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ARTICLE

# Use of semiochemical-baited traps to monitor the range expansion of the invasive *Sitona lineatus* (Curculionidae: Coleoptera) and the presence of associated ground beetles

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## Abstract
The invasive pea leaf weevil, *Sitona lineatus* (Linnaeus) (Coleoptera: Curculionidae), damages field peas, *Pisum sativum* Linnaeus (Fabaceae), and faba beans, *Vicia faba* Linnaeus (Fabaceae), on the Canadian prairies. We used semiochemical-baited pitfall traps to monitor and detect *S. lineatus* range expansion and capture associated predaceous ground beetles (Coleoptera: Carabidae) in pulse-growing regions across Alberta. Traps captured male and female *S. lineatus* in all pulse-growing regions in the spring and fall, including a first record of *S. lineatus* in the Peace River region of northwestern Alberta. Pheromone-baited traps captured more weevils than unbaited traps did, and the addition of host plant volatiles did not increase the catch. More weevils were captured in traps in pea fields compared to in faba bean fields. Rubber septa lures released more pheromones and attracted a similar number or more weevils to traps than microcentrifuge tube lures did. Ground beetle capture was not affected by semiochemical baits targeting *S. lineatus*. Ground beetle diversity varied by region and collection period, but the most frequently collected species was *Pterostichus melanarius*, a potential predator of *S. lineatus*. This study shows that pitfall traps baited with rubber septa pheromone lures can be used to monitor new and expanding *S. lineatus* populations, as well as potential natural enemy communities.

## Introduction

Canada is a global leader in pulse crop production, with the majority of production occurring in the provinces of Alberta and Saskatchewan (Bekkering 2014; Knodel and Shrestha 2018; Bhat *et al.* 2022). Growing conditions in the Canadian Prairie Provinces are well suited for pulse production, including crops such as field peas, *Pisum sativum* Linnaeus (Fabaceae), lentils, *Lens esculenta* Moench (Fabaceae), field beans, *Phaseolus vulgaris* Linnaeus (Fabaceae), chickpeas, *Cicer arietinum* Linnaeus (Fabaceae), and faba beans, *Vicia faba* Linnaeus (Fabaceae) (Miller *et al.* 2002; Vankosky *et al.* 2009; Gan *et al.* 2015; Zander *et al.* 2016). Pulses are valuable for

Subject editor: Tharshinidevy Nagalingam human consumption and livestock nutrition because they are a significant source of protein in seed dry matter (Zander *et al.* 2016). Pulses also enhance nitrogen availability in the soil, which helps to reduce reliance on inorganic fertilisers (Koutika *et al.* 2004) and can positively contribute to the quality and yield of future cereal crops (Miller *et al.* 2003; Angus *et al.* 2015; Gan *et al.* 2015).

Cereal–oilseed crop rotations that incorporate pulses are common on the Canadian prairies and can decrease pest pressure by breaking disease and pest cycles that cause economic damage (MacWilliam *et al.* 2014; Zander *et al.* 2016). Increased production of pulse crops, however, can attract specialist pulse-feeding insect herbivores, although pest status varies regionally (Knodel and Shrestha 2018). Pulse pests on the Canadian prairies include both generalist insect herbivores, such as wireworms (Coleoptera: Elateridae), cutworms (Lepidoptera: Noctuidae), grasshoppers, *Melanoplus* spp. Stål (Orthoptera: Acrididae), and Lygus bugs, *Lygus* spp. Hahn (Hemiptera: Miridae), and pulse specialists, including alfalfa caterpillar, *Colias eurytheme* Boisduval (Lepidoptera: Pieridae), alfalfa looper, *Autographa californica* Speyer (Lepidoptera: Noctuidae), pea aphid, *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae), clover root curculio, *Sitona hispidulus* (Fabricius) (Coleoptera: Curculionidae), and pea leaf weevil, *Sitona lineatus* (Linnaeus) (Coleoptera: Curculionidae) (Philip *et al.* 2015; Knodel and Shrestha 2018).

*Sitona lineatus* represents a considerable threat to field pea and faba bean production (Cárcamo *et al.* 2018). Since its detection in southern Alberta in 1997, *S. lineatus* has become a significant pest in the pulse-growing regions of the Canadian Prairie Provinces (Vankosky *et al.* 2009; Cárcamo and Vankosky 2011). In North America, *S. lineatus* is univoltine but undergoes two periods of adult activity throughout the growing season (Schotzko and O'Keeffe 1986). In early spring, adults emerge from overwintering sites and fly to pea and faba bean plants to reproduce (Nielsen and Jensen 1993; Landon *et al.* 1995). Adults feed on the foliage of young plants and leave characteristic U-shaped notches around the leaf margin (Stein 1972; Fisher and O'Keeffe 1979; Hamon *et al.* 1987; Landon *et al.* 1995). Females lay eggs on the soil surface near the base of plants, and larvae hatch and move through the soil to feed on root nodules containing symbiotic *Rhizobium leguminosarum* var. *viciae* Frank (Rhizobiaceae) bacteria (George *et al.* 1962; Johnson and O'Keeffe 1981). Economic damage occurs mostly as the result of larval feeding on root nodules, which reduces the capacity of plants to fix nitrogen (Corre-Hellou and Crozat 2005; Cárcamo *et al.* 2015) and contributes directly to yield loss (Williams *et al.* 1995; Vankosky *et al.* 2011a, Cárcamo *et al.* 2012). Damage is more prominent on main roots than on lateral roots (Verkleij *et al.* 1992) and can completely destroy root nodules during severe infestations (Jackson 1920; Cantot 1989). In the fall, nonreproductive, new-generation adults migrate to shelter belts, where they consume foliage of secondary legume hosts (*e.g.*, alfalfa, *Medicago sativa* Linnaeus (Fabaceae)) before overwintering in perennial leguminous crops or field margins (Jackson 1920; Schotzko and O'Keeffe 1986). As more habitats become suitable, further range expansion of *S. lineatus* in Canada is anticipated (Marsico *et al.* 2010; Olfert *et al.* 2012).

Host and mate location by adult *S. lineatus* requires response to semiochemical cues (Blight *et al.* 1984; Landon *et al.* 1997). Reproductively active male *S. lineatus* produce an aggregation pheromone, 4-methyl-3,5-heptanedione, in the spring that attracts both male and female weevils (Blight *et al.* 1984, 1991). In the fall, male and female weevils respond electrophysiologically (Blight *et al.* 1991) and behaviourally (Evenden *et al.* 2016; St. Onge *et al.* 2018) to aggregation pheromone, although newly eclosed reproductively immature males do not produce pheromone (Blight *et al.* 1991). Weevils also detect and respond to host volatiles released by leguminous host plants during both the spring and fall dispersal periods (Blight *et al.* 1984; Landon *et al.* 1997), but host volatile-baited traps do not attract weevils in the field (Evenden *et al.* 2016). Host volatiles do, however, enhance the attractiveness of synthetic aggregation pheromone lures but only during the fall dispersal period when weevils are reproductively immature (Evenden *et al.* 2016; St. Onge *et al.* 2018). These behaviours, mediated by intra- and interspecific chemical communication, can be exploited for the development of semiochemical-baited traps to monitor weevils during both adult dispersal periods (Evenden 2018) and detect the range expansion of this invasive species.

