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Limoniic Acid is a Sex Attractant Pheromone Component of *Limonius agonus* (Coleoptera: Elateridae)

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Abstract

Recently, (*E*)-4-ethyloct-4-enoic acid (limoniic acid) has been reported as the major sex attractant pheromone component of *Limonius canus* LeConte and *Limonius californicus* (Mannerheim) (Coleoptera: Elateridae) in western North America. Our objective was to determine whether limoniic acid is also a sex attractant pheromone component of the eastern field wireworm, *Limonius agonus* (Say). In gas chromatographic-electroantennographic detection (GC-EAD) analyses of headspace volatiles from *L. agonus* females, antennae from male *L. agonus* responded to limoniic acid as a trace component. In field experiments, traps baited with synthetic limoniic acid, or its analog (*E*)-5-ethyloct-4-enoic acid, afforded captures of male *L. agonus* 3.6- to 8.9-times greater than unbaited control traps. In long-term field trapping studies, emergence and captures of *L. agonus* males fluctuated with temperature for more than 5 weeks, with distinctively different emergence patterns at the two study sites. Compared to previous studies with *L. canus* and *L. californicus*, limoniic acid as a trap lure afforded relatively low captures of *L. agonus* males, suggesting that *L. agonus* populations were low or that other *L. agonus* pheromone components are yet to be identified.

Keywords Click beetles, wireworms, *Limonius agonus*, sex pheromone, integrated pest management

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Research to identify the pheromones of pestiferous click beetle (Coleoptera: Elateridae) species native to North America has only recently resumed after several decades of limited activity. Sex pheromones have now been identified for one or more species of *Agriotes, Cardiophorus, Idolus, Limonius, Melanotus, Parallelostethus,* and *Selatosomus* (Serrano et al. 2018, 2022; Williams et al. 2019; Gries et al. 2021, 2022; Millar et al. 2022; Singleton et al. 2022a, 2023). Deployment of synthetic sex pheromones for these species will allow pest management professionals to determine the presence and relative abundance of native pest elaterids, map their distributions, and eventually, when used in mass trapping or mating disruption tactics, reduce populations of crop-damaging larvae (Blackshaw and Vernon 2008, Tóth 2013, Vernon et al. 2014, Traugott et al. 2015, Vernon and van Herk 2022).

The sex attractant pheromone (*E*)-4-ethyloct-4-enoic acid ('limoniic acid') of *Limonius canus* LeConte and *Limonius californicus* (Mannerheim) and its analog, (*E*)-5-ethyloct-4-enoic acid, attracted not only male beetles of these two species, but also males of the eastern field wireworm *Limonius agonus* (Say) (Gries et al. 2021, van Herk et al. 2021a), suggesting that female *L. agonus* may also produce a sex attractant pheromone similar or identical to limoniic acid. This inference was supported by electrophysiological recordings showing that antennae of male *L. agonus* strongly responded to the pheromone analog (van Herk et al. 2021a). However, potential electrophysiological responses of male *L. agonus* to limoniic acid and its production by female *L. agonus* have never been investigated.

Limonius agonus is an important pest in central Canada and the northeastern United States (Rawlins 1940, Glen et al. 1943, Lanchester 1946, Vernon and van Herk 2022). In a recent survey (2014–2017) in southern Ontario, *L. agonus* was the predominant species of elaterids of economic importance to agriculture, being present in

45% of all samples collected from more than 1,300 locations (Smith et al., unpublished). In a similar survey (2011–2015) in southern Quebec, *L. agonus* was the main *Limonius* spp. collected (Saguez et al. 2017). The abundance of adult beetles is monitored to assess the risk of wireworm damage in subsequent years (Vernon and van Herk 2018, Furlan et al. 2020, Poggi et al. 2021), and to inform potential control measures (Vernon and van Herk 2022).

