Sex pheromone identification, spectral sensitivity, and colour preferences of select native and invasive click beetles (Coleoptera: Elateridae) in Canada

by
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Declaration of Committee

Name:	**Kendal Singleton**
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Title:	**Sex pheromone identification, spectral sensitivity, and colour preferences of select native and invasive click beetles (Coleoptera: Elateridae) in Canada**
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Abstract

My objectives were (1) to identify the sex pheromones of the click beetles *Agriotes ferrugineipennis* and *A. mancus* (Coleoptera: Elateridae), (2) analyze the spectral sensitivity of nine elaterids in electrophysiological recordings, and (3) analyze the color preference of two invasive *Agriotes* species in field and laboratory-behavioural bioassays. Captures of *A. ferrugineipennis* males in traps baited with 7-Methyloctyl 7-methyloctanoate exceeded those of unbaited control traps, on average by nearly 1,200 times. While in a second experiment, lures containing geranyl butanoate and geranyl hexanoate captured significantly more *A. mancus* males than the unbaited controls. All beetles proved most sensitive to green (515–538 nm) and ultra-violet (UV) light (~360 nm) and in four-choice bioassay arenas beetles were preferentially attracted to green and blue LEDs. In field experiments, Vernon Pitfall Traps® fitted with a green, blue or white LED captured significantly more male and female *Agriotes lineatus* and *A. obscurus* than dark control traps.

Keywords: Click beetles; Elateridae; sex pheromone identification; monitoring; electroretinogram; spectral sensitivity

For my mom,

Your unwavering support and zest for the gift of everyday has shaped me into the person I am today. You've encouraged me to go on every adventure I set my heart on and this one was no exception.

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Chapter 1.

Introduction

With almost 10,000 described species, elaterids (Coleoptera: Elateridae) are regarded as one of the most diverse insect families (Johnson, 2002). The beetles' multi-year life cycle – influenced by latitude, temperature, soil moisture, and food availability (Barsics et al., 2013) – render multiple species important and challenging agricultural pests (Vernon and van Herk, 2013). In the Holarctic region alone, over 100 elaterid species are economically important pests of potato (Vernon and van Herk, 2022), with many other species being significant pests of grains, forage, small fruits, and other assorted vegetables (Traugott et al., 2015; Poggi et al., 2021; Vernon & van Herk, 2022). Wireworms, the larvae of pest elaterids, feed on roots, seeds, stems, and harvestable plant parts, causing major damage (Traugott et al, 2015). Individual fields may be infested with millions of wireworms per hectare (Miles and Cohen 1941), which impact both the quality and yield of crops.

Following the advent of the highly effective chlorinated hydrocarbon soil and seed insecticides (e.g., lindane, aldrin and heptachlor) in the 1940s, followed in subsequent decades by organophosphate and carbamate insecticides, elaterids were considered relatively minor pests (Wilkinson et al., 1976; Parker, 2005; van Herk et al., 2008). Single applications of some highly persistent insecticides, namely aldrin and heptachlor, were found to control wireworm populations for up to 13 years (Wilkinson et al. 1964, 1976), making elaterid research a low priority for research for the next 50 years. However, bioaccumulation of these highly persistent insecticides evoked environmental concerns, and eventually resulted in their deregistration on global markets.

In the last 30 years, the pest status of wireworms has shifted, possibly due to the deregistration of many of the most effective wireworm insecticides (van Herk et al. 2021a), to insecticidal residues declining in arable land, increased quality expectations of consumers, and agricultural practices such as no-tillage farming and 'land set-aside' schemes (Parker and Howard, 2001; Jedlička and Frouz 2007; Traugott et al. 2015; Vernon and van Herk 2022). Renewed interest in the study of elaterid biology and management has also placed increased focus on the more visible and accessible adult

beetle stage, since the wireworm stage is soil-dwelling and thus more difficult to monitor and control.

Pheromone- and light-based trapping (Chapters 2-4) of adult beetles are two tactics that could become part of integrated click beetle management.

1.1. Sex pheromones

Sex pheromones are important communication signals in many insects. The low molecular weight and volatility of pheromones enable long-distance communication between prospective mates (Phillips, 1997), benefitting both signal emitters and signal recipients. By definition, sex pheromones are produced by one sex, typically females, and elicit a behavioural response by signal recipients (Witzgall et al., 2010; Tóth, 2013). Widespread use of synthetic pheromones in contemporary integrated pest management (IPM) programs is attributed to three main criteria: (1) species-specificity, 2) effectiveness at low lure dose, and (3) no toxic effects on non-targets (Witzgall et al., 2010).