Several parameters influence the capture of *S. lineatus* in semiochemical-baited traps, which could affect the efficacy of a monitoring system. Trap placement and location in the field do not appear to influence the capture of *S. lineatus*: similar numbers were captured in traps placed on the edge and 25 m into pea fields (St. Onge *et al.* 2018) and in traps positioned at the north and south field edges (Reddy *et al.* 2018). It is not known, however, how many traps per unit area are required to get an accurate estimate of *S. lineatus* presence and activity, but at least three traps per field are recommended because of variation in the number of weevils captured between traps (Biddle *et al.* 1996). Furthermore, although weevils respond to a wide range of semiochemical release rates (St. Onge *et al.* 2018), over what distance response occurs is not known and therefore neither is the trap density required regionally to detect expanding populations known. Although *S. lineatus* fly to access host plants in the spring (Fisher and O'Keeffe 1979; Hamon *et al.* 1987), ground-based traps most efficiently capture weevils within pea crops (Reddy *et al.* 2018; St. Onge *et al.* 2018). Further research is needed to determine the number of traps necessary to estimate activity and over what distance *S. lineatus* orients to traps.

The use of semiochemical-baited pitfall traps to monitor *S. lineatus* results in the capture of other ground-dwelling arthropods that could be assessed as potential biological control agents for this invasive herbivore (Petsopoulos *et al.* 2021). Ground-dwelling natural enemies of *S. lineatus* include ground beetles (Coleoptera: Carabidae), ladybird beetles (Coleoptera: Coccinellidae), ants (Hymenoptera: Formicidae), rove beetles (Coleoptera: Staphylinidae), and spiders (Araneae) (Vankosky *et al.* 2011b; Knodel and Shrestha 2018). Ground beetles are among the most diverse and numerous arthropods present in agroecosystems and can reduce populations of agricultural pests (Kromp 1999; Holland *et al.* 2009; Boetzl *et al.* 2018). Therefore, identifying potential biological control assemblages in the expanded range of *S. lineatus* gives added utility to nontarget trap bycatch (Spears *et al.* 2016; Grocock *et al.* 2020) and is essential for the development of conservation biocontrol tactics for pest suppression (Altieri 1999; Symondson *et al.* 2002; Snyder and Straub 2006; Boreau de Roincé *et al.* 2012).

In the current study, a semiochemical-based monitoring trap developed in western Canada (Evenden *et al.* 2016; St. Onge *et al.* 2018) was tested as a tool to detect the range expansion of *S. lineatus* in Alberta, Canada. The capture of weevils in traps positioned in fields of the two reproductive hosts, field pea and faba bean, was compared. Using a mark–recapture approach, attempts were made to test the attractive radius (Hamon *et al.* 1987) of traps baited with two types of pheromone lures. Finally, the abundance and community structure of ground beetles captured as trap bycatch were assessed to determine potential predators that could contribute to the biological control of *S. lineatus* in its expanded range.

## Materials and methods

### *Monitoring* Sitona lineatus *across Alberta*

In April 2017, semiochemical-baited traps were positioned at 43 sites across five different pulse-growing regions of Alberta (Fig. 1). Geographical regions consisted of the Peace region in the northwest (n = 5 sites), the capital region around Edmonton (n = 8 sites), the east region towards the Saskatchewan border (n = 8 sites), the central region in the middle of the province (n = 17 sites), and the south region consisting of sites south of Calgary (n = 5 sites; Fig. 1). Individual sites were separated by at least 10 km to ensure independence of sampled weevil populations. To make direct comparisons of weevil activity in the two crop types, six faba bean sites were established in close proximity (< 5 km) to six pea fields (see paired analysis). At each site, a transect was established 3 m into the field in the northwest corner once host plants had germinated and cotyledons were visible. Transects were parallel to the field edge, with three pitfall traps separated by 25 m. Pitfall traps consisted of 473-mL plastic cups (Solo, Lakeforest, Illinois, United States of America) sunk into the soil so that the rims were flush with the soil surface.

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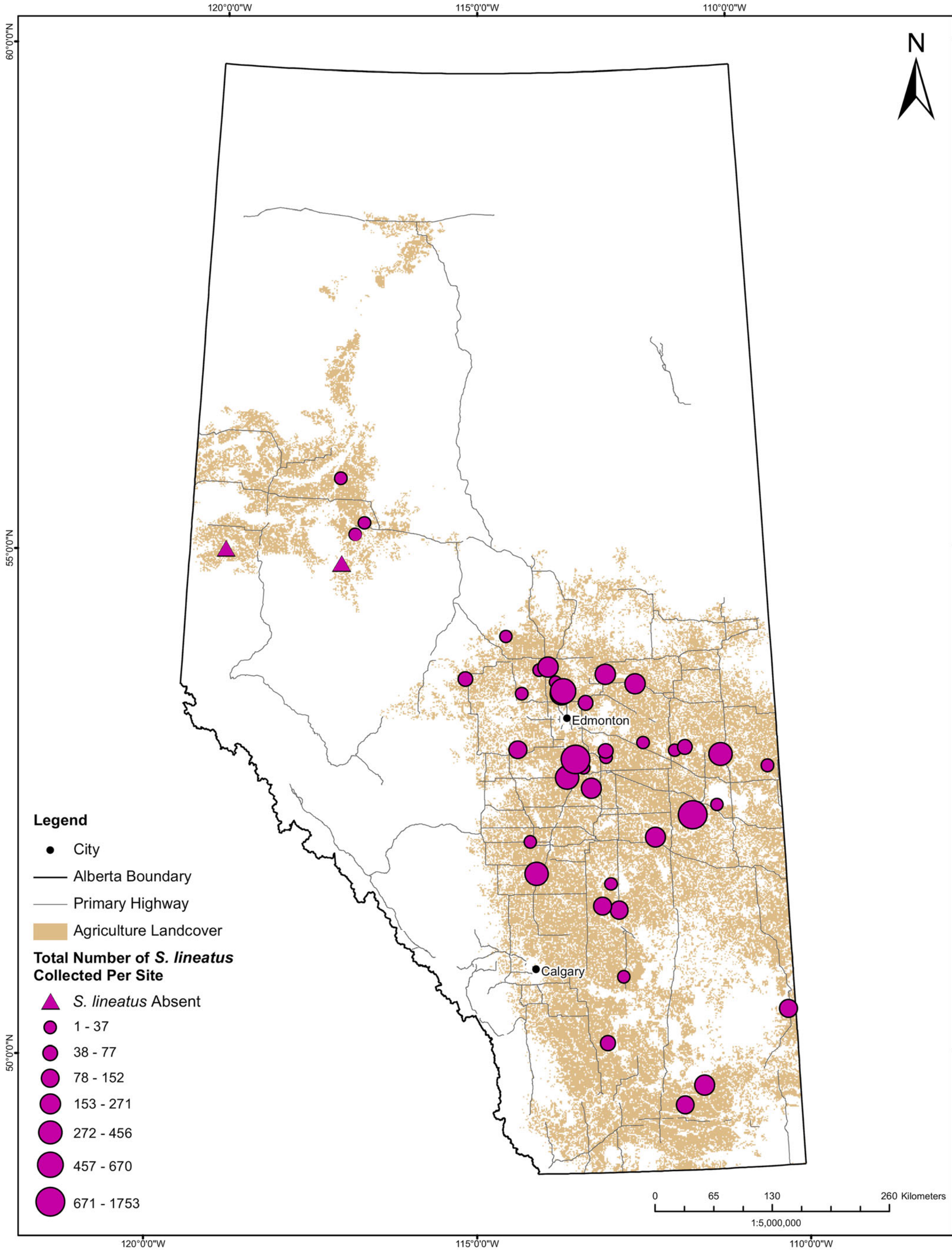