The life history, ecology, and seasonal behaviour of *L. agonus* (Begg 1956, 1962; Kring 1957, 1959) resemble those of several other elaterid pests in Canada such as *Agriotes mancus* (Say), *Agriotes lineatus* (L.), and *L. canus*. *Limonius agonus* has a 4- to 5-year life cycle, with larvae spending multiple years underground. Similar to other elaterids, the long-lived subterranean larvae ('wireworms') of *L. agonus* cause economic damage by feeding on seedlings, tubers, and other underground plant parts. Larvae may also need to feed on insects to complete their development (Kring 1959), and seem to preferentially inhabit fine, sandy soils (Rawlins 1940, Kring 1957). Their preference for sandy soil has made them serious pests of tobacco (Kulash 1943). Larvae move up and down the soil profile in response to food availability and soil temperature and moisture, overwintering below the frost level (Olson 1946, Begg 1956) and pupating in late-July and early-August. Adults overwinter and emerge in late April and early May (in western New York and Connecticut; Lacroix 1935, Hawkins 1936, Olson 1946).

Research on *L. agonus* has focussed on evaluating the efficacy of insecticides (e.g., Begg 1959) but the behaviour, ecology, and communication biology of *L. agonus* remain largely unknown. Here, we report limoniic acid as a sex attractant pheromone component of *L. agonus*.

Materials and Methods

Collection of beetles and headspace volatiles for pheromone identification. In April 2022, four male and two female *L. agonus* were collected at a hoop house on an organic vegetable farm in St. Thomas, Ontario (42.711452, –81.107655). For pheromone analysis, beetles were shipped to Simon Fraser University (SFU, Burnaby, BC) in 50-mL Falcon tubes containing a few blades of grass and a small piece of moistened tissue paper. A shipping permit was not required because *L. agonus* is not a quarantine pest and insects were shipped between provinces within Canada. Volatiles were collected following a protocol previously detailed (Gries et al. 2021). Briefly, at SFU, female beetles were placed into a Pyrex® glass chamber (8 cm high × 8 cm diameter) fitted with a moist cotton wick (Richmond Dental, Charlotte, NC, USA) as a source of water and walk-on substrate. A mechanical pump (Neptune Dyna-pump, Model 2, Dover, NJ, USA) drew charcoal-filtered air at a flow rate of 0.5 L · min^{-1} for 24 h through the chamber and through a glass column (6 mm outer diameter × 150 mm) containing 200 mg of manufacturer-preconditioned Porapak-Q™ adsorbent (50–80 mesh; Waters Associates, Milford, MA, USA). The Porapak-Q volatile trap was desorbed with pentane:ether (2 mL, 50:50) and concentrated to 100 µL for analyses.

Gas chromatography with electroantennographic detection (GC-EAD) analyses. Aliquots of Porapak-Q extracts were analyzed by GC-EAD, with equipment and procedures previously detailed (Gries et al. 2002). Briefly, the GC-EAD setup employed a Hewlett-Packard 5890 gas chromatograph (GC) (Agilent Technologies Inc., Santa Clara, CA, USA) fitted with one of four GC columns (DB-5, DB-210, DB-23, FFAP; all 30 m × 0.32 mm ID; film thickness 0.25 µm; Agilent J & W column). Helium served as the carrier gas (35 cm · s^{-1}) with the following temperature programs: 50 °C for 1 min, then 20 °C · min^{-1} to 220 °C (DB-210, DB-23) or 280 °C (DB-5); 100 °C for 1 min, then 20 °C · min^{-1} to 180 °C (held for 15 min) (FFAP). The injector port and flame ionization detector (FID) were set to 260 °C and 280 °C, respectively. For each GC-EAD recording, an antenna—with its very tip ablated—was carefully dislodged from a male's head and suspended between two glass capillary electrodes (1.0 × 0.58 × 100 mm; A-M Systems, Carlsborg, WA, USA) prepared to accommodate the antenna and filled with a saline solution (Staddon and Everton 1980). Antennal responses to compounds in the column effluvium—that was directly released into a stream of medical air (250 mL · min^{-1} flow) continuously passing over the electrode-suspended antenna—were amplified with a custom-built amplifier and recorded on an HP 3392A integrator (Agilent Technologies Inc.). The amplifier was fitted with a low-pass filter (< 0.02 Hz) and a high-pass filter (> 10 kHz) to improve the signal-to-noise ratio, and with signals sent to the output terminal through a 100-Ω resistor.

