Received: 10 March 2023 | Revised: 24 May 2023 | Accepted: 14 June 2023

DOI: 10.1111/jen.13158

ORIGINAL ARTICLE

JOURNAL OF APPLIED ENTOMOLOGY WILEY

# Mixed sex pheromone lures for combined captures of *Agriotes* and *Limonius* pest click beetles in North America

Emily Lemke[1,2] | Willem G. van Herk[1] | Kendal Singleton[1,2] | Julien Saguez[3] | Graeme Fowler[4] | Doug Pepper[4] | Kathleen Furtado[1,2] | Gerhard Gries[2]

[1]Agassiz Research and Development Centre, Agriculture and Agri-Food Canada, Agassiz, British Columbia, Canada

[2]Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada

[3]Centre de recherche sur les grains, Saint-Mathieu-de-Beloeil, Quebec, Canada

[4]British Columbia Ministry of Agriculture, Courtenay, British Columbia, Canada

**Correspondence**
Emily Lemke, Agassiz Research and Development Centre, Agriculture and Agri-Food Canada, 6947 Highway 7, Agassiz, BC V0M 1A0, Canada.
Email: elemke96@gmail.com

## Abstract

Sex pheromone lures are effective tools for monitoring and potentially controlling populations of pest click beetles (Coleoptera: Elateridae). To date, these lures are genus-specific (e.g., *Limonius* spp.) or species-specific (e.g., *Agriotes lineatus* Linnaeus). However, if sympatric heterogeners were not to be repelled by each other's pheromones, trap lures effective for multiple elaterid genera could be developed, improving cost efficiency in elaterid pest management programs. In both western and eastern North America, several species of *Agriotes* spp. and *Limonius* spp. co-occur and inflict similar crop damage. We investigated whether the sex pheromones of these species can be combined in a mixed lure without reducing its attractiveness to all target species. In western Canada, we show that the pheromones of *A. lineatus* (geranyl butanoate & geranyl octanoate) and *Limonius* spp. [(*E*)-4-ethyloct-4-enoic acid (limoniic acid)] can be combined without significantly reducing captures of male *A. lineatus*, *L. canus* (LeConte), *L. californicus* (Mannerheim) and *L. infuscatus* (Motschulsky) relative to traps baited with species-specific lures for *A. lineatus* and *Limonius* spp.. Similarly, the pheromone of *A. obscurus* (Linnaeus) (geranyl hexanoate & geranyl octanoate) and limoniic acid can be combined without significantly reducing trap captures of male *L. canus*, *L. infuscatus* and *L. californicus* but reduced *A. obscurus* captures relative to traps baited only with the *A. obscurus* pheromone. In eastern Canada, combining pheromones for *Agriotes mancus* (Say) (geranyl butanoate & geranyl hexanoate) and limoniic acid reduced captures of *A. mancus* but not *A. pubescens* (Melsheimer) and *A. sputator* (Linnaeus). These data imply that pheromones of select elaterid heterogeners can be combined in a 'catch-more' pheromone lure to effectively monitor for, or possibly control, multiple elaterid pests, but that such mixed lures should be evaluated for each species combination.

**KEYWORDS**
Elateridae, insect chemical communication, integrated pest management, pheromone-based monitoring, species detection, wireworms

## 1 | INTRODUCTION

Pheromone-based trapping is an effective tool in integrated pest management (IPM) for monitoring and estimating population densities, detecting endangered or pest species, mapping species distributions, and timing pest management tactics (Howse et al., 1998; Vernon & van Herk, 2022). Synthetic sex pheromones of click beetles (Coleoptera: Elateridae) are deployed to monitor the presence and abundance of pest species (Furlan et al., 2020), and to curtail pest populations by mass trapping (Arakaki et al., 2008; Vernon, Blackshaw, et al., 2014) and mating disruption (Reddy & Tangtrakulwanich, 2014; Vernon & van Herk, 2022). In North America, sex pheromones are now identified for multiple pest click beetles, including *Agriotes ferrugineipennis* and *A. mancus* (Singleton et al., 2023; Singleton, Gries, et al., 2022), *Limonius canus* and *L. californicus* (Gries et al., 2021; van Herk, Lemke, Gries, et al., 2021), *Selatosomus aeripennis destructor* (Gries et al., 2022), *Cardiophorus tenebrosus* and *C. edwardsi* (Serrano et al., 2018), and *Melanotus communis* (Williams et al., 2019).

In agricultural fields in North America, multiple elaterid pest species and genera commonly co-occur, such as *Glyphonyx recticollis* (Say) and *Melanotus communis* (Gyllenhal) in the southeastern USA (Vernon & van Herk, 2022), *Limonius infuscatus*, *L. californicus* and *Selatosomus pruininus* in the Pacific Northwestern USA (Milosavljević et al., 2016), *Selatosomus aeripennis destructor* (Brown) and *Hypnoidus bicolor* (Eschscholtz) on the Canadian Prairies (van Herk, Vernon, Labun, et al., 2021), *A. obscurus* and *A. lineatus* in coastal British Columbia (BC), and *L. canus*, *L. infuscatus*, and *A. lineatus* in central BC (Vernon & van Herk, 2022). In the province of Quebec, the abbreviated wireworm, *Hypnoidus abbreviatus* (Say), often co-occurs with *Melanotus*, *Agriotes* and *Limonius* species (Saguez et al., 2017). In Canada to date, populations of elaterid species are monitored with species-specific pheromone lures, which makes pheromone-based monitoring and mass trapping cumbersome and expensive (Vernon & van Herk, 2022). With sex pheromones of key elaterid pests becoming known and commercially available, pheromone lures that attract more than one elaterid species or genus would improve cost efficiency and practicality of pheromone-based tactics for elaterid pest management. As previously shown, (*E*)-4-ethyloct-4-enoic acid (limoniic acid) as a single-component sex pheromone is highly attractive to each of the four major North American *Limonius* pests (*L. californicus*, *L. canus*, *L. infuscatus* and *L. agonus*) (Gries et al., 2021; Lemke et al., 2022; van Herk et al., 2023; van Herk, Lemke, Gries, et al., 2021). Conversely, the sex pheromones of *A. obscurus* (geranyl hexanoate & geranyl octanoate) and *A. lineatus* (geranyl butanoate & geranyl octanoate) could not be combined in coastal BC without reducing the lure's attractiveness to *A. obscurus*. When synthetic sex pheromones of *A. lineatus* and *A. obscurus* were combined in a mixed lure, captures of *A. lineatus* remained unaffected but captures of *A. obscurus* decreased by 76%–77% (van Herk, Vernon, Bourassa-Tait, et al., 2022; Vernon, van Herk, & Tanaka, 2014), indicating a deterrent effect of the *A. lineatus* pheromone on attraction and capture of male *A. obscurus*. Responses of male *A. lineatus* and *A. obscurus* to limoniic acid, and responses of male *Limonius* spp. to pheromones of *Agriotes* heterogeners, have not yet been tested. With the distinctively different types of pheromone produced by *A. lineatus* and *A. obscurus* (terpenoid esters), and by *Limonius* spp. (ethyl-branched aliphatic acid), we predicted that a mixed ester/acid lure would still be attractive to both *Agriotes* spp. and *Limonius* spp.

The concept of combining synthetic pheromones of multiple pest species in a mixed pheromone lure has previously been explored with varying degrees of success in several integrated pest management programs. The concept was tested with mealybugs (Waterworth et al., 2011), longhorn beetles (Fan et al., 2019; Nakamuta et al., 1997; Rice et al., 2020; Wong et al., 2012), moths (Brockerhoff et al., 2013; Jones et al., 2009; Preti et al., 2020) and true bugs (Kim et al., 2015; Yasuda et al., 2010). Mixed pheromone lures are effective if no pheromone in that lure adversely affects attraction and capture of all target species.