Identification of elaterid sex pheromones was initiated in the 1980s, with the expectation that pheromone-based trapping of beetles could be as successful as pheromone-based moth control in orchard settings (Tóth, 2013). Since the 1980s, pheromones have become increasingly used in IPM programs for click beetles. Synthetic sex pheromone lures are useful tools to (*i*) monitor population trends of pest and endangered beetle species (Svensson et al., 2012; Vernon & van Herk, 2022), (*ii*) help predict crop damage by correlating beetle captures in pheromone-baited traps with larval abundance in the soil and with crop damage (Furlan et al., 2020; Vernon et al., 2020), (*iii*) time seasonal abundance and insecticidal control measures (Ester & van Rozen, 2005; Vernon & van Herk, 2022), (*iv*) delineate the geographic distribution of species (Vernon et al., 2001; Subchev et al., 2006; Musa et al., 2013), (*v*) detect the presence and monitor the spread of invasive species (Singleton et al., 2022a), and (*vi*) track displacement of native species by invasive species (e.g., van Herk et al., 2021b). Also, in jurisdictions such as Canada where insecticides are being deregistered, or in organic production systems where synthetic insecticides must not be applied, synthetic sex pheromones are being developed for control of adult beetle populations through mass trapping, mating disruption, and attract & kill tactics (e.g., Vernon et al., 2014a,b;

Reddy & Tangtrakulwanich, 2014; Kabaluk et al., 2015; van Herk et al., 2022; Vernon & van Herk, 2022).

While sex pheromones have been identified for most species of economic importance in Europe and have been utilized in IPM programs in many countries, only a few pheromones of North American native elaterids have been identified, all of which in the last five years. In North America, sex pheromones are now known for *Cardiophorus tenebrosus* and *C. edwardsi* (Serrano et al., 2018), *Melanotus communis* (Williams et al., 2019), *Limonius canus* and *L. californicus* (Gries et al., 2021; van Herk et al., 2021c), *Selatosomus aeripennis destructor* (Gries et al., 2022), *Idolus californicus* (Serrano et al., 2022), *Agriotes ferrugineipennis* (Singleton et al., 2022b; Chapter 2), *Parallelostethus attenuatus* (Millar et al., 2022), and *A. mancus* (Chapter 3).

In chapters 2 and 3, I report the identification of sex pheromones of *A. ferrugineipennis* and *A. mancus*.

1.2. Visual system of elaterids

Spectral sensitivity studies of representative insect orders suggest that the ancestor of all pterygote insects was trichromatic and possessed three r-opsin genes, conferring sensitivity in the ultraviolet (UV), short-wavelength (SW), and long-wavelength (LW) regions (Briscoe and Chitka, 2001). Opsin duplications and losses have been reported several times across the Insecta, including several families within the Odonata, Lepidoptera, Diptera and Coleoptera (Briscoe, 2008; Futahashi et al., 2015; Sharkey et al., 2017; van der Kooi et al., 2021), illustrating remarkably different visual capabilities of insects. Molecular studies on numerous beetle lineages have been unable to recover an SW opsin protein, suggesting that the short-wavelength opsin class has been lost before the divergence of modern beetles (Sharkey et al. 2017). However, some beetle species do possess blue-sensitive photoreceptors (e.g.; Lin, 1993; Lord et al. 2016; Sharkey et al. 2017; Meglič et al., 2020), suggesting that another mechanism underlies blue-sensitivity (Sharkey et al., 2017). Molecular analyses of 62 beetle species revealed that duplication events of the UV and LW opsin genes, and subsequent sub functionalization, have restored the blue-sensitivity in some families (Sharkey et al., 2017,2021).

Many beetle taxa rely on vision to locate mates (Szentesi et al. 2003; Yang et al. 2017; Wang et al. 2022), forage for hosts (Szentesi et al. 2003) or other

resources (Harmon et al. 1998; Szentesi et al. 2003; Wang et al. 2022), and to initiate or continue flight (Boiteau, 2005). This reliance on vision presents a unique opportunity for IPM and will be discussed below, and in chapter 4, where I report the spectral sensitivity of nine elaterid species and their responses to coloured light in laboratory and field-based experiments.