GC-mass spectrometric (GC-MS) analyses. In headspace volatile extracts, compounds that elicited antennal responses were deemed candidate pheromone components (CPCs) and were identified, if possible, by GC-MS, using both a Varian Saturn 2000 Ion Trap GC-MS and a 5977 Series 96MDS (both Agilent Technologies Inc., Santa Clara, CA, USA) coupled to a 7890 GC. Both instruments were operated

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Table 1. Names and geographic coordinates of field study sites, as well as trapping periods at each site, established to test the effect of limoniic acid and an analog as trap lures on captures of *Limonius agonus* in southern Ontario, in 2021.

Site name	Site location (latitude, longitude)	Trapping period	No. beetles captured	Proportion male *L. agonus*
Dawn Mills	42.5938, –82.1146	09 April–17 June	2210	0.992
Ridgetown	42.4500, –81.8813	07 April–17 June	240	1.00
Rodney	42.5612, –81.6487	14–28 April	188	0.984

in full-scan electron ionization mode and fitted with a DB-5MS column (30 m × 0.25 mm ID; Agilent J&W GC), using helium as the carrier gas (35 cm $\cdot s^{-1}$). The injector port of both instruments was set to 250 °C, the Varian ion trap was at 200 °C, the Agilent Quadrupole and MS source were at 150 °C and 230 °C, respectively, and the transfer line of both instruments was set to 280 °C. The temperature program was as follows: 50 °C for 5 min, 10 °C $\cdot min^{-1}$ to 280 °C (held for 10 min). To identify CPCs in Porapak-Q headspace volatile extract, their retention indices (Van den Dool and Kratz 1963) and mass spectra were compared with authentic standards available from a previous study (Gries et al. 2021).

Source of chemicals. All chemicals needed for compound identification and field testing were available from a previous project (Gries et al. 2021).

Field trapping experiments. Candidate pheromone components were field tested in southern Ontario (2021) at three locations: Dawn Mills, Ridgetown, and Rodney (Table 1). Each experiment was set up in a complete randomized block design with eight replicates (blocks) and four treatments. Vernon Pitfall Traps® (Intko Supply, Chilliwack, BC, Canada) were placed at ground level along a field's edge, with 10-m spacing between treatments in each replicate, and with 10-m spacing between replicates. Traps were placed in early-mid April and checked weekly until late April (Rodney) or mid-June (Dawn Mills, Ridgetown) (Table 1). The trapping period at Rodney was terminated early as beetle numbers there appeared to be in decline, and as a long (1 hr) commute made the site inconvenient to access. Traps (all experiments) were either left unbaited (control treatment), or baited with limoniic acid (4 mg), the analog (4 mg), or both (2 mg each). A low 4-mg lure dose was chosen because it was highly attractive, and as attractive as a 0.4-mg lure dose, in a previous study with *L. californicus* and *Limonius infuscatus* (Mots.) (van Herk et al. 2021a). These compounds were pipetted onto 100% cotton pellets (size #0; Richmond Dental, Charlotte, NC, USA) placed inside of 1-mL low-density polyethylene (LDPE) containers (diameter: 8 mm, height: 32 mm; wall thickness: 0.98 mm; Kartell Labware, Noviglio, IT) which were closed and suspended from the roof of traps. Lures were prepared from the same batch of chemicals used in previous studies (Gries et al. 2021, van Herk et al. 2021a, Lemke et al. 2022), and were not replaced during the entire trapping period. Captured beetles were identified (Table 1) and their sex determined using a taxonomic key (Al Dhafer 2009, Etzler 2013). Voucher specimens are retained at the Agassiz Research and Development Centre (Agassiz, BC, CA). Weather data from the nearest weather station (Ridgetown RCS, World Meteorological organization ID: 71307; 42.4500°, –81.8833°) were used to determine the mean daily temperature and accumulated precipitation during the trapping periods. Soil temperature data were collected hourly until 13 May near the Ridgetown study site, using two probes (WatchDog A150, Spectrum Technologies Inc., Aurora, IL) placed approx. 100 m apart, at a 15-cm depth. Soil and air temperatures were not recorded at sites other than Ridgetown.

Data analyses. Data were analyzed 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. All analyses were performed on the total number of click beetles captured (collection dates, species, and sexes combined), using SAS Enterprise Guide v.7.1 (SAS Institute, Cary, NC, USA). Seasonal emergence patterns of beetles at Dawn Mills and Ridgetown were determined by taking the mean of the total number of beetles collected per replicate (treatments combined). The proportion of beetles collected before the midpoint of the collection period (i.e., 13 May) was compared between the two sites with a Chi-square test.