**Figure 1.** Map of Alberta showing season-long trap capture of *Sitona lineatus* in pheromone-baited pitfall traps in the pulse-growing regions of Alberta in 2017 overlaid on agriculture landcover extent (Wall-to-wall, Landcover Inventory, Alberta Biodiversity Monitoring Institute).

Traps contained propylene glycol (Home Hardware, Edmonton, Alberta, Canada) to preserve captured arthropods. Following a randomised block design, pitfall traps were baited with one of three treatments: (1) aggregation pheromone (10 mg 4-methyl-3,5-heptanedione) released from a

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grey rubber septa lure (186.55.2; Chemtica Internacional S.A., Santo Domingo, Costa Rica); (2) aggregation pheromone released from a grey rubber septa lure and bean volatiles released from five 250-μL microcentrifuge tubes (AM12350; ThermoFisher Scientific, Austin, Texas, United States of America): one tube loaded with 21 mg (*Z*)-3-hexen-1-yl acetate, one tube loaded with 34 mg (*Z*)-3-hexen-1-ol acetate, and three tubes, each loaded with 50 mg linalool (Contech Enterprises Inc., Delta, British Columbia, Canada; St. Onge *et al.* 2018); and (3) blank control (no lure present). Baited pitfall traps (Evenden *et al.* 2016; St. Onge *et al.* 2018) were baited with lures suspended with wire from a 15 × 15-cm piece of white corrugated plastic (Home Depot, Canada). The white corrugated plastic cover was positioned directly above each trap and secured to the ground with 15-cm nails. The corrugated plastic also served as a canopy to protect the trap from rain and to keep out large, unwanted organisms. Traps were checked, captured arthropods were removed, and the propylene glycol was refreshed biweekly throughout the duration of the spring (May–June) and fall (late July–September) adult *S. lineatus* activity periods. Before the fall adult activity period in late July, trap placement was re-randomised along the transect and traps were baited with new lures.

Weevils and the associated arthropod bycatch were removed from the propylene glycol and counted, and a subsample of 20 *S. lineatus* was separated by sex, following Jackson (1920). Predatory and omnivorous ground beetles (Carabidae) were separated from other arthropod bycatch and were identified using taxonomic keys (Lindroth 1968) and pre-existing carabid collections at the E.H. Strickland Museum at the University of Alberta (Edmonton, Alberta, Canada). Ground beetles were identified to species, except for *Poecilus* spp., *Amara* spp., *Harpalus* spp., and *Agonum* spp. *Pterostichus adstrictus* and *Pterostichus pensylvanicus* were grouped as *Pterostichus* spp. Three to four voucher specimens per identified taxa were deposited at the E.H. Strickland Entomological Museum.

### Mark–release recapture experiments

To determine the number of traps required on the landscape to monitor weevil range expansion, two mark–release recapture experiments were conducted in 2015 and 2016 to assess the attractive radius of semiochemical-baited traps targeting *S. lineatus*. For the first experiment, adult *S. lineatus* were collected on 10 August 2015 in sweep nets from an alfalfa stand near Lethbridge, Alberta (49.69° N, 112.84° W). Approximately 10 000 weevils were collected and transported to the laboratory, where 5250 weevils were marked and used in this experiment. Weevils were kept under cool conditions (5 °C) and provided with alfalfa as a food source during the marking period (three days) before release. Weevils were individually marked with a small dot of nail polish on the prothorax. Nail polish colour corresponded to the release distance either upwind or downwind of the semiochemical-baited traps. Preliminary experiments determined that the nail polish mark was not toxic to weevils and would not degrade or change colour in the propylene glycol used to capture weevils in the pitfall trap.

Weevils were released in three pea fields after harvest during the week of 10 August 2015. A subset of the released weevils (200) was separated by sex to confirm that both male and female weevils were released in approximately equal numbers. A trap line was positioned parallel to the field edge at each site along a north–south transect starting 100 m from the field edge, with 75 m between each of the eight traps. Two hundred and fifty weevils were released at each of seven distances – 10, 25, 50, 100, 500, and 1000 m downwind and 100 m upwind – from the trap line at each site in 2015. Within the trap line, two pitfall traps were baited with each treatment: (1) aggregation pheromone (10 mg 4-methyl-3,5-heptanedione) released from a grey rubber septa lure (186.55.2, Chemtica Internacional S.A.); (2) aggregation pheromone (21 mg 4-methyl-3,5-heptanedione) released from a 250-μ; microcentrifuge tube (AM12350; ThermoFisher Scientific); (3) aggregation pheromone released from a gray rubber septa lure and bean volatiles released from five 250-μL microcentrifuge tubes – one tube loaded with 21 mg (*Z*)-3-hexen-1-yl acetate (Blight *et al.* 1984), one tube loaded with 34 mg (*Z*)-3-hexen-1-ol acetate (Blight *et al.* 1984), and three tubes each loaded with 50 mg linalool (Contech Enterprises Inc.; St. Onge *et al.* 2018) – and (4) blank control (no lure present). Treatments were randomly assigned to traps along the transect at each of the three sites. Weevils were released in the evening (20h 00–24h 00, local time), and traps were checked 24 hours after release and weekly for one month following release. The semiochemical lures attracted unmarked *S. lineatus* over the month-long trapping period, which permitted us to compare the attractiveness of the differently baited semiochemical-baited traps. These weevils were collected, counted, and separated by sex.

Because only two of 5250 marked weevils released at the three field sites were recaptured in the 2015 experiment (0.04% recapture rate), the experimental design was altered in 2016. The experiment used 5100 marked *S. lineatus* and was conducted at only two field sites, and weevils were released at only three distances downwind of the trap line (10, 25, and 100 m), to allow for more weevils to be released ($n = 850$) per distance from the trap line. In 2016, weevils were collected on 29 July 2016 from a spring pea field during the day near Lethbridge, Alberta (49.46° N, 112.30° W). Weevils were collected, stored, and marked in a similar manner as in 2015. Weevils were released along the transect directly upwind of the traps and covered with alfalfa foliage for protection, under similar conditions to 2015. Traps were checked 24 hours after release and weekly for one month following release. The semiochemical lures did not capture any marked weevils in 2016 but attracted unmarked *S. lineatus* and were collected, counted, and separated by sex.