1.2.1. Spectral sensitivity of beetles

Electroretinogram (ERG) and intracellular recordings are routinely used to determine the sensitivity of photoreceptor cells in an insect's compound eye to different wavelengths of light. Beetles are commonly sensitive in the green and ultra-violet (UV) regions (e.g.; Lall et al., 2010; Katsuki et al., 2013; van der Kooi et al., 2021), although great variation exists (van der Kooi et al. 2021). For example, both males and females of the emerald ash borer, *Agrilus planipennis*, have sensitivity peaks in the UV (340 nm), violet (420-430 nm), blue (460 nm), and green (540-560 nm) regions (Crook et al., 2009). In contrast to most other beetles, female *A. planipennis* also have a potential red photoreceptor sensitivity in the 640-nm to 670-nm range. The bumble-bee scarab beetle, *Pygopleurus* i*sraelitus*, and the flathead oak borer, *Coraebus undatus,* are the only other beetle species currently known to possess red receptors (Martínez-Harms et al., 2012; Meglič et al., 2020). As *P. israelitus* commonly visits red flowers, photoreceptors conferring sensitivity in the red (628 nm), UV (352 nm) and green (536 nm) regions seem adaptive (Martínez-Harms et al., 2012). *Coraebus undatus* is sensitive in the red range (600nm) as well as the UV (335-350 nm), blue (430 nm), and green (540 nm) ranges (Meglič et al., 2020). The discovery of a blue photoreceptor in *C. undatus* supports previous reports that jewel beetles have restored sensitivity in the blue region (Meglič et al., 2020). The scarab beetle *Anomala corpulenta* differs from most beetles in that it does not have the distinctive primary green sensitivity (Yue-Li, 2015). Instead, the sensitivity curve of the ERG peaks in the near UV (400 nm), with secondary and tertiary peak sensitivities observed in the yellow green (498-562 nm) and blue green (460 nm) regions (Yue-Li 2015).

1.2.2. Exploitation of visual cues for beetle mass trapping and population monitoring

Electroretinogram and intracellular recordings can inform the selection of light stimuli/lures for light traps, and the wavelength and intensity of light emitting diodes (LEDs) as trap lures can be adjusted to capture target species (Cohnstaedt et al., 2008). Routinely, light traps are used as sampling tools for many insect species, particularly flies and moths (e.g.; Steinbauer, 2003; Hoel et al., 2007; Costa-Neta et al., 2018). Light traps allow captures of specific nocturnal insects, and thus are popular sampling tools (Szentkiralyi, 2002). Within the order Coleoptera, light-based trapping or other light-based technologies have been deployed to determine the flight periods of adult beetles (Amorós et al. 2022), monitor population dynamics of agricultural pest beetles (Al-Deeb et al. 2012; Amorós et al. 2022), assess beetle diversity (Hebert et al., 2000; Pablo-Cea et al. 2022), measure vertical stratification of beetles in their inhabited ecosystem (Stork et al., 2016), repel stored-produce pest beetles (Kim et al., 2013; Miyatake et al. 2016), curtail population size (Arakaki et al., 2015; Santi et al. 2022), capture and identify invasive beetle species (Cruz-López et al. 2022), and serve as a push-pull system in timber processing plants (Pawson et al., 2009). Sex pheromone-baited traps are used to monitor pest beetle populations in the field but sex pheromone lures primarily target signal recipients which often are males. Light traps alone or in combination with sex pheromone-baited traps might attract both males and females, and could be used to optimally monitor populations of pest species. For example, red flour beetles, *Tribolium castaneum*, were most strongly attracted to dome traps baited with both light stimuli and a species-specific chemical lure (Duehl et al., 2011).

Various trap designs have been explored for monitoring beetle populations. Most commonly, some type of a funnel trap is placed above or below (pitfall) ground (Hebert, 2000; Arakaki et al., 2015). Bowl and fabric light traps have been used to capture pollen beetles, *Astylus atromaculatus*, and red pumpkin beetles, *Aulacophora africana*, respectively (Van den Berg et al., 2008; Chukwu et al., 2020). Placement of light lures above or below the trap's lid greatly affects beetle captures (Duehl et al., 2011).

The optimal light type for maximizing capture rates may be species- and context-specific. Compared to black lights (Arakaki et al., 2015), fluorescent lights (Mohammed et al., 2017), and incandescent bulbs (Chukwu et al., 2020), LEDs are advantageous in that their wavelengths are adjustable (Cohnstaedt et al., 2008). In Saudi Arabia, solar-

powered light traps were used to monitor pest populations of various date palm pests, including the longhorned trunk borer, *Jebusaea hammerschmidti,* and frond borer, *Phonapate frontalis* (Mohammed et al., 2017). When capture rates of date palm pests afforded by different light sources were compared, traps baited with black fluorescent lights captured significantly more beetles than traps baited with compact fluorescent lights or LEDs (Mohammed et al., 2017). In Japan, effects of UV LEDs and black lights on captures of green chafer beetles, *Anomala albopilosa*, varied with site (Arakaki et al. 2015). At two sites, black lights were most effective but at a third site the UV LED was superior (Arakaki et al., 2015).