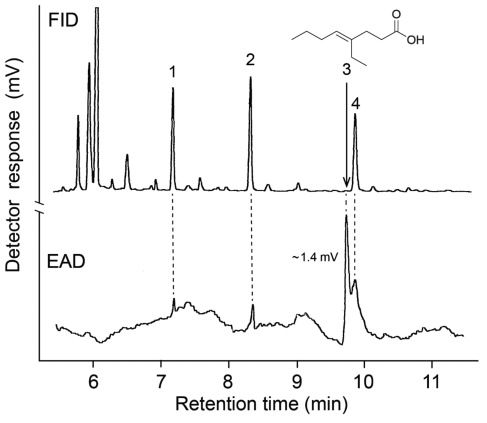

Figure 1. Representative responses of a gas chromatographic flame ionization detector (FID) and an electroantennographic detector (EAD: antenna of a male *Limonius agonus*) to an aliquot of Porapak-Q headspace volatile extract from conspecific females. Compounds were identified as octanoic acid (**1**), nonanoic acid (**2**), (*E*)-4-ethyloct-4-enoic acid (limoniic acid) (**3**), and decanoic acid (**4**). Chromatography: free fatty acid phase (FFAP) column (100 °C for 1 min, then 20 °C · min^{-1} to 180 °C (held for 15 min).

Results

Identification of candidate pheromone components. In GC-EAD analyses of extracts of headspace volatiles collected from female *L. agonus,* antennae from male beetles responded to multiple compounds but most strongly to a trace compound (**3** in Fig. 1), with retention characteristics identical to those of limoniic acid. As compound **3** occurred below detection threshold of the mass spectrometer and a mass spectrum of the compound could not be obtained, its molecular structure was ascertained by comparing retention times of antennal responses to female-produced compound **3** and to synthetic standards on each of four GC columns. Moreover, to unequivocally determine that female *L. agonus* indeed produce (*E*)-4-ethyl-4-oct-4-enoic acid, rather than (*Z*)- or (*E*)-5-ethyloct-4-enoic acid with retention indices identical to those of (*E*)-4-ethyloct-4-enoic acid on three out of four GC columns (see Table 1 in Gries et al. 2021), we also analyzed headspace volatile extract and synthetic (*E*)-4-ethyloct-4-enoic acid as well as (*Z*)- and (*E*)-5-ethyloct-4-enoic acid isothermally (120 °C). To improve the separation of compounds, a 10:1 split injection was used. On the DB 23 column, but not on any of the other three columns, (*E*)-4-ethyloct-4-enoic acid [retention time (RT): 16.36] was suffiently well separated from (*Z*)-5-ethyloct-4-enoic acid (RT: 16.16) and (*E*)-5-ethyloct-4-enoic (RT: 16.65), allowing us to determine that the antennae of *L. agonus* males responded to (*E*)-4-ethyloct-4-enoic acid in headspace volatile extracts of females. GC-MS analyses of EAD-active **1**, **2** and **4** (Fig. 1), and of authentic acids, revealed that they were octanoic acid (**1**), nonanoic acid (**2**) and decanoic acid (**4**).

