of the yellow cup to kill captured insects. Vapona insecticide strips, the yellow Coroplast lid, and semiochemicals were secured to the traps with steel wire. Traps were serviced by opening the Coroplast lid and removing captured insects with forceps.

Figure B-6. Yellow bucket trap placed in the soil.

8. *Solo cup pitfall trap with metal mesh to exclude large bycatch*

The Solo pitfall cups were used for the experiments testing semiochemical lures in the field, previously described in this paper. In these experiments, some traps captured plenty of bycatch. To limit the amount of bycatch in these traps, particularly the less appealing vertebrate bycatch (frogs, salamanders and mice), Solo cups were tested with 6 mm x 6 mm chicken wire meshed added. A 45 cm x 3 cm strip of this mesh was cut, stapled into a circle with 12cm diameter, then stapled to the Coroplast trap lid. These traps were serviced by removing the trap lid to replace the propylene glycol in the trap.

Figure B-7. Solo cup pitfall trap modified with metal mesh to exclude large bycatch.

9. *Yellow pan trap*

A yellow rectangular pan trap (6.5 cm x 20 cm x 27 cm) was dug into the ground so that the upper rim was level with the soil surface. The yellow pan trap was then half-filled with propylene glycol to serve as a killing agent.

Figure B-8. Yellow pan trap.

10. *Legget cone trap + modification to prevent PLW escapes*

In experiments conducted by Evenden *et al.* (2016), PLWs were suspected to be escaping from the Legget cone traps, which were designed for the larger cotton boll weevil. To prevent PLW escapes, Legget cone traps were modified by sealing all holes larger than 2mm with hot glue. Traps were serviced by removing the insect collection vial, collecting insects, and returning the vial.

Figure B-9. Legget boll weevil trap.

11. *PVC pitfall cup with small holes*

These PVC pitfall cups were constructed as a possible alternative to the Solo pitfall traps. Pitfall traps constructed out of PVC piping are sturdier than Solo pitfall traps and were expected to last longer than Solo pitfall traps without breaking. To construct these PVC pitfall traps, a 4” length of 4” diameter PVC pipe and two 4” diameter PVC lids were used. Each lid was lined with a ring of hot glue 1.5cm from the base of the lid to prevent the PVC pipe from fully entering the lids. On one end of the 4” length of PVC pipe, a PVC cap was permanently attached with hot glue to form the base of the trap. On the other end of the trap, twenty 2mm diameter holes were drilled to allow PLW entry. Semiochemical lures were secured to the inside of the remaining PVC cap and used as the trap lid. Traps were serviced by removing the lid and pouring the insects in propylene glycol into a Whirl-Pak collection bag.

Figure B-10. PVC pitfall cup with small holes, opened to see trap inside and lure.

12. *PVC pitfall cup with large holes*

These PVC pitfall traps were similar to the previously described PVC trap except with larger holes for PLW entry. Instead of twenty 2mm diameter holes, six 20mm x 10mm ovals were cut into the side of the PVC pipe.

Figure B-11. PVC pitfall trap with large entry holes, placed in the ground.

13. *Omnidirectional sticky flight trap*

To increase PLW captures on sticky card traps, three 18 cm x 14 cm were stapled together in a cylinder, with the 14cm ends overlapping by 1-2 cm, similar to the omnidirectional sticky flight trap used by Fisher and O'Keeffe (1979). This cylinder of yellow sticky cards was then stapled to a 20 cm wooden stake which held the sticky cards above the pea crop stubble.

Figure B-12. Omnidirectional sticky trap.

14. *Vernon beetle trap*

The Vernon beetle trap was developed for wireworms. The box portion of this trap is placed on the soil surface with two ramp portions leading into the box. The lower edges of the ramps are buried in the soil. Insects walk up the ramp portions and are captured on the yellow sticky card lining.

Figure B-13. Vernon beetle trap with ramp slightly removed to show sticky card lining.

15. *Vernon pitfall trap*

The Vernon pitfall trap is structurally very similar to the Solo cup pitfall traps but has a slightly smaller capacity (200 mL). It is commercially manufactured for $1/trap. Vernon pitfall traps are also available in green and yellow.

Figure B-14. Vernon pitfall trap with trap lid removed and attached.

Appendic C: Lolitrack analysis of olfactometer video data

Olfactometer videos were first analyzed with the program Lolitrack (version 2; Loligo Systems Inc.). Each 30 minute olfactometer trial was recorded using a Logitech HD 1080p webcam suspended above the olfactometer arena. The software Total Video Converter (EffectMatrix Ltd.) was used to convert videos to .avi format at 5 frames per second and 720 x 404 resolution which is readable by Lolitrack. Converted videos were then analyzed using Lolitrack. In Lolitrack, for each video, the olfactometer arena was divided up into four labelled quadrants, each corresponding to a different volatile source. In each video, pea leaf weevils were identified by a human observer for tracking. Lolitrack then searches for pixels of the same colour as the identified 'pea leaf weevil'search image. Pea leaf weevil tracking is double-checked by a human user who manually blocks non-PLW pixels from being identified as PLW. Lolitrack then tracked and recorded the movement of each PLW within the olfactometer arena. The following variables were recorded for each trial: 1) time spent moving or not moving in each quadrant of the olfactometer; 2) velocity of movement in each quadrant of the olfactometer; 3) distance moved in each quadrant of the olfactometer. Lolitrack provides useful data such as weevil velocity, that would be labour-intensive for a human to calculate, but was abandoned due to time constraints.