In 2016, we measured the release rate of pheromone from the two different pheromone lures (*i.e.,* microcentrifuge tubes loaded with 21 mg of pheromone and gray rubber septa loaded with 10 mg of pheromone) tested in the mark–recapture experiment. Three lures of each type were aerated individually in the laboratory. Charcoal-filtered air was drawn through glass chambers (5 cm high × 10 cm outer diameter) containing an individual lure. Air was drawn at 0.4 L/min through the aeration chamber using Dyna pumps (Neptune Dyna-Pump Model #2; Neptune Products Inc, Dover, New Jersey, United States of America). Released pheromone was captured on Porapak-Q (23151817; Waters Associated, Milford, Massachusetts, United States of America) and extracted with 2 mL of pentane/diethyl ether (50:50), internal standard (dodecyl acetate 10 μg) was added to the sample, and the sample was concentrated under a stream of ultrapure nitrogen to 500 μL. The sample was analysed using gas chromatography–mass spectrometry using an Agilent 7890B Gas Chromatograph coupled to a 5977A Mass Selective Detector (Agilent Technologies Inc., Santa Clara, California, United States of America) fitted with a DB-5 GC-MS column (30m × 0.25-mm ID, film thickness 0.25 μm). The injector port was set to 250 °C, the mass spectrometry source was set to 230 °C, and the mass spectrometry quadrupole was set to 150 °C. The transferline was set to 280 °C. Helium was used as a carrier gas with the following temperature program: 50 °C held for 5 minutes, 10 °C $min{-1}$ to 280 °C (held for 10 minutes). The injection split was 10:1, with a 1-μL injection volume. The area counts of the pheromone were quantified using an internal standard (dodecyl acetate 10 μg). Split injection was used to improve the peak shape of the dione. The release rate of three lures of each type were measured over three days at 23–24 °C, and the amount of 4-methyl-3,5-heptanedione released from each lure (μg/24 hours) was quantified.

## Statistical analyses

All analyses were conducted in R, version 4.1.3 (R Core Team 2022). All figures displaying analysed data were made with the *ggplot2* package (Wickham 2016). Separate statistical analyses were conducted for weevil capture at paired pea and faba bean sites (paired analysis) and for all pea sites across the province in the province-wide monitoring experiment. Weevil abundance data were separated into spring and fall collection periods and analysed using generalised linear mixed effects models with negative binomial distributions from the *lme4* package (Bates *et al.* 2015).

The sex of weevils collected in the variously baited traps was compared separately for the two collection periods and analysed using generalised linear mixed effects models (*lme4*) with binomial distributions. Site nested within the region was specified as a random effect and semiochemical treatment as the fixed effect for weevil abundance models.

Ground beetle abundance data from all field sites across Alberta were separated into spring and fall collection periods and analysed using generalised linear mixed effects models (*lme4*) with negative binomial distributions. The number of specimens of predatory or omnivorous ground beetles was included as the responding variable of predator abundance. Site nested within the trapping region was used as a random effect, and semiochemical treatment was used as the fixed effect. Ground beetle species diversity data were transformed using the Hellinger transformation and analysed using nonmetric multidimensional scaling and analysis of similarities from the *vegan* (Oksanen *et al.* 2017) and *ecodist* packages (Goslee and Urban 2007).

As only two and no (0) marked weevils were recaptured in 2015 and 2016, respectively, the number of unmarked *S. lineatus* captured in the variously baited traps in the mark–recapture experiments was compared. Each year was analysed separately using generalised linear mixed effects models (*lme4*) with negative binomial distributions. Semiochemical treatment was treated as the fixed effect. Generalised linear mixed-effects models (*lme4*) with binomial distributions were used to compare the sex of weevils captured in the variously treated traps.

Model validation was conducted for each analysis using the *DHARMa* package (Hartig 2022). This package creates interpretable scaled (quantile) residuals for fitted generalised linear mixed models to test assumptions of normality, homogeneity of variance, and over- and underdispersion.

## Results

### *Monitoring* Sitona lineatus *across Alberta*

Semiochemical-baited traps in all five pulse-growing regions of the province captured *S. lineatus* in both the spring and fall dispersal periods in 2017 (Fig. 1). Trap capture was highest in the capital region during the spring activity period and in the central region during the fall activity period. Although not compared statistically, more weevils were captured in the fall collection period than in the spring collection period (Fig. 2A). Trap catch in the Peace region (n = 5 sites) was low, and most captures occurred in only one pea field east of the Smoky River (Fig. 1). Capture of *S. lineatus* in the Peace region represents the first record of this species in this most northern pulse-growing region of the province. The identification of weevils captured in the Peace region was confirmed as *S. lineatus* (Canadian National Collection of Insects, Agriculture and Agri-Food Canada, Ottawa, Ontario).

For the unpaired analysis of all pea sites (n = 37), *S. lineatus* abundance was significantly affected by semiochemical treatment in the spring ($\chi^2 = 77.83$, $df = 2$, $P < 0.001$) and fall ($\chi^2 = 102.2$, $df = 2$, $P < 0.001$) collection periods. More individuals were caught in traps with lures than in control traps without lures (Fig. 2A). There was no significant difference in the number of weevils captured in traps baited with aggregation pheromone alone compared to aggregation pheromone and host bean volatiles in either collection period (spring: n = 86, fall: n = 95; Fig. 2A). Similar numbers of male and female *S. lineatus* were captured ($\chi^2 = 0.326$, $df = 2$, $P = 0.850$). In paired sites (n = 12), slightly more weevils were captured in pea fields than in faba bean fields ($\chi^2 = 4.03$, $df = 2$, $P = 0.05$; Fig. 3).

At all pea sites across the province, ground beetle abundance did not differ significantly with semiochemical treatment in spring or fall collection periods ($\chi^2 = 4.43$, $df = 2$, $P = 0.109$), but significantly more ground beetles were captured in the fall period than in the spring period ($\chi^2 = 134.1$, $df = 2$, $P < 0.001$; Fig. 2B). For the paired analysis, ground beetle abundance did not differ significantly between pea and faba bean fields ($\chi^2 = 2.08$, $df = 1$, $P = 0.150$). Due to