Field experiments. As many as 2210, 240, and 188 click beetles were collected at the Dawn Mills, Ridgetown, and Rodney study sites, respectively, nearly all of which (>98%) were identified as male *L. agonus* (Table 1). Beetle captures differed significantly between treatments (Dawn Mills: $\chi^2 = 17.73$; df = 3,21; $P = 0.0006$; Ridgetown: $\chi^2 = 8.50$; df = 3,21; $P = 0.03$; Rodney: $\chi^2 = 11.85$; df = 3,21; $P = 0.0079$), and between replicates (Dawn Mills: $\chi^2 = 23.28$; df = 7,21; $P = 0.0015$; Ridgetown: $\chi^2 = 40.98$; df = 7,21; $P < 0.0001$; Rodney: $\chi^2 = 21.45$; df = 7,21; $P = 0.0032$). Traps baited with limoniic acid captured significantly more beetles than unbaited control traps at Dawn Mills [mean (SE): 75.6 (19.9) *versus* 19.5 (7.2)] and Ridgetown [8.9 (4.5) *versus* 1.0 (0.7)] but not at Rodney [4.5 (1.8) *versus* 1.3 (0.8)] (Fig. 2). At each study site, beetle captures in traps baited with limoniic acid or the analog did not differ ($P > 0.05$) (Fig. 2), but numerically analog-baited traps captured 1.2-times more beetles (Dawn Mills), 0.4-times fewer beetles (Ridgetown) and 2.1-times more beetles (Rodney) than limoniic acid-baited traps (Fig. 2). Captures in traps baited with both limoniic acid and the analog were consistently (but not significantly; $P > 0.05$) higher than in traps baited with limoniic acid alone (1.2-, 1.8-, and 1.8-times, respectively), were similar to those of analog-baited traps at Dawn Mills (1.1-times) and Rodney (0.8-times), and higher than those in analog-baited traps at Ridgetown (4.4-times) (Fig. 2).

Beetle emergence periods at Ridgetown and Dawn Mills differed ($\chi^2 = 328.7$; df = 1; $P < 0.0001$; Fig. 3). By 13 May (i.e., the 5th of 10 weekly collections), nearly all beetles (231/240 = 96%) had been collected at Ridgetown, whereas only 26% (570/2210) had been collected at Dawn Mills.

Due to warm air temperatures during the first 2 weeks of April, followed by 2-week periods of cold weather in both April and May, the mean daily soil temperatures at Ridgetown rose above 10 °C early in the season (i.e., 7–14 April), but did not reach this threshold again until 19, 27–29 April and 2–5, 7, and 13 May (Fig. 3C).

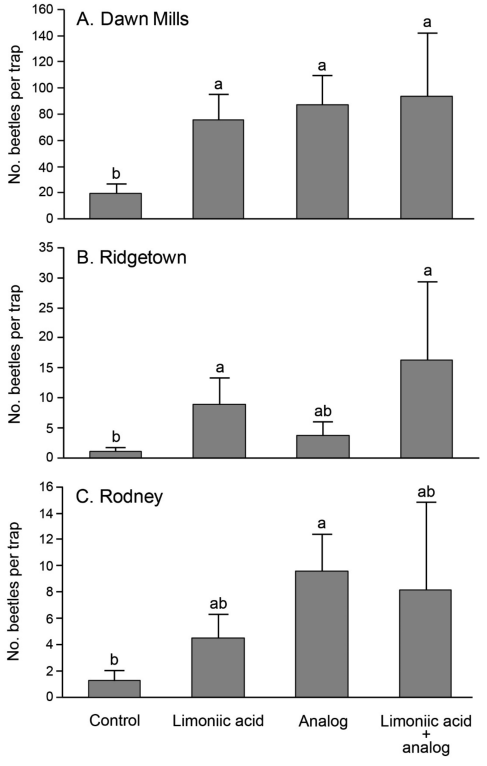

Figure 2. Mean (+ SEM) captures of male *Limonius agonus* in field experiments run near Dawn Mills (A), Ridgetown (B), and Rodney (C), in southern Ontario, in 2021. Four treatments were tested: (1) an unbaited (control); (2) (*E*)-4-ethyloct-4-enoic acid (limoniic acid), 4 mg; (3) (*E*)-5-ethyloct-4-enoic acid (an analog of limoniic acid), 4 mg; (4) limoniic acid (2 mg) *plus* analog (2 mg). Means with different letters indicate statistically significant differences in trap captures; P<0.05 (SAS Proc GENMOD, followed by Tukey's HSD).

Discussion

Our data show that limoniic acid is a sex attractant pheromone component of female *L. agonus*. The compound is produced by females, elicits antennal responses from males (Fig. 1), and limoniic acid-baited traps attract and capture male beetles in field settings (Fig. 2).