Here, we investigated whether synthetic sex pheromones of *Agriotes* spp. and *Limonius* spp. (Figure 1) can be combined in a mixed lure without affecting its attractiveness to any *Agriotes* and *Limonius* species. Mixed lures were designed to capture *Limonius* spp. and *A. lineatus* and *A. obscurus* in western Canada, and *L. agonus* and *A. mancus* in eastern Canada. These species were selected because *Limonius* spp. and *Agriotes* spp. commonly co-occur in Canada with generally overlapping seasonal swarming periods (Begg, 1959; Jakubowska et al., 2018; Lemke et al., 2022; Levesque & Levesque, 1993; van Herk, Lemke, Gries, et al., 2021; van Herk, Vernon, Acheampong, et al., 2021; Vernon & van Herk, 2022). As *A. mancus* may also co-occur with *A. pubescens* and *A. sputator*, the effect of mixed lures on captures of *A. pubescens* and *A. sputator* was also of interest. The biology and life history of *Limonius* spp. and native *Agriotes* spp., such as *A. pubescens*, remain hardly studied, but these species inhabit the same microhabitat (i.e., unfarmed grassy margins surrounding crops), making them ideal heterogenera to study together (Traugott et al., 2015).

## 2 | MATERIALS AND METHODS

### 2.1 | General methods

Five experiments were conducted with *L. canus*, *L. californicus*, *L. infuscatus* and *A. lineatus* or *A. obscurus* in western Canada, and one experiment with *L. agonus* and *A. mancus* in eastern Canada. All six experiments ($n=8$ each) used Vernon Pitfall Traps® (VPT) (van Herk, Vernon, Borden, et al., 2022) and tested four or six treatments in a randomized complete block design. Traps were placed at 10- and 20-m spacing between treatments and blocks (replicates), respectively, in grassy field edges, following a general protocol previously detailed (Gries et al., 2021). Trap lures consisted of a closed, 1-mL low-density-polyethylene receptacle (Kartell Labware) containing a cotton pellet (Richmond Dental #0), onto which the pheromones were dispensed. Lures were suspended from trap roofs and not replaced over the trapping period of each study (van Herk, Vernon, Acheampong,

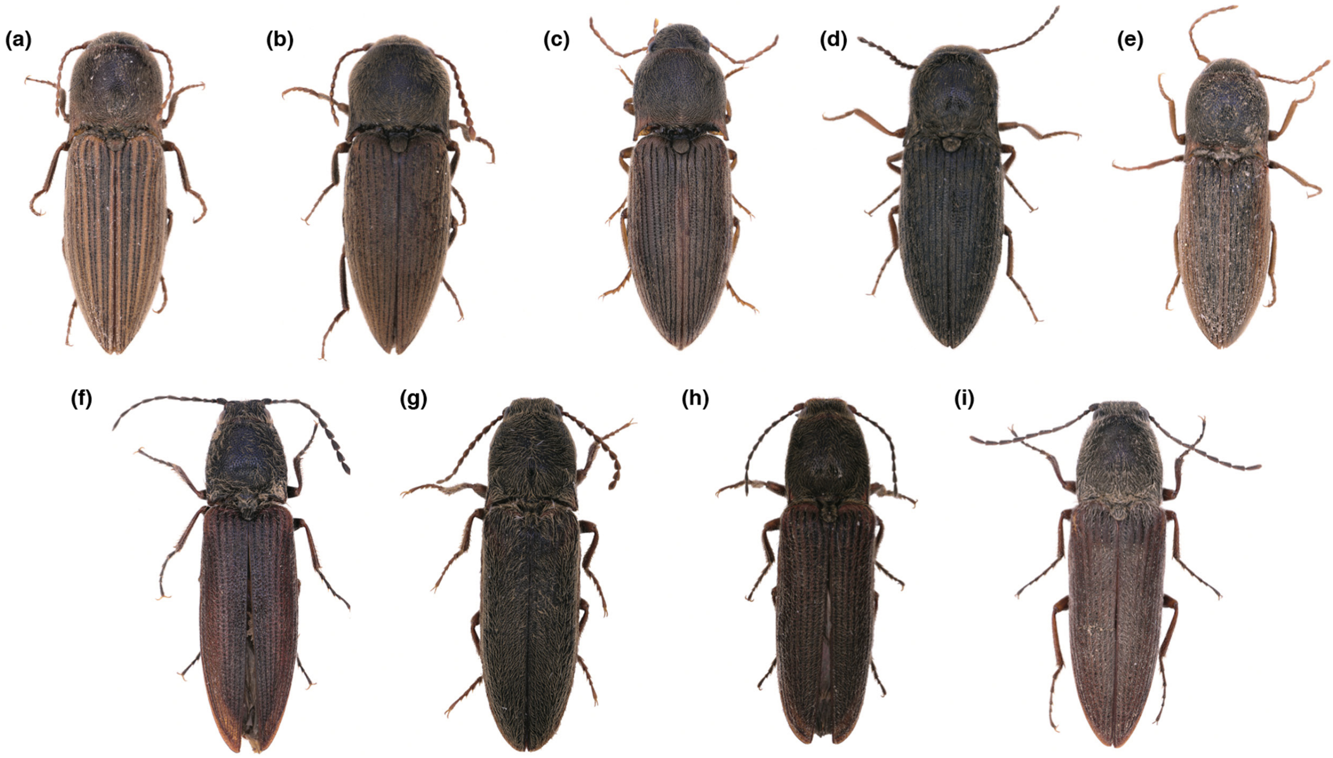



FIGURE 1 Photographs of (a) *Agriotes lineatus*, (b) *A. obscurus*, (c) *A. mancus*, (d) *A. pubescens*, (e) *A. sputator*, (f) *Limonius canus*, (g) *L. californicus*, (h) *L. infuscatus*, (i) *L. agonus*. Photo credits: Julien Saguez. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 List of experiment numbers (Exp. #), cities near study locations, geographic coordinates (latitude, longitude) of study sites in British Columbia (BC) and Quebec (QC), trapping durations (in 2020* and 2021), numbers of Vernon pitfall traps deployed, and total numbers of *Limonius* spp. and Agriotes spp. captured in all traps.

| Exp. # | Cities | Geographic coordinates | Trapping period (days) | Traps | Beetles captured |
|---|---|---|---|---|---|
| Study 1 | | | | | |
| 1 | Kelowna, BC | 49.821284, −119.441011 | 25 April – 16 May (21)* | 16 | 10,063 |
| 2 | Courtenay, BC | 49.732937, −125.018113 | 04 May – 01 June (28) | 32 | 67,982 |
| 3 | Duncan, BC | 48.735409, −123.669593 | 03 May – 02 June (30) | 32 | 31,567 |
| Study 2 | | | | | |
| 4 | Kelowna, BC | 49.868836, −119.443256 | 14 April – 18 May (34) | 42 | 29,627 |
| 5 | Pemberton, BC | 50.429281, −122.914445 | 12 April – 13 May (31) | 48 | 6506 |
| Study 3 | | | | | |
| 6 | Saint-Mathieu-de-Beloeil, QC | 45.585074, −73.2464447 | 3 May – 18 June (46) | 32 | 365 |

et al., 2021). Studies were placed in locations with historic wireworm damage and were terminated once total beetle captures declined.

## 2.2 | Sources of pheromones

Limoniic acid was synthesized from a previous study (detailed in Gries et al., 2021), and geranyl octanoate, geranyl hexanoate and geranyl butanoate were purchased from Penta Manufacturing Corporation (Fairfield).