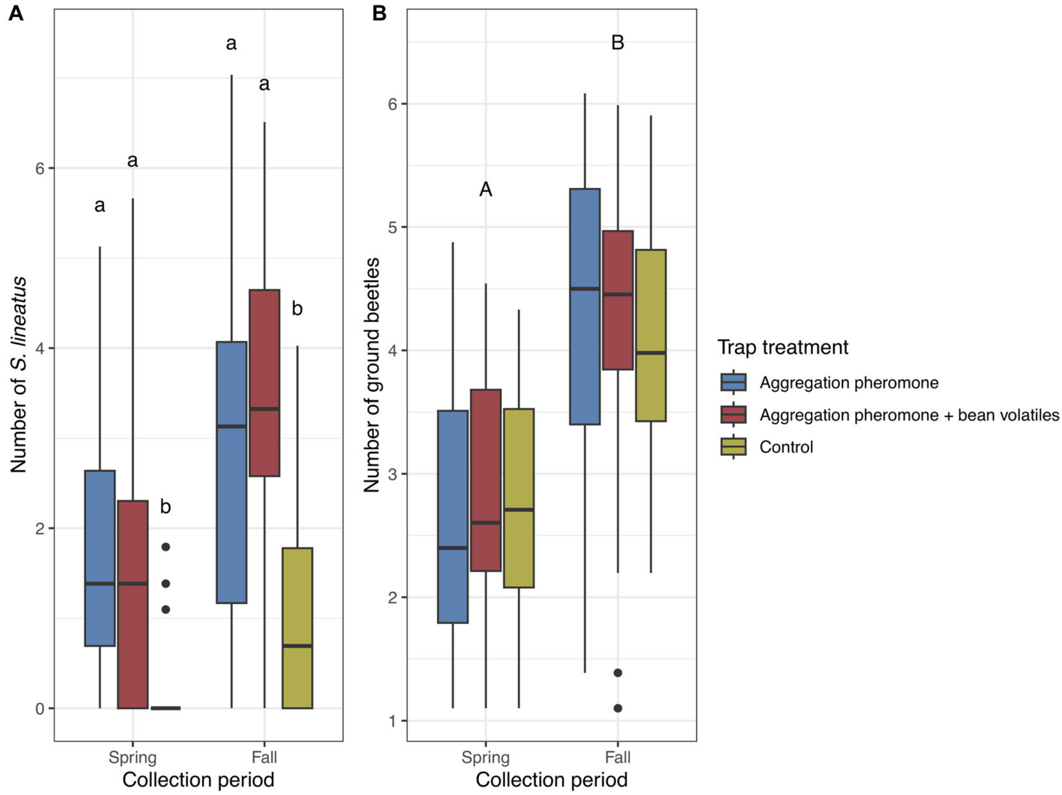


**Figure 2.** Number of **A**, *Sitona lineatus* and **B**, ground beetles (Carabidae) captured in pitfall traps in field pea during the spring and fall collection periods of 2017. Box plot is shown with medians (horizontal line), 25th and 75th percentiles (upper and lower box limits), minimum and maximum (whiskers), outliers (black circles), and statistical significance (letters). Lowercase letters indicate significant differences ($P < 0.05$) among trap treatments for *S. lineatus* capture, uppercase letters indicate significant difference ($P < 0.05$) over collection periods (ground beetles). Generalised linear mixed model analysis included a log-link function, and y-axes are log-transformed (y + 1) to display data on a logarithmic scale.

differences in the number of sites between regions, ground beetle abundance was not compared directly between regions; however, ground beetle diversity differed significantly by region (R = 0.123, $P = 0.001$; Fig. 4A) and collection period (R = 0.05, $P = 0.001$; Fig. 4B). Some species were trapped exclusively in the South and Peace regions, whereas other species were found consistently in all regions (Table 1). Species trapped in the spring collection period differed from species trapped in the fall collection period (R = 0.078, $P = 0.014$; Fig. 4B). *Chlaenis* spp. and *Pasimachus elongatus* were collected in the spring, and *Calathus ingratus* was collected only in the fall. For the paired analysis of sites, diversity did not differ significantly between crop type (R = –0.248, $P = 1$) or semiochemical treatment (R = –0.003, $P = 0.789$).

## Mark–release recapture experiments

In 2015, only two of 5250 marked and released weevils were recaptured at the three field sites (0.04% recapture rate). One of each of the recaptured weevils was released at the two closest release distances (10 and 25 m) downwind of the trap line. Despite the modifications made to the experimental design in 2016, none of the 5100 marked weevils released at the two field sites in 2016 were captured in the semiochemical traps. The semiochemical lures attracted unmarked *S. lineatus* over the month-long trapping period in both 2015 (n = 30 per treatment) and 2016

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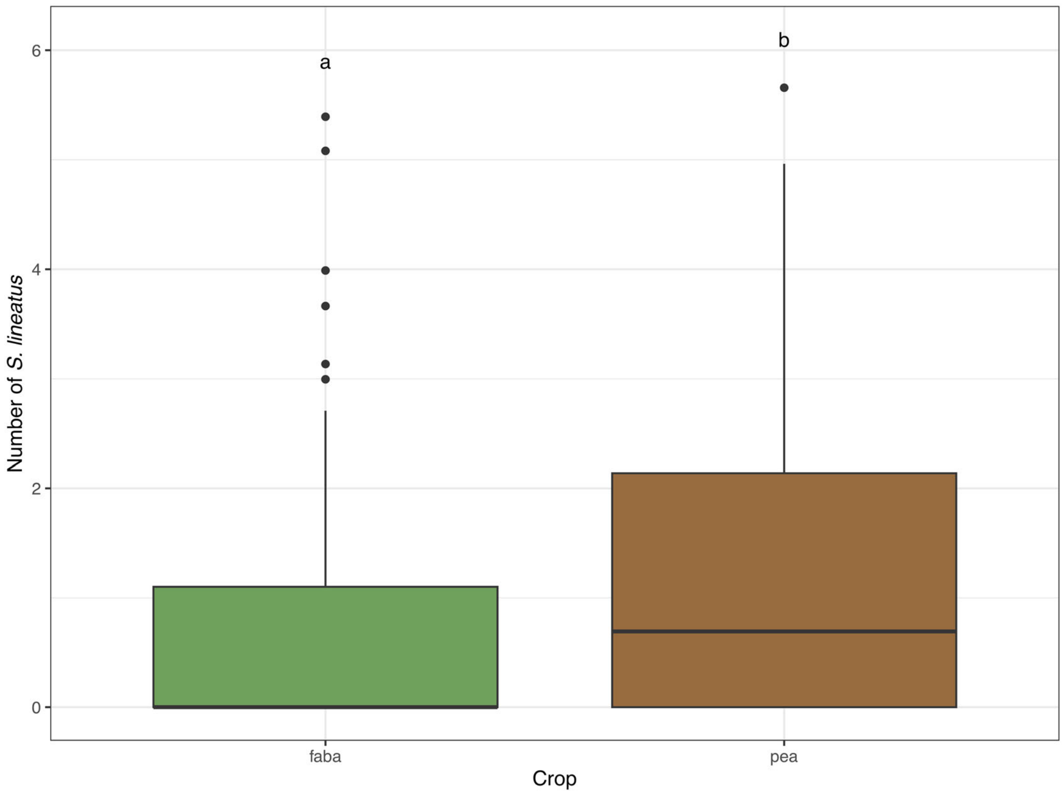


**Figure 3.** Number of *Sitona lineatus* captured in pitfall traps in faba bean and field pea fields in 2017. Box plot is shown with medians (horizontal line), 25th and 75th percentiles (upper and lower box limits), minimum and maximum (whiskers), and outliers (black circles). Different letters above boxes indicate significant differences ($P < 0.05$). Generalised linear mixed model analysis included a log-link function, and y-axes are log-transformed (y + 1) to display data on a logarithmic scale.