Males of *L. agonus* are also attracted to (*E*)-5-ethyloct-4-enoic acid, an analog of limoniic acid (Fig. 2). The relative attractiveness of limoniic acid and the analog varied with location, possibly due to varying population densities at the study sites. Surprisingly, the analog was least attractive at Ridgetown, where—in 2020—it was 2.1-times more attractive than limoniic acid (van Herk et al. 2021a). The attractiveness of limoniic acid and its analog also varied in field studies with the three western congeners *L. canus, L. californicus* and *L. infuscatus*, but limoniic acid was typically far more attractive than the analog (van Herk et al. 2021a). The same pattern does not seem to apply to *L. agonus*. The attraction of click beetles to compounds resembling their sex pheromone is intriguing, and was demonstrated previously for *Cardiophorus* spp. Males of both *C. edwardsi* (Horn) and *C. tenebrosus* (LeConte) are attracted to analogs resembling their sex pheromone, and *C. edwardsi* males are even more strongly attracted to the analog than to the pheromone (Serrano et al. 2020).

In Canada, many pest elaterid larvae pupate at a soil depth of 5–15 cm in late summer, with adult beetles emerging in spring when the soil temperature reaches approx. 10 °C (Lafrance 1963, van Herk and Vernon 2014). Similarly, below 10 °C the movement of beetles on the soil surface generally ceases (WvH, unpublished data). These temperature-dependent activity patterns may explain why beetle captures peaked earlier in the season at Ridgetown than at Dawn Mills (Fig. 3), despite the close proximity (~ 25 km) of both study sites. We surmise that in early April, temperatures of the clay loam soil at Ridgetown, but not of the loamy sand soil at Dawn Mills, were already sufficiently high to prompt beetle emergence and thus allowed beetle captures. As the onset and extent of beetle emergence periods may temperature-dependently fluctuate even within a small geographic range (Fig. 3), pheromone-based monitoring programs must stay in place sufficiently long to encompass the entire swarming period.

Compared to a previous study with *L. canus* and *L. californicus,* limoniic acid as a trap lure afforded relatively low captures of *L. agonus* males, suggesting that *L. agonus* populations were low or that other *L. agonus* pheromone components are yet to be identified. Traps baited with limoniic acid or its analog afforded captures of *L. agonus* only 3.6- to 8.9-times and 12- to 26-times greater than unbaited control traps (this study; van Herk et al. 2021a) but afforded captures of *L. canus, L. californicus* and *L. infuscatus* up to 100-times greater than control traps (van Herk et al. 2021a). The comparatively low captures of *L. agonus* in traps baited with limoniic acid suggest that other pheromone components may still be missing. These components could be the fatty acids that elicited antennal responses in EAD recordings (Fig. 1) but that were not field tested in this study. Even though these fatty acids reduced, rather than enhanced, attraction of western *Limonius* congeners to limoniic acid (van Herk et al. 2021a), these fatty acids may still be pheromone components in *L. agonus*.