## 2.3 | Trapping in western Canada

Three experiments were run for captures of *Limonius* spp. and *A. lineatus*, and two experiments were run for captures of *Limonius* spp. and either *A. lineatus* or *A. obscurus*. Experiment 1 was run at an organic farm in Kelowna, BC (Table 1), where three *Limonius* spp. and *A. lineatus* had been found in abundance in previous years (Lemke et al., 2022; van Herk, Lemke, Gries, et al., 2021; van Herk, Vernon, Acheampong, et al., 2021). Traps were installed on 25 April 2020 and checked on 02, 06 and 16 May 2020. Experiments 2 & 3 were run in Courtenay and

Duncan, BC (Table 1) on farms historically infested with *L. infuscatus* and *A. lineatus* (van Herk, personal obs.). In Courtenay, traps were installed on 04 May 2021 and checked on 10, 17, 24 May and 01 June. In Duncan, traps were installed on 03 May 2021 and checked on 12, 19, 26 May and 02 June. Experiment 4 was run in Kelowna, BC (Table 1) on two organic vegetable farms with historical presence of *L. infuscatus*, *L. canus*, *L. californicus* and *A. lineatus*, and a low population of *A. obscurus* (van Herk, personal obs). Experiment-4 traps were installed at two locations (less than 5 km apart) on 14 April and checked on 20, 28 April and on 04, 11, 18 May 2021. Experiment 5 was run in Pemberton, BC (Table 1) in a fallow field near a potato farm where *L. canus*, *A. lineatus* and *A. obscurus* had previously been found (van Herk, Vernon, Acheampong, et al., 2021). Experiment-5 traps were installed 12 April and checked on 19, 26 April and on 06, 10, 13 May 2021.

Experiments 1–3 tested four treatments: (1) an unbaited control; (2) *A. lineatus* pheromone (geranyl octanoate & geranyl butanoate at a 1:1 ratio; 40 mg); (3) *Limonius* spp. pheromone (limoniic acid; 4 mg); and (4) *A. lineatus* pheromone (20 mg) *plus* limoniic acid (2 mg). Experiments 4 and 5 also tested treatments 1–4 as well as (5) *A. obscurus* pheromone (geranyl hexanoate & geranyl octanoate at a 1:1 ratio; 40 mg), and (6) *A. obscurus* pheromone (20 mg) *plus* limoniic acid (2 mg).

## 2.4 | Trapping in Eastern Canada

One experiment (Exp. 6) was run at the grain research centre (CEROM) in Saint-Mathieu-de-Beloeil, Quebec (Table 1), where *L. agonus*, *A. mancus*, *A. pubescens* and *A. sputator* had previously been found (Singleton et al., 2023; Singleton, van Herk, et al., 2022). Traps were installed on 03 May and checked on 07, 11, 13, 17, 19, 21, 25, 27, 31 May and on 04, 07, 10, 14, 18 June 2021. Each experimental replicate consisted of four treatments: (1) an unbaited control; (2) *A. mancus* pheromone (geranyl hexanoate & geranyl butanoate at a 1:1 ratio; 40 mg); (3) limoniic acid (4 mg); and (4) *A. mancus* pheromone (20 mg) *plus* limoniic acid (2 mg).

The dose of esters and of limoniic acid in all mixed lures (compared to ester- or acid-only lures) was erroneously halved. This error, however, is deemed to have little bearing on beetle captures and the interpretation of experimental results. As limoniic acid is similarly attractive at 0.4 mg, 4 mg and 40 mg (van Herk et al. 2021), testing limoniic acid here at 2 mg or 4 mg would not have significantly affected trap captures. Similarly, although the ester dose in mixed and ester-only lures (20 mg and 40 mg, respectively) for attraction of *Agriotes* spp. differed by 2-fold, ester release rates from both types of lures were virtually identical (as determined by laboratory release rate studies over 7 days), suggesting no effect of ester dose on trap captures, at least not in the first one or 2 weeks of the trapping period.

## 2.5 | Identification of captured beetles

In experiments 1 & 6, all captured beetles were identified to species and their sex was determined using taxonomic keys (Al Dhafer, 2009; Etzler, 2013; Johnson, 2002) and an identification guide for northwestern *Limonius* species found on agricultural land (Frank Etzler, unpublished). In experiments 2–5, all beetles were counted and sorted by genus until further species identification. In experiments 2, 3 and 5, large beetle captures made it necessary to identify beetles in only subsamples. At each location per collection date, up to 50 *Limonius* and 50 *Agriotes* beetles per treatment were identified to species and their sex determined from two of eight randomly selected replicates (Exps. 2, 3) and from all eight replicates (Exp. 5; up to 800 beetles per collection date). In experiment 4 with a greater diversity of captured species, at each collection date up to ~100 *Limonius* and 100 *Agriotes* beetles per treatment were identified and their sex determined from all eight experimental replicates (up to 1400 beetles per collection date).

## 2.6 | Statistical analyses

All data were analysed using SAS Enterprise Guide v.7.1 (SAS Institute, Cary, NC, USA). The mean proportion of species per treatment and replicate was used to calculate the number of each species collected. These numbers were then analysed with a two-factor generalized linear model (Proc GENMOD), using a log-link function and a negative binomial distribution. Model factors were 'replicate' and 'treatment'. Pairwise comparisons between treatments used the 'lsmeans' statement with Tukey's adjustment. Data were not analysed for species with low captures (i.e., <0.1 proportion of total captures), and for the few female beetles that were captured in traps (i.e., <0.02 proportion of total captures). For experiment 4, initial analyses indicated that data from the two study sites were not significantly different, and these data were therefore combined for final analysis.

# 3 | RESULTS

## 3.1 | Trapping in Western Canada

More than 145,000 click beetle males were collected in Exps. 1–5, with the proportion of target species ranging from 0.00 to 0.75 (Table 2). Captures of *L. canus*, *L. infuscatus*, *L. californicus* and *A. lineatus* were not significantly reduced ($p > 0.05$) when limoniic acid was mixed with the *A. lineatus* pheromone [capture ratio of limoniic acid to mixed lure: *L. canus* = 0.59 and 1.24 in experiments 1 and 4, respectively (Figures 2a and 3i); *L. infuscatus* = 1.39, 1.38, 1.08 and 1.17 in experiments 1, 2, 3 and 4, respectively (Figures 2b,e,g and 3j); *L. californicus* = 1.54 in experiment 4 (Figure 3k)]; capture ratio *A. lineatus* pheromone to mixed lure: *A. lineatus* = 1.17, 0.85, 0.83, 1.23 and 1.18 in experiments 1, 2, 3, 4 and 5, respectively (Figures 2d,f,h and 3l,n). Similarly, when limoniic acid was mixed with the *A. obscurus* pheromone, captures of *L. canus*, *L. infuscatus*, *L. californicus* and *A. lineatus* were not significantly reduced [capture ratio of limoniic acid to mixed lure: *L. canus* = 1.07 and 2.11 in experiments 4 and 5, respectively (Figure 3i,m); *L. infuscatus* = 1.03 in experiment 4 (Figure 3j); *L. californicus* = 1.54 in experiment 4

**TABLE 2** Mean proportion of male *Limonius canus*, *L. californicus*, *L. infuscatus*, *Agriotes lineatus*, *A. obscurus*, *A. mancus*, *A. pubescens*, and *A. sputator* captured per pheromone treatment and control in each study and experimental location in British Columbia (BC) and Quebec (QC) (Exps. 1–6).