(n = 20 per treatment), but fewer weevils were captured overall in 2016 than in 2015 (Fig. 5). In 2015, baited traps attracted significantly more weevils than unbaited control traps did ($\chi^2 = 123.1$, $df = 3$, $P < 0.0001$; Fig. 5A). More weevils were captured in traps with microcentrifuge tube lures releasing aggregation pheromone and host plant volatiles than in traps with microcentrifuge tubes or septa lures releasing pheromone alone (Fig. 5A). As has been previously documented for weevil capture in the fall (Evenden *et al.* 2016; St. Onge *et al.* 2018), more weevils were captured in traps baited with pheromone and host plant volatiles than in traps baited with pheromone alone ($P < 0.0001$) in 2015 (Fig. 5A). In 2016, all semiochemical-baited traps captured more *S. lineatus* than the unbaited control traps did (${\chi^2}_3 = 25.52$, $P < 0.0001$), but there was no difference in captures of weevils in traps baited with either pheromone alone or with combined pheromone and host plant volatiles (Fig. 5B).

Male and female weevils were equally attracted to pitfall traps baited with the aggregation pheromone alone or with the combined pheromone and bean volatiles released from microcentrifuge tubes in the mark–recapture experiments. A slight female bias in trap capture occurred in pitfall traps baited with the pheromone released from rubber septa and in the unbaited control traps in 2015 but not in 2016.

Although *S. lineatus* trap capture was similar in the traps baited with the different types of pheromone lures in the mark–recapture experiments, the release rate of pheromone differed between the lure types. At least over the first three days, rubber septa lures released approximately

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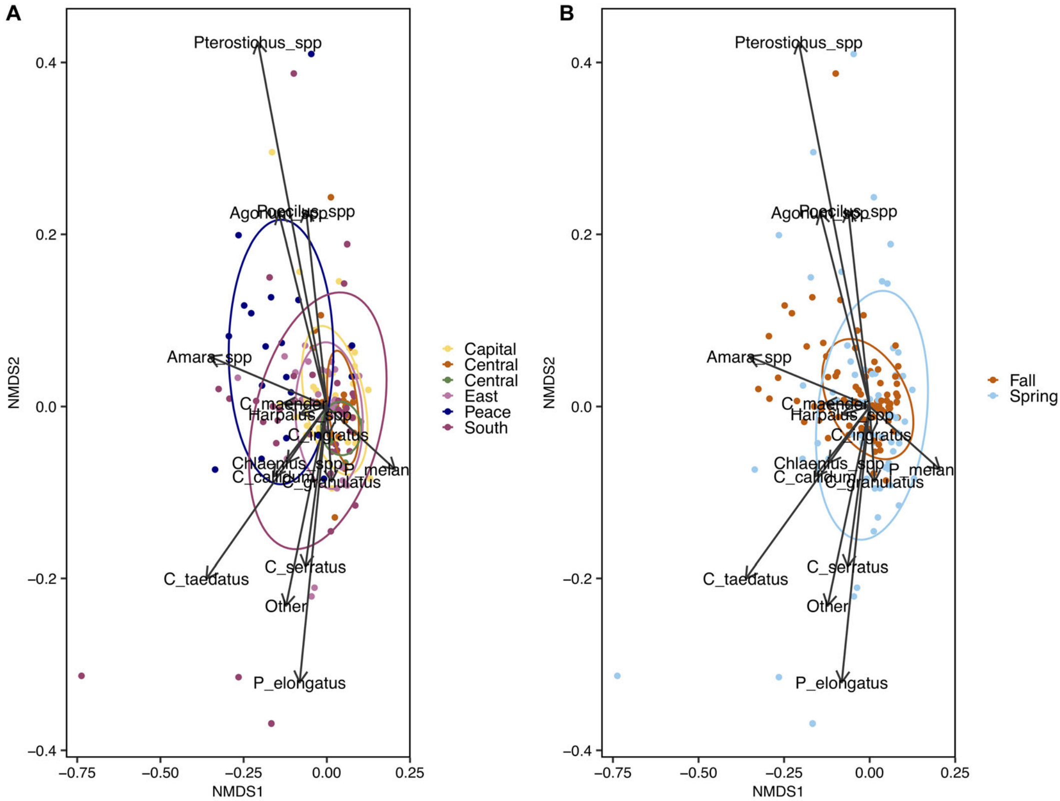


**Figure 4.** Nonmetric multidimensional scaling ordination of ground beetle community data across **A**, regions and **B**, collection periods in 2017 (Stress = 0.118). Ellipses represent **A**, region and **B**, collection period sampled, and vectors represent species.

10 times more pheromones than the microcentrifuge lures did. The average release rate (µg/day) for the septa lure was 193.67 (standard error = 41.94) for septum 1, 274.33 (standard error = 161.31) for septum 2, and 322.00 (standard error = 194.51) for septum 3. For the microcentrifuge tubes, the release rate (µg/day) was 20.33 (standard error = 2.58) for tube 1, 25.33 (standard error = 0.55) for tube 2, and 18 (standard error = 1.63) for tube 3. The microcentrifuge tube lures loaded with 21 mg of pheromone released less pheromone than the septa lures loaded with 10 mg did. The pheromone release rate was more similar between lures for the microcentrifuge tube than for the septa lures.

## Discussion

*Sitona lineatus* was captured in semiochemical-baited pitfall traps in pea and faba bean fields surveyed throughout the south, central, capital, and east regions of Alberta in 2017. This study is the first to report *S. lineatus* from field pea in the Peace region of Alberta, representing significant range expansion into Canada's northernmost agricultural region. Since its first detection in southern Alberta in 1997, *S. lineatus* has expanded its range to include all major pulse-growing regions in the province (Vankosky *et al.* 2009; Cárcamo *et al.* 2018). Outside of Alberta, *S. lineatus* has also continued an eastward expansion, first recorded in Saskatchewan in 2007 (Hartley 2007) and more recently in Manitoba in 2019 (Gavloski 2019). In 2016, pheromone-baited pitfall traps placed in pea fields near Saskatoon, Saskatchewan captured *S. lineatus* (Cárcamo and Mori,