UV light baits were favored by various beetle species (Pawson et al., 2009; Duehl et al., 2012; Katsuki et al., 2013; Duehl et al., 2011). UV- and black light-baited traps captured 2- to 4-times more bark beetles (*Hylurgus ligniperda, Hylastes ater*) than traps baited with other wavelengths of light. Similarly, UV- and black light-baited traps as well as UV-, black light- and blue-baited traps captured significantly more *Arhopalus ferus* longhorned beetles than traps baited with yellow, green or white lights (Pawson et al., 2009). Moreover, all three longhorned beetle species responding to light baits avoided yellow lights (Pawson et al. 2009). Small hive beetles, *Aethina tumida*, and red flour beetles, *Tribolium* castaneum, were more strongly attracted to UV light (390 nm) than to human-visible and other UV wavelengths (Duehl et al., 2011, 2012).

1.2.3. Visual system in Elateridae

Although many elaterids are major pests, their visual system has hardly been studied. Prior to my study, the spectral sensitivity was not known for any Holarctic species, and only a few reports have documented the spectral sensitivity of elaterids or their captures in light-based traps. In the earliest study (Genung, 1972), aerial black light traps deployed in Southern Florida captured moderate numbers of the corn wireworm, *Melanotus communius,* the southern potato wireworm, *Condoderus fali*, and the elaterid *Glyphonyx bimarginatus*. In 2010, Lall et al. reported the spectral sensitivity of four neotropical bioluminescent click beetles, documenting that all four species have a strong preference for green light (520-560 nm) and a moderate preference for light in the near UV range (360–390nm). In a 2015 field study in Atlantic Canada, white-light solar powered “Norhona Elaterid Light Traps” (NELTs) were effective at capturing both male and female *Agriotes sputator* and *Hypnoidus abbreviatus* (Noronha, unpublished data).

Attraction of elaterids to lights has repeatedly been documented (Johnson et al., 2002; Wells, 2007; Kirmse & Johnson, 2020) but the wavelength and intensity of light, and the species responding, have not always been reported. Bioluminescent beetles mostly in the tribe Pyrophorini are believed to use flash patterns of bioluminescent signals in mate attraction (Johnson et al. 2002). Whereas elaterids in North America are not bioluminescent, observations of North American native elaterids at lights (Johnson et al., 2002) provides impetus to study spectral sensitivities of diverse elaterid taxa.

1.3. Click beetles

Click beetles are among the world's most damaging agricultural pests. Larval wireworms of click beetles feed on potatoes, grains, and cereals, significantly reducing crop yield and quality. In North America, crop losses from wireworms have been estimated between 5-25% (Jansson & Seal, 1994), and on Prince Edward Island the wireworm-inflicted annual damage on potato crops, and the associated costs for wireworm control, were estimated at 10 million dollars (King, 2018).

Adult click beetles are routinely identified by their characteristic elongated narrow body, and well defined pro-thoracic hind angles. Most recognizable, however, is the ability of adult beetles to produce an audible click as they catapult themselves into the air. Their freely articulating pro-thorax enables beetles to rapidly move their prosternal spine into their mesosternal cavity, creating a jackknife body posture (Evans, 1972; Bolmin et al., 2017). During this pre-jump stage, the body posture is held by friction, and when the prosternal spine releases from the lip of the mesosternal lip, the beetles take off headfirst into the air, summersaulting vertically through the air (Bolmin et al., 2017) and landing some 50 cm away from their starting point (Evans, 1972; Ribak & Weihs, 2011; Bolmin et al., 2017). The click mechanism is believed to enable self-righting (Bolmin et al., 2017) but may also serve in predator escape behavior (Evans, 1972).

1.3.1. Distribution of elaterids in Canada

Three European invasive *Agriotes* species, *A. lineatus, A. obscurus* and *A. sputator,* have become increasingly important since their accidental introduction into Canada in the early 1900s. In British Columbia, *A. lineatus* and *A. obscurus* are now the two

dominant pest species (King et al., 1952; Wilkinson, 1963), and in Eastern Canada, *A. sputator, A. lineatus* and *A. obscurus* are well established (Brown 1940; Eidt, 1943).