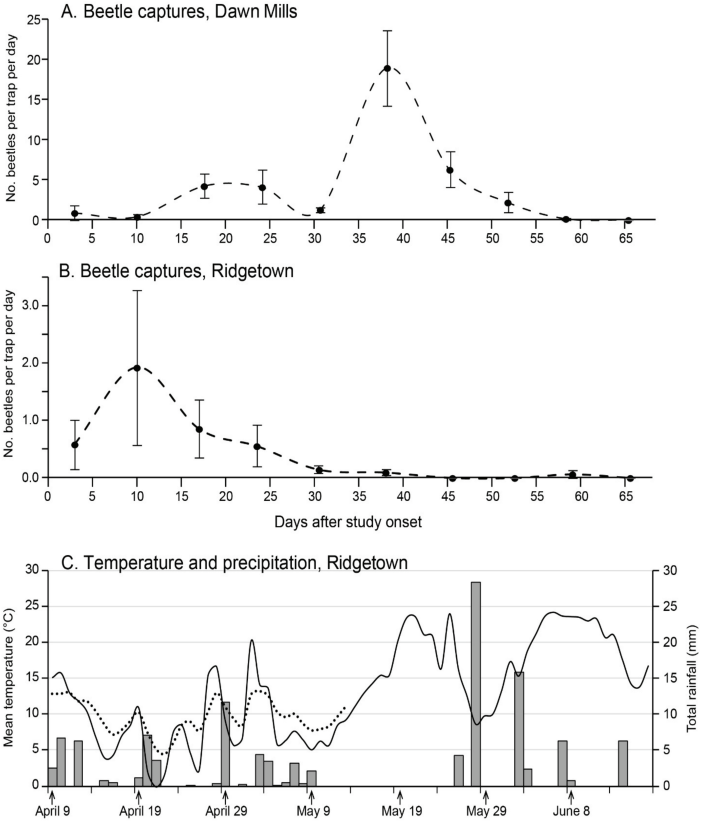

Figure 3. Mean (+ SEM) captures of male *Limonius agonus* per replicate (n = 8) per week at Dawn Mills (09 April–17 June) (A) and Ridgetown (07 April–17 June) (B) in southern Ontario in 2021 indicate distinct differences in peak beetle capture periods between locations. Mean daily air temperature (solid line), mean daily soil temperature (dashed line), and daily precipitation (mm) (bar diagrams) were recorded only for Ridgetown (C).

That click beetle congeners share sex pheromone components while using additional components to impart specificity to their sex pheromone, has been documented. For example, the pheromone blends of *Agriotes obscurus* L. (geranyl hexanoate and geranyl octanoate; Tóth et al. 2003) and *A. lineatus* (geranyl butanoate & geranyl octanoate; Tóth et al. 2003) share geranyl octanoate but differ in the second constituent of the blend. Similarly, *Idolus picipennis* (Bach) and a cryptic congener ('*TR* type') share geranyl hexanoate but use additional components that render pheromone blends species-specific (König et al. 2015). Analogously, the fatty acids produced by female *L. agonus* may enhance attraction of conspecific males to limoniic acid while—concurrently—reducing cross-attraction of current or former sympatric congeners. If so shown, this may also imply that the distribution ranges of eastern *L. agonus* and some of the western congeners may have overlapped in former times.

Even if the relatively low captures of *L. agonus* males were to indicate weak attractiveness of limoniic acid or its analog, these compounds could still be used for monitoring populations of *L. agonus*. Irrespectively, further work is required to determine the limoniic acid dose that is needed to effectively monitor and manage *L. agonus* populations. The success of pheromone-based wireworm control tactics would also depend on whether male beetles mate multiple times. To date, little is known about the mating systems of *Limonius* spp. other than that male *L. canus* may be able to mate more than once, that unmated *L. canus* females produce infertile eggs (Woodworth 1942), and that

the responsiveness of *L. californicus* males to sex pheromone declines after repetitive pheromone exposures (Lilly and McGinnis 1968). All data combined suggest that pheromone-based mass trapping and mating disruption are conceivable for control of *Limonius* spp., provided that many mate-seeking males are captured or otherwise prevented from locating receptive females.

The development of pheromone-based tactics for click beetle (and thus wireworm) management in Canada and the northern USA hinges upon three important considerations. First, most pest elaterids in this region have multi-year life histories, with larvae taking several years to complete development to pupae and adults. Therefore, suppression of elaterid populations would require control tactics to be repeated over several consecutive years. Second, elaterids such as *Hypnoidus abbreviatus* (Say), *A. mancus*, *Melatonus* spp. and *L. agonus* that may co-occur in the same fields (Saguez et al. 2017; WvH, unpublished data) have contrasting life histories, including distinct spring emergence periods, sexual or parthenogenetic reproduction, and potential cross-attraction, -inhibition or indifference to each other's pheromones (Vernon and van Herk 2022) that all must be reconciled by any control measure(s). Third, invasive elaterids have become established in their invaded North American range (Douglas 2011, Singleton et al. 2022b) and will continue to change the composition of endemic elaterid communities. For example, the invasive *Agriotes sputator* (L.) has largely displaced the native *H. abbreviatus* and *A. mancus* in PEI and Nova Scotia (Eidt 1953), as the invasive *A. obscurus* and *A. lineatus* have displaced the native *L. canus* and *A. sparsus* (LeConte) in south-western British Columbia (van Herk et al. 2021b).

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Author contributions

WvH & GG conceived the study; WvH captured beetles for pheromone analyses; RG captured headspace volatiles and analyzed volatile extracts; IS and JS ran field experiments; WvH analyzed capture data statistically and wrote the first draft, and all authors reviewed and approved of the final draft.

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Conflict of interest/ competing interest

The authors declare no conflict of interest.

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