**Table 1.** Ground beetle (Coleoptera: Carabidae) species collected from pulse-growing regions of Alberta in 2017

| Species | Capital | Central | East | Peace | South |
|---|---|---|---|---|---|
| *Agonum* spp. (Bonelli) | 20 | 42 | 20 | 29 | 22 |
| *Amara* spp. Herbst | 611 | 259 | 278 | 208 | 549 |
| *Calathus ingratus* Panzer | 0 | 5 | 0 | 0 | 0 |
| *Calosoma calidum* Fabricius | 2 | 0 | 0 | 3 | 0 |
| *Carabus granulatus* Müller | 1 | 2 | 1 | 7 | 0 |
| *Carabus maender* von Waldheim | 11 | 0 | 0 | 2 | 2 |
| *Carabus serratus* Fabricius | 0 | 1 | 0 | 0 | 2 |
| *Carabus taedatus* Fabricius | 0 | 1 | 0 | 0 | 17 |
| *Chlaenius* spp. (Bonelli) | 0 | 0 | 0 | 1 | 0 |
| *Harpalus* spp. (Latreille) | 4 | 7 | 1 | 24 | 59 |
| *Pasimachus elongatus* Dejean | 0 | 0 | 0 | 0 | 3 |
| *Poecilus* spp. (Bonelli) | 21 | 80 | 34 | 20 | 15 |
| *Pterostichus melanarius* (Illiger) | 2721 | 8117 | 1482 | 175 | 3538 |
| *Pterostichus* spp. (Bonelli) | 28 | 17 | 21 | 49 | 13 |
| Other | 48 | 34 | 44 | 25 | 74 |

unpublished data). Monitoring local movements and range expansion of adult *S. lineatus* is vital for an integrated pest management strategy to help producers make appropriate and informed management decisions to improve crop yield and decrease pest damage (Thomas 1999; Vankosky *et al.* 2009; Evenden *et al.* 2016; St. Onge *et al.* 2018). The Prairie Pest Monitoring Network (https://prairiepest.ca/) is a coordinated insect surveillance programme that uses unified insect monitoring protocols to coordinate and conduct insect population monitoring of pests, including *S. lineatus,* in field crops (Cárcamo *et al.* 2018; Prairie Pest Monitoring Network 2023). Currently, however, no unified pest management strategy for *S. lineatus* incorporates monitoring and various control tactics (Vankosky *et al.* 2009; Cárcamo *et al.* 2018).

Results from the province-wide survey in 2017 confirm that male and female *S. lineatus* are attracted to semiochemical-baited pitfall traps throughout both adult activity periods, corroborating results obtained in southern Alberta in previous studies (Evenden *et al.* 2016; St. Onge *et al.* 2018). The increased number of weevils captured in the fall is likely due to a longer fall activity period compared to the spring period (Fisher and O'Keeffe 1979) and the proximity of newly emerging weevils to baited pitfall traps. In a previous study, St. Onge *et al.* (2018) found that a microcentrifuge tube loaded with the aggregation pheromone (21 mg of 4-methyl-3, 5-heptanedione) deployed within a wet pitfall trap was the most effective lure and trapping method for *S. lineatus*. The addition of host plant volatiles occasionally increased the capture of *S. lineatus*, but results were not consistent enough to justify including host volatiles in lures for future monitoring tactics (St. Onge *et al.* 2018). Our 2017 results were similar to St. Onge *et al.*'s (2018) findings, with more weevils caught in traps baited with host volatiles and aggregation pheromone than in traps baited with pheromone alone during the fall trapping period. Despite low recapture rates of marked weevils in the mark–recapture experiments, lures attracted unmarked weevils during the one-month trapping period in the fall. The addition of host bean volatile increased attractiveness in 2015, supporting conclusions drawn by Evenden *et al.* (2016) and St. Onge *et al.* (2018); however, this trend was not observed in 2016. In addition to the semiochemical blend used in lures, lure type and dose can affect the number of insects captured in

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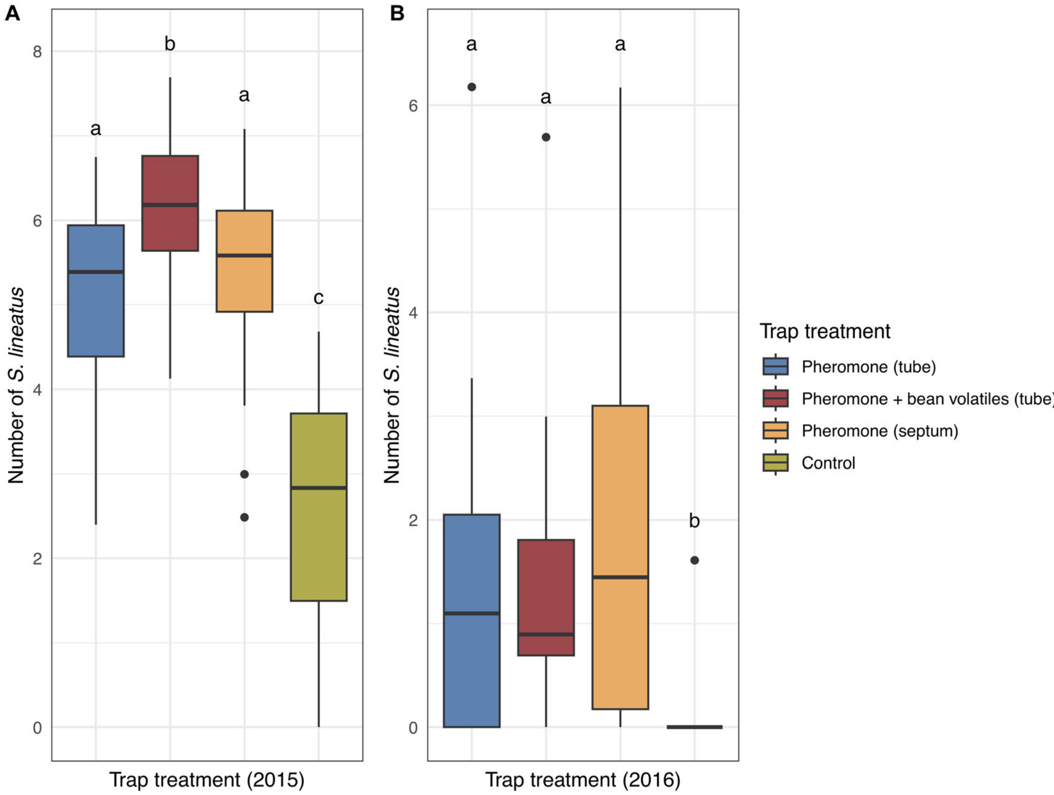


**Figure 5.** Number of unmarked *Sitona lineatus* captured in baited and control pitfall traps during **A**, 2015 and **B**, 2016 mark–release recapture experiment. Box plot is shown with medians (horizontal line), 25th and 75th percentiles (upper and lower box limits), minimum and maximum (whiskers), and outliers (black circles). Different letters above boxes indicate significant differences of *S. lineatus* captured in variously baited traps ($P < 0.05$). Generalised linear model analysis included a log-link function, and y-axes are log-transformed (y + 1) to display data on a logarithmic scale.

traps (Jansson *et al.* 1993; Byers 2013; Luo *et al.* 2020; Ebbenga *et al.* 2022; Batallas and Evenden 2023). Surprisingly, pheromone dose (10 mg versus 21 mg) and lure type (septa versus microcentrifuge tube, respectively) had no effect on the abundance of *S. lineatus* captured in 2016. The septa lure with a lower pheromone dose had a higher release rate measured over the first three days than the microcentrifuge tube lures did, and despite this distinction, trap capture was similar or slightly higher in the septa-baited traps. These results are consistent with previous studies that compared weevil capture by various release devices and pheromone doses, which also found no differences in weevil response to various pheromone release rates (St. Onge *et al.* 2018).