In British Columbia, range expansions of *A. lineatus* and *A. obscurus* were initially slow. At first, *A. lineatus* was located only at Cobble hill on Vancouver Island, whereas *A. obscurus* was located only in Agassiz, British Columbia (Wilkinson, 1963). By the late 90s, the beetles' range had expanded considerably. *Agriotes lineatus* was already present on the mainland, and *A. obscurus* was steadily moving westward towards Vancouver (Vernon and Päts, 1997). As of today, the beetles' range expansions continue and both species have been collected throughout Southern British Columbia, throughout the Okanagan valley and into the Kootenay region (van Herk et al., 2021b). Since their introduction, both invasive species have been displacing native pest elaterids. For example, in the 1940s and 1950s, crop damage in the Fraser Valley was attributed to *A. sparus, Corymbitodes lobata, Limonius canus* and *L. infuscatus* (Wilkinson 1963; Wilkinson et al., 1977). Today, these species have almost entirely been displaced by *A. lineatus* and *A. obscurus* and are rarely collected (van Herk et al., 2021b). With continued spread and establishment of *A. lineatus* and *A. obscurus,* more native elaterid species are likely to be displaced.

In the prairie provinces, there are at least 182 click beetle species (Vernon and van Herk 2013), but only 11 are considered pests (van Herk and Vernon 2014; Catton et al., 2021). Importantly, the three invasive European *Agriotes* species have not yet been found in this region but continued monitoring will be necessary. Of primary focus are the four dominant wireworm species *Hypnoides bicolor*, the prairie wheat wireworm, *Selatosomus aeripennis destructor*, the sugarbeet wireworm, *L. californicus*, and the flat wireworm, *Aeolus mellilus* (van Herk et al. 2021d).

In the Maritime Provinces, there are at least 129 click beetle species (Majka & Johnson, 2008). Since its arrival, the invasive *A. sputator* has become the predominant wireworm pest of high value crops, such as potatoes, in Prince Edward Island (PEI) and Nova Scotia (Fox 1961, Noronha 2011, Vernon & van Herk 2018, van Herk et al. 2021e). In Eastern Canada and in Quebec, *Hypnoidus abbreviatus* and *A. mancus* are the predominant pest species but are considered less damaging to crops (Saguez et al. 2017). Recently, *A. sputator* was found in Quebec, indicating its range expansion into Central Canada (Chapter 5; Singleton et al., 2022a). With the establishment of *A.*

sputator, the elaterid species composition in the Maritime Provinces will likely change analogous to changes recorded in PEI and Nova Scotia.

Continued spread of these three invasive species is likely and poses a substantial threat. The range expansion of *A. sputator* into Central Canada demonstrates a viable pathway from the Maritime provinces and suggests that the spread of the other two invasive species, *A. lineatus* and *A. obscurus,* is also likely.

In Chapter 5, I discuss the spread of *A. sputator* into Central Canada and discuss the implications of its arrival.

1.4. Overview of research chapters

In Chapter 2, I report the identification and field testing of the sex pheromone of *Agriotes ferrugineipennis*. Headspace volatiles from female beetles were collected on Porapak Q adsorbent, and aliquots of Porapak extract were analyzed by gas chromatographic - electroantennographic detection (GC-EAD) and GC-mass spectrometry. We found that 7-methyloctyl 7-methyloctanoate (7Me7Me) emitted by females elicited the strongest response from male antennae. In a field experiment, captures of *A. ferrugineipennis* males in traps baited with synthetic 7Me7Me exceeded those of unbaited control traps by nearly 1,200 times. Our data support the conclusion that 7Me7Me is the major, and possibly the only, sex attractant pheromone component of female *A. ferrugineipennis*.

In Chapter 3, I report the identification and field testing of the sex pheromone of *A. mancus.* We collected headspace volatiles from female beetles on Porapak Q, and analyzed aliquots of Porapak extract by GC-EAD and GC-MS. In GC-EAD recordings, two esters – geranyl butanoate and geranyl hexanoate – elicited antennal responses from *A. mancus* males. In field experiments, trap lures containing both geranyl butanoate and geranyl hexanoate afforded large captures of *A. mancus* males, which were – on average – 30-fold higher than captures in traps baited with a single ester. Traps baited with geranyl butanoate as a single-component lure captured a significant number of Palearctic *A. sputator.* Our data support the conclusion that geranyl butanoate and geranyl hexanoate comprise the sex pheromone of *A. mancus* and that *A. sputator* is well established in its invaded Nearctic range.