| | | | Mean proportion of species captured per control and pheromone treatment | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Exp. #** | **Location** | **Species captured** | **Control** | ***A. lineatus* pheromone** | ***Limonius* spp. pheromone** | ***A. lineatus + Limonius* spp. pheromone** | ***A. obscurus* pheromone** | ***A. obscurus + Limonius* spp. pheromone** |
| Study 1 | | | | | | | | |
| 1 | Kelowna, BC | *L. canus* | 0.03 | 0.43 | 0.20 | 0.34 | | |
| | | *L. californicus* | 0.00 | 0.03 | 0.65 | 0.32 | | |
| | | *L. infuscatus* | 0.00 | 0.02 | 0.57 | 0.41 | | |
| | | *A. lineatus* | 0.00 | 0.54[a] | 0.00 | 0.46 | | |
| | | *A. obscurus* | 0.00 | 0.25 | 0.00 | 0.75 | | |
| 2 | Courtenay, BC | *L. infuscatus* | 0.00 | 0.00 | 0.58 | 0.42 | | |
| | | *A. lineatus* | 0.01 | 0.45 | 0.01 | 0.53 | | |
| 3 | Duncan, BC | *L. infuscatus* | 0.00 | 0.00 | 0.52 | 0.48 | | |
| | | *A. lineatus* | 0.02 | 0.44 | 0.01 | 0.53 | | |
| Study 2 | | | | | | | | |
| 4 | Kelowna, BC | *L. canus* | 0.06 | 0.07 | 0.31 | 0.25 | 0.02 | 0.29 |
| | | *L. californicus* | 0.01 | 0.00 | 0.43 | 0.28 | 0.00 | 0.28 |
| | | *L. infuscatus* | 0.00 | 0.01 | 0.35 | 0.30 | 0.00 | 0.34 |
| | | *A. lineatus* | 0.00 | 0.54 | 0.02 | 0.44 | 0.00 | 0.00 |
| | | *A. obscurus* | 0.00 | 0.00 | 0.00 | 0.00 | 0.71 | 0.29 |
| 5 | Pemberton, BC | *L. canus* | 0.05 | 0.05 | 0.19 | 0.53 | 0.09 | 0.09 |
| | | *A. lineatus* | 0.00 | 0.53 | 0.00 | 0.45 | 0.01 | 0.01 |
| | | *A. obscurus* | 0.00 | 0.00 | 0.01 | 0.00 | 0.61 | 0.37 |
| Study 3 | | | | | | | | |
| 6 | Saint-Mathieu-de-Beloeil, QC | *A. mancus* | 0.00 | 0.63 | 0.00 | 0.37 | | |
| | | *A. pubescens* | 0.00 | 0.56 | 0.01 | 0.43 | | |
| | | *A. sputator* | 0.00 | 0.57 | 0.00 | 0.43 | | |

[a]We have no explanation as to why the *A. lineatus* lure captured some male *L. canus*.

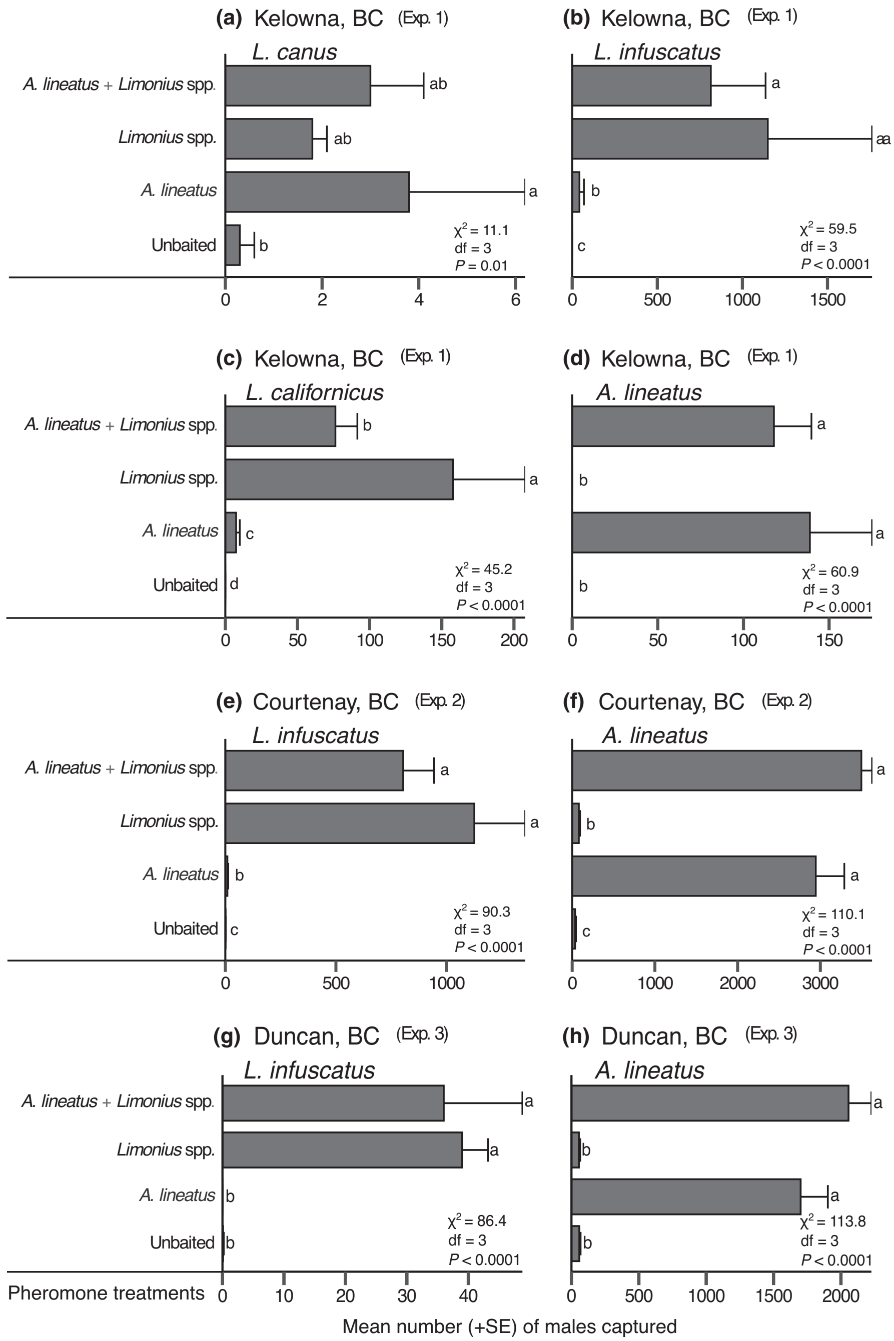



**FIGURE 2** Mean (+SE) captures of three *Limonius* spp. and *Agriotes lineatus* in traps baited with *Limonius* spp. pheromone ((*E*)-4-ethyloct-4-enoic acid (limoniic acid); 4 mg), *A. lineatus* pheromone (geranyl octanoate & geranyl butanoate at a 1:1 ratio; 40 mg) or both (2 mg limoniic acid +20 mg *A. lineatus* pheromone). For each of subpanels A–H, bars with different letters indicate statistically significant differences between in trap captures ($\chi^2$ tests); low capture data of *A. obscurus* in Kelowna, BC (<2 mean beetle captures per treatment) were not statistically analysed and did not warrant graphical illustrations.

(Figure 3k)]. Male *A. obscurus* were captured only in traps baited with either the *A. obscurus* pheromone alone or in combination with limoniic acid but capture data were too low to warrant analysis.