Adult weevils were captured in traps positioned in fields of both reproductive host plants, with slightly higher weevil capture occurring in field pea compared to faba bean. This is contrary to expectations because faba bean is a preferred host of *S. lineatus* in the spring (Bernstein and Jervis 2008); however, in the fall adults will feed on pea and faba beans without preference (Wijerathna 2021). Field peas are commonly grown in the south, central, and Peace regions of the province, whereas faba bean is grown to a much lesser extent across the same regions (Vankosky *et al.* 2009; Alberta Pulse Growers 2019). The results of our experiment indicate that both pea and faba bean should continue to be monitored closely for *S. lineatus* because adult abundance does not always reflect historical trends. Recently, a nominal threshold for *S. lineatus* in faba beans was determined by Wijerathna *et al.* (2021) to help growers estimate pest levels and assess management strategies. In field pea, the economic threshold is half of what it is in faba bean, with

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30% of the seedlings with damage on the terminal leaves during the second to fifth unfolded leaf stages of the seedlings (El-Lafi 1977; Vankosky *et al.* 2011a). Adult feeding damage has an economic injury level of 13.7% in faba bean, and the nominal threshold is rounded to 15% of seedlings with terminal leaf damage at the third unfolded leaf stage (Wijerathna *et al.* 2021). Damage to older plants is less detrimental to yield compared to damage to younger plants, which demonstrates that early adult monitoring with semiochemical traps for *S. lineatus* could help to detect adult presence, with feeding damage then prevented through the use of systemic insecticides (Wijerathna *et al.* 2021).

The use of nontarget arthropod bycatch in semiochemical-baited traps to monitor and survey pest species is common (Spears *et al.* 2016; Grocock *et al.* 2020). Through the collection and identification of bycatch, it is possible to incidentally monitor the communities of beneficial arthropods present in the system. Bycatch data can be collected in monitoring programmes with extensive spatial and temporal coverage and hold valuable potential for application in conservation studies (Wieten *et al.* 2012; Hung *et al.* 2015; Mester *et al.* 2020; Petsopoulos *et al.* 2021). Despite the tremendous potential, bycatch data are often ignored and excluded from analyses and publications because of the additional work required to sort and identify specimens of perceived secondary value than the target pest (Petsopoulos *et al.* 2021). Due to high capture rates and documented predatory behaviour of insect pests (Kromp 1999; Boetzl *et al.* 2018; Lemay *et al.* 2018), ground beetles were selected as a beneficial arthropod focus taxon in bycatch samples. Pitfall trapping is a simple method to sample ground-dwelling beetles in agroecosystems and is the most widely used technique for sampling ground beetles (Kromp 1999). Differences in activity density and trappability lead to large-bodied ground beetles being overrepresented and small-sized ground beetle species being underrepresented (Kromp 1999; McCravy 2018). Larger species are more likely to move farther distances, and smaller species are often able to evade capture upon reaching the lip of the cup of the pitfall trap (Drift 1951; Greenslade 1964; Spence and Niemelä 1994). Although this is a limitation of the pitfall trapping method, our interest is in the larger-bodied ground beetles because they can act as biological control agents and consume larger prey, including adult insects (Baines *et al.* 1990; Holopainen and Helenius 1992; Vankosky *et al.* 2011b), compared to small and intermediate-sized counterparts (Grafius and Warner 1989; Finch and Elliott 1992).

In the current study, the diversity of ground beetles varied between regions and between the spring and fall collection periods. Assemblages that were identified from all regions consisted of *P. melanarius, Amara* spp., *Poecilus* spp., *Pterostichus* spp., *Agonum* spp., *Carabus* spp., and *Harpalus* spp. Other studies have also found similar groups of ground beetles in agroecosystems in Saskatchewan (de Heij *et al.* 2022). Unique species identified from the Peace, south, and central regions included *Chlaenius* spp., *Pasimachus elongatus*, and *Calathus ingratus*, respectively. Typically, North American agroecosystems exhibit low ground beetle diversity, with only a small number of characteristic species. These species are usually found in greater abundance (Hance *et al.* 1990; Fan *et al.* 1993; Tonhasca 1993; Ellsbury *et al.* 1998; Holland and Luff 2000). Our results for *Amara* spp. and *P. melanarius* beetles, medium- and large-sized species that were collected in large numbers, are consistent with this pattern.

Ground beetles represented the majority of arthropod bycatch in the *S. lineatus* pitfall traps, and similar numbers of ground beetles were captured in the baited and control pitfall traps and in pea and faba bean fields. The dominant species collected was the nonnative *Pterostichus melanarius*. Populations of this species were heavily concentrated in the south, central, and capital regions, whereas they were less commonly recovered from samples collected in the Peace region. More ground beetles were captured during the fall collection period, which coincided with peak populations of *P. melanarius* in mid-July–August, when mating occurs (Busch *et al.* 2021). The dominant presence of *P. melanarius* in our study coincides with findings in other studies that sampled ground beetle assemblages in different ecosystems in Alberta (Cárcamo *et al.* 1995; Hartley *et al.* 2007). Since reaching Edmonton in 1959 (Madge 1959), the presence of *P. melanarius* is associated with disturbed habitats such as urban areas and agroecosystems (Niemelä and Spence 1991). Studies have shown that ground beetles can have a significant impact on insect pests and can help to contribute to the predator complex (Frank 1971; Edwards *et al.* 1979; Tyler and Ellis 1979; Hance 1987; Chiverton 1988; Cárcamo *et al.* 1995).

Predation of arthropods by ground beetles usually occurs on the soil surface (Holland and Luff 2000). Both adults and larvae are predatory, but ground beetle larvae are considered to be more carnivorous and predatory in diet than the adults because food availability is limited (Thiele 1977). Several species of ground beetle consume *S. lineatus* eggs, including *P. melanarius* and *Bembidion quadrimaculatum* (Linnaeus) (Vankosky *et al.* 2011b). Living and dead *S. lineatus* positioned in mesocosms containing pea plants, soil, and stubble were consumed mostly by *Poecilus lucublandus* (Say) and *P. melanarius* (Hanavan 2016) in Moscow, Idaho, United States of America. Predation in mesocosms resulted in less feeding damage per plant by adult *S. lineatus* (Hanavan 2016). This demonstrates that, under laboratory conditions, ground beetles have the potential for use as biological control agents of *S. lineatus*.

A comprehensive understanding of the potential natural enemies of *S. lineatus* in its expanded range in North America is crucial for the development of an integrated pest management programme for the species. The results of the current study indicate that pheromone-baited pitfall traps can survey the range expansion of *S. lineatus* and the presence and abundance of ground-dwelling arthropods in pea and faba bean fields. Furthermore, our bycatch data provide insight into which ground beetle species are present in the pulse-growing regions of Alberta and into how these community assemblages change over the growing season. Future research should focus on the potential effect of the identified ground beetles as biological control agents of *S. lineatus* and on identifying factors that influence the success of ground beetles as natural enemies in pulse agroecosystems.

**Competing interests.** The authors declare that they have no competing interests.

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