In Chapter 4, we analyzed the spectral sensitivity and color preference of nine elaterids across six genera in electrophysiological recordings and in behavioural

bioassays. In electroretinogram recordings (ERGs), dark-adapted beetles were exposed to 10-nm bandwidths of light, ascending in 10-nm increments from 330 nm to 650 nm, and then descending back to 330 nm. We found that all beetles were most sensitive to green (515–538 nm) and UV light (~360 nm). In four-choice bioassay arenas with three LEDs [green (525 nm), blue (470 nm), red (655 nm)] and a dark control as test stimuli, beetles discriminated between test stimuli, orienting towards green and blue LEDs. In field experiments, modified Vernon pitfall traps fitted with a green, blue or white LED captured significantly more male and female *Agriotes lineatus* and *A. obscurus* than dark control traps. Traps baited with green or blue LEDs at a light intensity of 2.7E+16 photons/cm^2/s captured numerically more beetles than traps baited with green or blue LEDs at a lower light intensity (2.7E+15 photons/cm^2/s), but trap catch data in accordance with light intensity did not differ statistically. We conclude that light-based trapping may be viable for monitoring elaterid species known not to have sex pheromones.

In Chapter 5, I present the first record of *A. sputator* in Quebec, documenting its range expansion into Central North America. We captured 32 and 217 *A. sputator* males in two consecutive years, indicating that the population is already well established and is increasing in size. *Agriotes sputator* is the first of three invasive non-native *Agriotes* pests in North America (*A. sputator*, *A. lineatus*, *A. obscurus*) to arrive in Quebec and the central lowlands of North America, which are among the world's largest agricultural growing areas. *Agriotes* sputator is likely to displace the currently predominant native pest wireworm *Hypnoidus abbreviatus* (Saguez et al., 2017), and to become the primary elaterid pest in Quebec.

1.5. References

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Chapter 2.

Identification of the major sex pheromone component of the click beetle *Agriotes ferrugineipennis*[1]

[1]A near identical version of this chapter is published in the Journal of Chemical Ecology (2022, https://doi.org/10.1007/s10886-022-01367-1) with the following authors: Singleton, K, Gries, R., van Herk, R.G., Alamsetti, S.K., Lemke, E., Furtado, K., Gries, G.

KS, WvH & GG conceived the study; KS, EL & WvH captured beetles for pheromone analyses; KS & RG captured headspace volatiles; RG analyzed volatile extract as well as model compounds by GC-EAD and GC-MS; SA synthesized chemicals; KS, EL & WvH ran field experiments; KS & KF identified and determined the sex of beetles captured in traps; WvH analyzed capture data statistically; KS & GG wrote the first draft, and all authors reviewed and approved of the final draft.

2.1. Abstract

Synthetic sex pheromone lures are useful tools to monitor and control populations of adult click beetles (Coleoptera: Elateridae). However, sex pheromones for *Agriotes* click beetle species native to North America have yet to be identified. Here we report the identification and field testing of the sex pheromone of *Agriotes* ferrugineipennis. Headspace volatiles from female beetles were collected on Porapak Q, and aliquots of Porapak extract analyzed by gas chromatographic-electroantennographic detection (GC-EAD) and GC-mass spectrometry. 7-Methyloctyl 7-methyloctanoate (7Me7Me) emitted by females was more abundant and elicited much stronger responses from male antennae than the aldehydes octanal and nonanal and the ketone 6,10,14-trimethyl-2-pentadecanone. In a field experiment, captures of *A. ferrugineipennis* males in traps baited with candidate pheromone components exceeded those of unbaited control traps, on average by nearly 1,200 times. Neither the ketone nor the aldehydes as lure constituents appeared to alter captures of males in 7Me7Me-baited traps. We conclude that 7Me7Me is the major, and possibly the only, sex attractant pheromone component of female *A. ferrugineipennis.*

2.2. Introduction

Wireworms, the larvae of click beetles (Coleoptera: Elateridae), are ubiquitous soil-dwelling pests (Poggi et al. 2021), feeding on many agricultural crops (Traugott et al. 2008, 2015). In recent years, populations of pestiferous click beetles have regained economic importance, possibly due to the deregistration of insecticides (van Herk et al. 2021a), insecticidal residues leaching out of arable land, and agricultural practices such as no-tillage farming and 'land set-aside' schemes (Jedlička and Frouz 2007; Traugott et al. 2015; Vernon and van Herk 2022). Tillage alters the soil microclimate, destroys beetle eggs and larvae, and brings them to the soil surface where they desiccate or fall prey (Lees 1943; Saussure et al. 2015).