Overall, the data reveal no significant differences in captures of *L. infuscatus* or *A. lineatus* when traps were baited with either the species-specific pheromone alone or in combination with the heterogeneric pheromone (Figure 2b,d–h). Trap captures of *A. lineatus* increased >15% when limoniic acid was added to the *A. lineatus* pheromone (Figure 2; Exps. 2 & 3). Significantly more *L. infuscatus* males and *A. lineatus* males were captured in traps baited with the *A. lineatus* pheromone and with limoniic acid, respectively, than in unbaited control traps (Figure 2; Exp. 2).

## 3.2 | Trapping in Eastern Canada

Predominantly *A. mancus* males but also some *A. pubescens* and *A. sputator* males were captured in traps baited with the *A. mancus* pheromone (Figure 4; Exp. 6). Mixing the *A. mancus* pheromone with limoniic acid significantly reduced captures of *A. mancus* males capture ratio of *A. mancus* pheromone to mixed lure: 1.70 (Figure 4p) but not of *A. pubescens* and *A. sputator* males [capture ratio: 1.30 and 1.33 for *A. pubescens* and *A. sputator* (Figure 4q,r), respectively. Captures of the three *Agriotes* species in traps baited with limoniic acid and left unbaited did not differ (Figure 4)]. Captures of *L. agonus* beetles were too low for analysis.

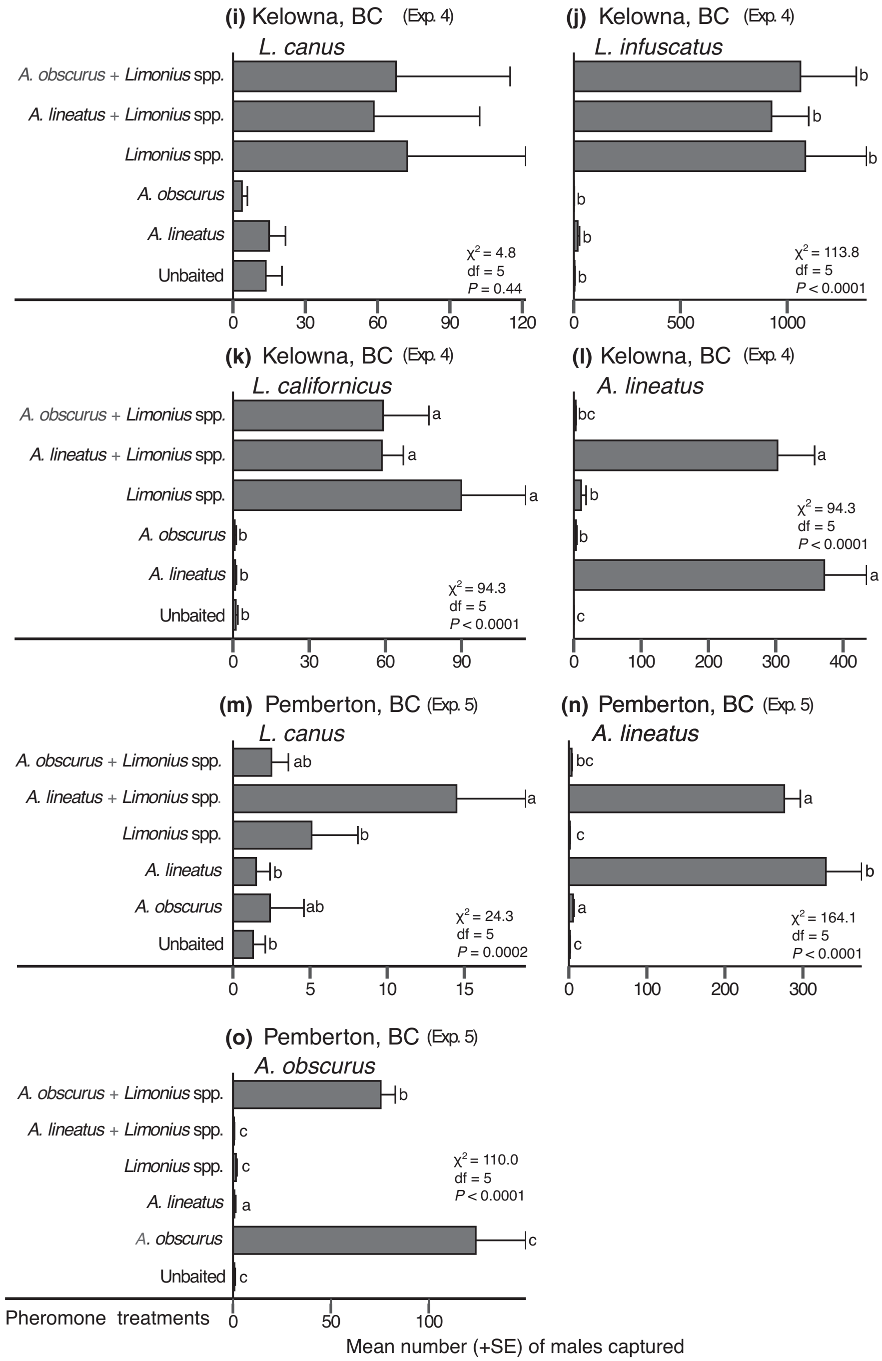


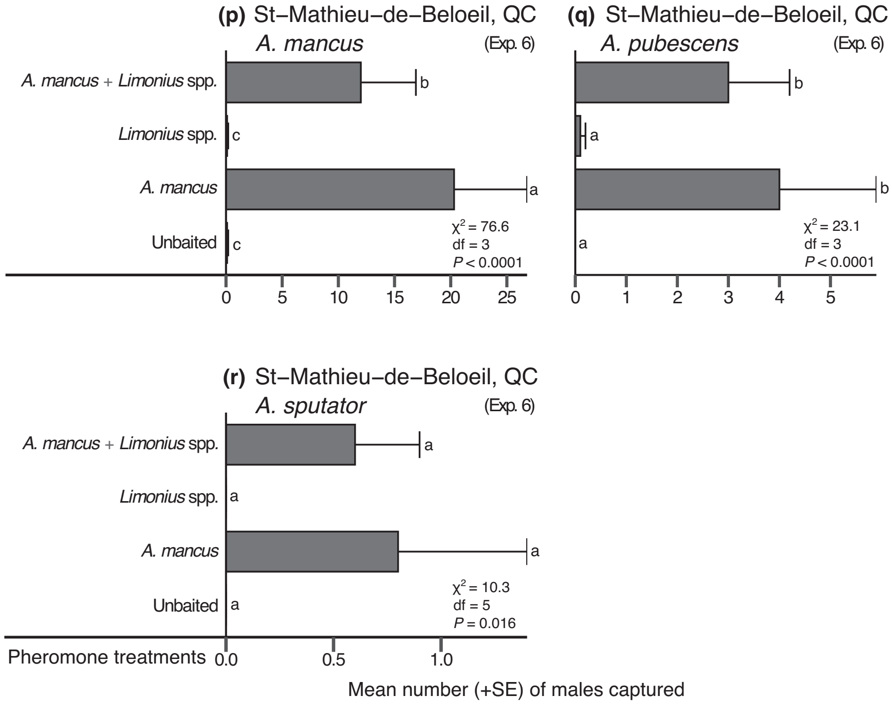

**FIGURE 3** Mean (+SE) captures of three *Limonius* spp. and two *Agriotes* spp. in traps baited with *Limonius* spp. pheromone ((*E*)-4-ethyloct-4-enoic acid (limoniic acid); 4 mg), *Agriotes lineatus* pheromone (geranyl octanoate & geranyl butanoate at a 1:1 ratio; 40 mg), *A. obscurus* pheromone (geranyl hexanoate & geranyl octanoate at a 1:1 ratio; 40 mg), and binary combinations of limoniic acid (2 mg) and either *A. lineatus* pheromone (20 mg) or *A. obscurus* pheromone (20 mg). For each of subpanels I–O, bars with different letters indicate statistically significant differences between trap captures ($\chi^2$ tests); low capture data of *A. obscurus* in Kelowna, BC (<2 mean beetle captures per treatment) were not statistically analysed and did not warrant graphical illustrations.

## 4 | DISCUSSION

In western Canada, mixed trap lures containing both *Agriotes* and *Limonius* pheromones were as effective as species-specific lures in attracting and capturing male *Limonius canus*, *L. californicus*, *L. infuscatus*, and either *Agriotes lineatus* or *A. obscurus*. In eastern Canada, mixed trap lures containing both *Limonius and Agriotes mancus* pheromones attracted 40% fewer male *A. mancus* than the *A. mancus* specific lure, whereas lure type (mixed or species-specific) did not alter captures of *A. pubescens*. As *L. agonus* males were nearly absent from the trapping location in eastern Canada, the effect of lure type on attraction and capture of *L. agonus* males could not be determined. The effect of mixed lures containing (*i*) the pheromones of all three eastern *Agriotes* spp. and limoniic acid, or (*ii*) the pheromones of *A. pubescens* and *A. sputator* and limoniic acid on attraction and captures of all target species including *L. agonus*, has yet to be determined. Overall, our data suggest that mixed pheromone lures can be developed for monitoring or mass trapping sympatric elaterid heterogeners, such as *A. lineatus* and *L. infuscatus* in the Interior of BC or on Vancouver Island. Compared to species-specific lures, mixed pheromone lures would require fewer traps and reduce the costs for lure preparation and trap deployment.

Prior to deploying mixed pheromone lures, multiple interrelated factors must be considered to optimize trapping efficiency,

**FIGURE 4** Mean (+SE) captures of two *Agriotes* spp. in traps baited with *Limonius* spp. pheromone ((*E*)-4-ethyloct-4-enoic acid (limoniic acid); 4 mg), *Agriotes mancus* pheromone (geranyl hexanoate & geranyl butanoate at a 1:1 ratio; 40 mg) or both (2 mg limoniic acid +20 mg *A. mancus* pheromone). For each of subpanels P–R, bars with different letters indicate statistically significant differences between in trap captures ($\chi^2$ tests); low capture data of *Limonius agonus* (<2 mean beetle captures per treatment) were not statistically analysed and did not warrant a graphical illustration.




including dispenser type (e.g.; rubber septa, bubble caps, cotton pellet), lure dose, lure longevity and attractive range, as well as the mobility and activity period of target elaterid pests. The optimal dose for attraction of beetles would need to be determined for each specific dispenser type. Limoniic acid dispensed at a dose of 0.4–4 mg from a cotton pellet inside a low-density-polyethylene receptacle is highly attractive to *Limonius* species (van Herk, Lemke, Gries, et al., 2021), whereas a 40-mg lure dose optimally attracts *A. lineatus* and *A. obscurus* (van Herk unpublished). Both dispenser type and lure dose affect lure longevity which is particularly important for trapping beetles with separate, non-overlapping seasonal activity periods. The attractiveness range for *A. lineatus* lures is 5–20 m (Traugott et al., 2015) but is still unknown for *Limonius* spp. The distance males cover in search for mates is still largely unknown but could be determined in mark-release-recapture studies.

Low captures of male *A. obscurus* and *L. agonus* in various experiments did not allow us to assess whether lure type (mixed or species-specific) affects beetle capture rates. The lack of *A. obscurus* captures at two study sites was likely due to low abundance of *A. obscurus* rather than repellency caused by nearby *A. lineatus* lures, because traps baited with *A. obscurus* or *A. lineatus* pheromone lures can be placed as little as 3 m apart without compromising the attractiveness of either lure (Vernon, van Herk, & Tanaka, 2014). Low captures of male *L. agonus* could be attributed to underestimated beetle abundance in the particular trapping site, or to traps being deployed too late in the season. The onset and duration of seasonal swarming by *L. agonus* are still not known but may be short and take place as soon as soil temperatures rise above 10°C (van Herk et al., unpublished), as shown for western *Limonius* spp. (Lemke et al., 2022; van Herk, Lemke, Gries, et al., 2021).

Understanding lure dose-dependent attraction of target elaterids is essential for the preparation of optimally effective mixed pheromone lures. Although a 40-mg lure dose is currently used to delineate the distribution range of *A. mancus* in eastern Canada (van Herk unpubl.), the optimal lure dose has yet to be determined. Our findings that mixed lures containing both limoniic acid and *A. mancus* pheromone attracted 40% fewer male *A. mancus* than the *A. mancus* specific lure could be due to the lower amount of *A. mancus* pheromone in the mixed lure which may have reduced lure longevity, even though ester release rates from both types of lures were identical for at least the first 7 days. Follow-up studies should compare the attractiveness of mixed and species-specific lures when the amount of *A. mancus* pheromone is kept identical in either lure type. But even if mixed-pheromone lures for concurrent attraction of multiple species are somewhat less attractive for some target species than single-species pheromone lures (e.g., *L. californicus* in Kelowna; Figure 1; *A. mancus* in Quebec; Figure 4), mixed-pheromone lures could still be operationally viable. Diminished attractiveness of mixed-pheromone lures may be tolerable as long as trap captures reliably indicate species presence and threshold numbers that would trigger control measures.

Attraction of both male *A. pubescens* and male *A. sputator* to the *A. mancus* pheromone blend can be attributed to geranyl butanoate in that blend which is a pheromone component of both *A. sputator* (Singleton, van Herk, et al., 2022; Tóth, 2013) and *A. pubescens* (van Herk, unpubl), and of several other *Agriotes* species (Toth et al., 2008; Vuts et al., 2018).

In conclusion, our study provides proof of concept that mixed pheromone lures containing synthetic pheromones of elaterid heterogeners can be as effective as species-specific lures in attracting target species. While here we tested mixed pheromone lures comprising binary combinations of *Agriotes* and *Limonius* pheromones, many other binary or even ternary combinations of heterogeneric elaterid pheromones are conceivable for testing in future studies. If successful, mixed pheromone lures could be developed for cost-effective integrated elaterid pest management.

## AUTHOR CONTRIBUTIONS

**Emily Lemke:** Conceptualization; data curation; supervision; formal analysis; investigation; methodology; visualization; writing – original draft; writing – review and editing. **Willem G. van Herk:** Conceptualization; data curation; supervision; formal analysis; funding acquisition; investigation; writing – original draft; methodology; project administration; resources. **Kendal Singleton:** Investigation. **Julien Saguez:** Investigation. **Graeme Fowler:** Investigation. **Doug Pepper:** Investigation. **Kathleen Furtado:** Investigation. **Gerhard Gries:** Conceptualization; funding acquisition; methodology; project administration.

## ACKNOWLEDGEMENTS

We thank Russ Alcock (Sunshine Farms), Jordan Marr (Unearthed Farms), Harvey and Joe Wikkerink (Blue Diamond farms), Jim Casanave Jr. and Sr. (Casawood farms), Mark Kuurne (Kuurne farms Inc.), and John Beks (Shaw Creek farms) for access to their land in Kelowna, Duncan, Courtenay and Pemberton, BC. Tracy Hueppelsheuser for coordinating field sites, Emily Carmichael and Angela Boss for assistance in running studies on Vancouver Island, Alyssa Moller, Haley Schonhofer, Gaby Bourassa-Tait and Corey Lemke for assistance in beetle processing, and Frank Etzler for his invaluable and extensive help with *Limonius* identifications.