Synthetic sex pheromone lures of click beetles are useful tools to (i) monitor population dynamics of adult beetles, (ii) delineate the geographic distribution of species, (iii) help predict crop damage, (iv) time insecticidal control measures, (v) detect the presence and track the spread of invasive species, and (vi) surveil the displacement of native species by invasive species (e.g., Kudryavtsev et al. 1993; Tolasch et al. 2007; Musa et al. 2013; Traugott et al. 2015; Furlan et al. 2020; van Herk et al. 2021b). Also, synthetic sex pheromones are increasingly considered for control of adult beetle populations through mass trapping, mating disruption, and attract & kill tactics (e.g., Vernon and van Herk, 2022; Reddy and Tangtrakulwanich 2014; Vernon et al. 2014; Kabaluk et al. 2015).

To date, sex pheromones are known for only eight elaterid species native to North America, including *Melanotus communis* (Williams et al. 2019), *Cardiophorus tenebrosus* and *C. edwardsi* (Serrano et al. 2018), *Limonius canus* and *L. californicus* (Gries et al. 2021; van Herk et al. 2021d), *Selatosomus aeripennis destructor* (Gries et al. 2022), *Idolus californicus* (Serrano et al. 2022), and *Parallelostethus attenuatus* (Millar et al. 2022). Sex pheromones are not yet known for any North American-native *Agriotes* click beetles such as *Agriotes ferrugineipennis*.

The genus *Agriotes* is of particular agricultural importance, with about 20 of the >200 described species (Becker 1956) being significant agricultural pests in Europe (Tóth 2013; Traugott et al. 2015; Ritter and Richter 2013), North America, and Asia (Vernon and van Herk 2022). In the UK and northern Europe, *A. lineatus*, *A. obscurus*, and *A. sputator* are the predominant pest wireworm species in agricultural land (Parker

and Howard 2001). These three species established in Canada in the 1800s, with *A. lineatus* and *A. obscurus* now being important pests of field crops in southern British Columbia (BC) (Wilkinson 1963; van Herk et al. 2021c), and all three being pests in Eastern Canada since the 1800s (Eidt 1953, Vernon and van Herk 2022).

Adult beetles of *A. ferrugineipennis* are medium-sized (9–12 mm) with a distinct reddish hue on their antennae and legs, and pronounced hind angles of the pronotum (Becker 1956). The beetles occur throughout BC, Alberta, Washington, California, Idaho, Oregon, Nevada and Utah (Becker 1956; Wilkinson 1963; van Herk and Vernon 2021b). Although found in agricultural land, the pest status of *A. ferrugineipennis* is unclear (Glen 1944; Wilkinson 1963).

Males of *A. ferrugineipennis* reportedly respond to abdominal extracts of conspecific females but the compound(s) mediating the attraction responses of males remained unknown (Lilly and McGinnis 1965). Here we report the identification and field testing of the major sex pheromone component of female *A. ferrugineipennis*.

2.3. Materials and Methods

2.3.1. Field collection of beetles

In May and June 2020, click beetles were collected in sweep nets in Pemberton, BC, at potato fields with historically high *A. ferrugineipennis* populations. Crops were rotated every three years, and at the time of collection, the field was covered with grass from which beetles were collected with sweep nets. Captured beetles were separated by species and their sex was determined by careful extrusion of their genitalia. Due to a paucity of sweep-netted beetles, a group of only three females and 30 males were collected. These groups were maintained in separate plastic cups (140 mL; Fisher Scientific, Ottawa, ON, CA) with perforated lids to facilitate air exchange. Cups contained fresh grass for both moisture and walk-on substrate for beetles, and small pieces (2 × 2 cm) of apple for food. All cups were kept at a low temperature (~ 4 °C) to extend the beetles' longevity. Prior to collecting the beetles' headspace volatiles, cups were warmed to room temperature and the grass was replaced with a moist Kim wipe. Apple pieces were replaced once a week or when they had become soft and moldy, and Kim wipes (Fisher Scientific, Ottawa, ON, CA) were remoistened as needed. To reduce beetle mortality, cups were replaced every two weeks or when a beetle had died.