## FUNDING INFORMATION

This research was supported by an Agriculture and Agri-Food Canada (AAFC) Canadian Agricultural Partnership Cluster Project (Developing IPM tools for wireworm management in Canada) administered by the Canadian Horticulture Council and supported by the Potato Growers of Alberta, the BC Potato Industry Development Committee, and the BC Lower Mainland Horticultural Improvement Association.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in 'zenodo' at https://doi.org/10.5281/zenodo.7957907.

## ORCID

*Emily Lemke* https://orcid.org/0000-0002-6680-5237
*Willem G. van Herk* https://orcid.org/0000-0001-5412-8745
*Kendal Singleton* https://orcid.org/0000-0002-9366-8004
*Julien Saguez* https://orcid.org/0000-0002-8430-5842
*Gerhard Gries* https://orcid.org/0000-0003-3115-8989

## REFERENCES

Al Dhafer, H. M. (2009). Revision of the north American species of *Limonius* (Coleoptera: Elateridae). *Transactions of the American Entomological Society*, *135*, 209–352. https://doi.org/10.3157/061.135.0301

Arakaki, N., Nagayama, A., Kobayashi, A., Kishita, M., Sadoyama, Y., Mougi, N., Kawamura, F., Wakamura, S., & Yamamura, K. (2008). Control of the sugarcane click beetle *Melanotus okinawensis* Ohira (Coleoptera: Elateridae) by mass trapping using synthetic sex pheromone on Ikei Island, Okinawa, Japan. *Applied Entomology and Zoology*, *43*, 37–47. https://doi.org/10.1303/aez.2008.37

Begg, J. A. (1959). Control of the eastern field wireworm, *Limonius agonus* (say), in early potatoes in Ontario by application of insecticides to the soil. *Canadian Journal of Plant Science*, *39*, 342–349. https://doi.org/10.4141/cjps59-046

Brockerhoff, E. G., Suckling, D. M., Roques, A., Jactel, H., Branco, M., Twidle, A. M., Mastro, V. C., & Kimberley, M. O. (2013). Improving the efficiency of lepidopteran pest detection and surveillance: Constraints and opportunities for multiple-species trapping. *Journal of Chemical Ecology*, *39*, 50–58. https://doi.org/10.1007/s10886-012-0223-6

Etzler, F. E. (2013). Identification of economic wireworms using traditional and molecular methods. M.Sc. thesis, Montana State University-Bozeman, Montana.

Fan, J. T., Denux, O., Courtin, C., Bernard, A., Javal, M., Millar, J. G., Hanks, L. M., & Roques, A. (2019). Multi-component blends for trapping native and exotic longhorn beetles at potential points-of-entry and in forests. *Journal of Pest Science*, *92*, 281–297. https://doi.org/10.1007/s10340-018-0997-6

Furlan, L., Contiero, B., Chiarini, F., Benvegnù, I., & Tóth, M. (2020). The use of click beetle pheromone traps to optimize the risk assessment of wireworm (Coleoptera: Elateridae) maize damage. *Scientific Reports*, *10*, 1–12. https://doi.org/10.1038/s41598-020-64347-z

Gries, R., Alamsetti, S. K., van Herk, W. G., Catton, H., Meers, S., Lemke, E., & Gries, G. (2021). Limoniic acid – Major sex pheromone component of the click beetles *Limonius canus* and *L. californicus*. *Journal of Chemical Ecology*, *47*, 123–133. https://doi.org/10.1007/s10886-020-01241-y

Gries, R., van Herk, W., Alamsetti, S. K., Catton, H., Meers, S., Otani, J., & Gries, G. (2022). (*Z*, *E*)-α-Farnesene – Sex pheromone component of female click beetle *Selatosomus aeripennis destructor* with intra-and inter-sexual communication function. *Entomologia Experimentalis et Applicata*, *170*, 344–351. https://doi.org/10.1111/eea.13142

Howse, P. E., Stevens, I. D. R., & Jones, O. T. (1998). *Insect pheromones and their use in pest management*. Chapman & Hall. https://doi.org/10.1007/978-94-011-5344-7

Jakubowska, M., Bocianowski, J., & Nowosad, K. (2018). Seasonal fluctuation of *Agriotes lineatus*, *A. obscurus* and *A. sputator* click beetles caught using pheromone traps in Poland. *Plant Protection Science*, *54*, 118–127. https://doi.org/10.17221/39/2016-PPS

Johnson, P. J. (2002). Elateridae. In R. H. Arnett, M. C. Thomas, P. E. Skelley, & J. H. Frank (Eds.), *American beetles* (Vol. *2*, pp. 160–173). CRC Press LLC.

Jones, B.C., Roland, J. &, Evenden, M.L. (2009). Development of a combined sex pheromone-based monitoring system for *Malacosoma disstria* (Lepidoptera: Lasoicampidae) and *Choristoneura conflictana* (Lepidoptera: Tortricidae). *Environmental Entomology*, *38*, 459–471. https://doi.org/10.1603/022.038.0220

Kim, J., Lim, E., Roh, H. S., Cho, Y. S., & Park, C. G. (2015). A trap baited with multiple pheromones attracts sympatric hemipteran pests of sweet persimmon. *Journal of Asia-Pacific Entomology*, *18*, 465–470. https://doi.org/10.1016/j.aspen.2015.06.001

Lemke, E., van Herk, W. G., Singleton, K. S., & Gries, G. (2022). Seasonal and diel communication periods of sympatric pest *Limonius* click beetle species (Coleoptera: Elateridae) in western Canada. *Journal of Environmental Entomology*, *51*, 980–988. https://doi.org/10.1093/ee/nvac067

Levesque, C., & Levesque, G. Y. (1993). Abundance and seasonal activity of Elateroidea (Coleoptera) in a raspberry plantation and adjacent sites in southern Quebec, Canada. *The Coleopterists Bulletin*, *47*, 269–277.

Milosavljević, I., Esser, A. D., & Crowder, D. W. (2016). Effects of environmental and agronomic factors on soil-dwelling pest communities in cereal crops. *Agriculture, Ecosystems & Environment*, *225*, 192–198. https://doi.org/10.1016/j.agee.2016.04.006

Nakamuta, K., Leal, W. S., Nakashima, T., Tokoro, M., Ono, M., & Nakanishi, M. (1997). Increase of trap catches by a combination of male sex pheromones and floral attractant in longhorn beetle, *Anaglyptus subfasciatus*. *Journal of Chemical Ecology*, *23*, 1635–1640. https://doi.org/10.1023/B:JOEC.0000006427.56337.6c

Preti, M., Knight, A. L., & Angeli, S. (2020). Improved monitoring of *Grapholita molesta* (Lepidoptera: Tortricidae) in stone fruit orchards with a pheromone-kairomone combination lure. *Insects*, *11*, 412. https://doi.org/10.3390/insects11070412

Reddy, G. V. P., & Tangtrakulwanich, K. (2014). Potential application of pheromones in monitoring, mating disruption, and control of click beetles (Coleoptera: Elateridae). *ISRN Entomology*, *2014*, 1–8. https://doi.org/10.1155/2014/531061

Rice, M. E., Zou, Y., Millar, J. G., & Hanks, L. M. (2020). Complex blends of synthetic pheromones are effective multi-species attractants for longhorned beetles (Coleoptera: Cerambycidae). *Journal of Economic Entomology*, *113*, 2269–2275. https://doi.org/10.1093/jee/toaa157

Saguez, J., Latraverse, A., De Almeida, J., van Herk, W. G., Vernon, R. S., Légaré, J.-P., Moisan-De Serres, J., Fréchette, M., & Labrie, G. (2017). Wireworm in Quebec field crops: Specific community composition in North America. *Environnemental Entomology*, *46*, 814–825. https://doi.org/10.1093/ee/nvx116 PMID: 28881953.

Serrano, J. M., Collignon, R. M., Zou, Y., & Millar, J. G. (2018). Identification of sex pheromones and sex pheromone mimics for two north American click beetle species (Coleoptera: Elateridae) in the genus *Cardiophorus* Esch. *Journal of Chemical Ecology*, *44*, 327–338. https://doi.org/10.1007/s10886-018-0940-6

Singleton, K., Gries, R., van Herk, W. G., Alamsetti, S. K., Lemke, E., Furtado, K., & Gries, G. (2022). Identification of the major sex pheromone component of the click beetle *Agriotes ferrugineipennis*. *Journal of Chemical Ecology*, *48*, 491–501. https://doi.org/10.1007/s10886-022-01367-1

Singleton, K., van Herk, W. G., Saguez, J., Douglas, H. B., & Gries, G. (2022). First record of the invasive wireworm *Agriotes sputator* Linnaeus, 1758 (Coleoptera: Elateridae) in Quebec, Canada, and implications of its arrival. *The Pan-Pacific Entomologist*, *98*, 184–187. https://doi.org/10.3956/2022-98.3.184

Singleton, K., van Herk, W. G., Saguez, J., Scott, I., Gries, R., & Gries, G. (2023). Sex pheromone of Nearctic *Agriotes mancus*, and its similarity to that of three Palearctic *Agriotes* invasive in North America. *Agriculture and Forest Entomology*, *25*, 468–476. https://doi.org/10.1111/afe.12568

Tóth, M. (2013). Pheromones and attractants of click beetles: An overview. *Journal of Pest Science*, *86*, 3–17. https://doi.org/10.1007/s10340-012-0429-y

Toth, M., Furlan, L., Xavier, A., Vuts, J., Toshova, T., Subchev, M., Szarukan, I., & Yatsynin, V. (2008). New sex attractant composition for the click beetle *Agriotes proximus*: Similarity to the pheromone of *Agriotes lineatus*. *Journal of Chemical Ecology*, *34*, 107–111. https://doi.org/10.1007/s10886-007-9398-7

Traugott, M., Benefer, C. M., Blackshaw, R. P., van Herk, W. G., & Vernon, R. S. (2015). Biology, ecology, and control of elaterid beetles in agricultural land. *Annual Review Entomology*, *60*, 313–334. https://doi.org/10.1146/annurev-ento-010814-021035

van Herk, W. G., Gries, R., Smith, J., Scott, I., & Gries, G. (2023). Limoniic acid is a sex pheromone component of *Limonius agonus* (Coleoptera: Elateridae). *The Great Lakes Entomologiste* in review.

van Herk, W. G., Lemke, E., Gries, G., Gries, R., Serrano, J. M., Catton, H., Wanner, K., Landolt, P. J., Cooper, W. R., Meers, S., Nikoukar, A., Smith, J., Alamsetti, S. K., & Etzler, F. E. (2021). Limoniic acid and its analog as trap lures for pest *Limonius* species (Coleoptera: Elateridae) in North America. *Journal of Economic Entomology*, *114*, 2108–2120. https://doi.org/10.1093/jee/toab154

van Herk, W. G., Vernon, R. S., Acheampong, S., Otani, J. K., & Uloth, K. (2021). Distribution of two European elaterids, *Agriotes obscurus* and *A. lineatus* in British Columbia: New records, and potential implications of their dispersal. *Journal of Asia-Pacific Entomology*, *24*, 688–694. https://doi.org/10.1016/j.aspen.2021.06.001

van Herk, W. G., Vernon, R. S., Borden, J. H., Ryan, K., & Mercer, G. (2022). Comparative evaluation of pitfall traps for click beetles (Coleoptera: Elateridae). *Journal of Economic Entomology*, *115*, 582–591. https://doi.org/10.1093/jee/toab259

van Herk, W. G., Vernon, R. S., Bourassa-Tait, G., Tóth, M., & Kovacs, E. (2022). Field evaluation of selected plant volatiles and conspecific pheromones as attractants for *Agriotes obscurus* and *A. lineatus* (Coleoptera: Elateridae). *Insects*, *13*, 173. https://doi.org/10.3390/insects13020173

van Herk, W. G., Vernon, R. S., Labun, T. J., Sevcik, M. H., & Schwinghamer, T. D. (2021). Distribution of pest wireworm (Coleoptera: Elateridae) species in Alberta, Saskatchewan, and Manitoba (Canada). *Journal of Environmental Entomology*, *50*, 663–672. https://doi.org/10.1093/ee/nvab006

Vernon, R. S., Blackshaw, R. P., van Herk, W. G., & Clodius, M. (2014). Mass trapping wild *Agriotes obscurus* and *Agriotes lineatus* males with pheromone traps in a permanent grassland population reservoir. *Agricultural and Forest Entomology*, *16*, 227–239. https://doi.org/10.1111/afe.12058

Vernon, R. S., & van Herk, W. G. (2022). Wireworms as pests of potatoes. In P. Giordanengo, C. Vincent, & A. Alyohkin (Eds.), *Insect pests of potato: Global perspectives on biology and management* (2nd ed., pp. 103–164). Academic Press.

Vernon, R. S., van Herk, W. G., & Tanaka, J. A. (2014). Blending of pheromone lures for two exotic European pest elaterid beetles. *Journal of Pest Science*, *87*, 619–627. https://doi.org/10.1007/s10340-014-0596-0

Vuts, J., Furlan, L., & Tóth, M. (2018). Female responses to synthetic pheromone and plant compounds in *Agriotes brevis* Candeze (Coleoptera: Elateridae). *Journal of Insect Behaviour*, *31*, 106–117. https://doi.org/10.1007/s10905-018-9668-y

Waterworth, R. A., Redak, R. A., & Millar, J. G. (2011). Pheromone-baited traps for assessment of seasonal activity and population densities of mealybug species (Hemiptera: Pseudococcidae) in nurseries producing ornamental plants. *Journal of Economic Entomology*, *104*, 555–565. https://doi.org/10.1603/EC10317

Williams, L., Serrano, J. M., Johnson, P. J., & Millar, J. G. (2019). 13-Tetradecenyl acetate, a female-produced sex pheromone component of the economically important click beetle *Melanotus communis* (Gyllenhal) (Coleoptera: Elateridae). *Scientific Reports*, *9*, 16197. https://doi.org/10.1038/s41598-019-52199-1

Wong, J. C., Mitchell, R. F., Striman, B. L., Millar, J. G., & Hanks, L. M. (2012). Blending synthetic pheromones of cerambycid beetles to develop trap lures that simultaneously attract multiple species. *Journal of Economic Entomology*, *105*, 906–915. https://doi.org/10.1603/EC11434

Yasuda, T., Oku, K., Higuchi, H., Suzuki, T., Kashin, J., Kichishima, T., Watanabe, T., Takeda, A., Yasuda, M., Tabuchi, K., & Takahashi, A. (2010). A multi-species pheromone lure: A blend of synthetic sex pheromone components for two mirid species, *Stenotus rubrovittatus* (Matsumura) and *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridae). *Applied Entomology and Zoology*, *45*, 593–599. (In Japanese). https://doi.org/10.1303/aez.2010.593

**How to cite this article:** Lemke, E., van Herk, W. G., Singleton, K., Saguez, J., Fowler, G., Pepper, D., Furtado, K., & Gries, G. (2023). Mixed sex pheromone lures for combined captures of *Agriotes* and *Limonius* pest click beetles in North America. *Journal of Applied Entomology*, *147*, 592–601. https://doi.org/10.1111/jen.13158