2.3.2. Collection of Headspace Volatiles

Headspace volatiles of beetles were collected following a protocol previously detailed (Gries et al., 2021). Briefly, the three females and the 11 males of *A. ferrugineipennis* we had available for volatile captures were placed into separate Pyrex® glass chambers (8 cm high × 8 cm diameter), each 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 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). Porapak Q volatile traps was desorbed with pentane/ether (2 mL, 50:50) and concentrated to 100 µL for analyses.

2.3.3. Gas Chromatography with Electroantennographic Detection (GC-EAD) Analyses

Aliquots of the Porapak Q extract of female beetles, and of synthetic standards, 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) 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, Agilent Technologies Inc., Santa Clara, CA, USA). 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 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 flow) continuously passing over the electrode-suspended antenna – were amplified with a custom-built amplifier and recorded on an HP 3392A integrator. The voltage of antennal responses was derived from correlations between peak height and integrator attenuation, as tabulated in the recorder manual. Stable GC retention

times made it possible to direct the entire column effluent, in sequence, to the FID and the EAD, thus allowing us to align EAD responses to FID peaks while increasing the probability of detecting minor sex attractant pheromone components. Because only eight males were available for analyses, and not every antennal preparation was functional, just one or two usable GC-EAD recordings could be obtained on each of the four GC columns (see above).

2.3.4. GC-Mass Spectrometry and NMR Spectroscopy

Headspace volatiles that elicited antennal responses were deemed candidate pheromone components (CPCs) and were analyzed by GC-MS, using both a Varian Saturn 2000 Ion Trap GC-MS and a 5977A Series MSD (both Agilent Technologies Inc., Santa Clara, CA, USA) coupled to a 7890B GC. Both instruments were operated 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 · s−1). The injector port and ion trap were set at 250 °C and 200 °C, respectively, and the temperature program was as follows: 50 °C for 5 min, 10 °C · 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 those of authentic standards that were purchased or synthesized. The 1H-NMR spectra of a synthetic candidate pheromone component and of two model compounds, were recorded on a Bruker 500 MHz spectrometer using CDCl3 as solvent. Signal positions (δ) are given in ppm from tetramethylsilane (δ 0) and were measured relative to the signal solvent (1H NMR: CDCl3: δ 7.26). Coupling constants (J) are given in Hertz (Hz) and are reported to the nearest 0.1 Hz. 1H NMR spectral data are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br., broad), coupling constants, number of protons.

2.3.5. Chemicals

2.3.5.1. Synthesis of 7-methyloctyl 7-methyloctanoate (7Me7Me), 6-methyloctyl 6-methyloctanoate (6Me6Me), and 5-methyloctyl 5-methyloctanoate (5Me5Me)

All synthetic acid intermediates (7-methyl octanoic acid, 6-methyl octanoic acid, 5-methyl octanoic acid) were purchased (Toronto Research Chemicals; North York, ON, CA) and

the corresponding alcohols were produced by reduction of these acids with lithium aluminum hydride (LiAlH4) (Jones and Fleming 1997). Esters were obtained following a well-established method (Neises and Steglich 1978), using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as coupling reagent and catalyst, respectively, with yields ranging between 68–73%. 1H NMR data of 7Me7Me were consistent with those previously reported (Tolasch et al. 2007) and the mass spectrum is shown in Fig. 2.3. 1H NMR and GC-MS data of 6Me6Me: 1H NMR (500 MHz, CDCl3): δ 4.06 (t, J = 6.7 Hz, 2H), 2.30 (t, J = 7.5 Hz, 2H), 1.65-1.51 (m, 5H), 1.32 – 1.21 (m, 8H), 1.11 (dq, J = 12.7, 6.3, 5.8 Hz, 3H), 0.9 – 0.82 (m, 16H); the mass spectrum of 6Me6Me is shown in Fig. 2.33. 1H NMR and GC-MS data of 5Me5Me: 1H NMR (500 MHz, CDCl3): δ 4.06 (t, J = 6.7 Hz, 2H), 2.30 (t, J = 7.5 Hz, 2H), 1.65-1.51 (m, 5H), 1.32 – 1.21 (m, 8H), 1.11 (dq, J = 12.7, 6.3, 5.8 Hz, 3H), 0.9 – 0.82 (m, 16H); the mass spectrum of 5Me5Me is shown in Fig. 2.3.

All three esters were purified for NMR analyses by HPLC (Waters Corporation, Milford, MA, USA: 600 Controller, 2487 Dual Absorbance Detector, Delta 600 pump) fitted with a Spursil RP C18 column (3 µm, 250 mm × 4.6 mm; Dikma Technologies Inc., Lake Forest, CA; USA) eluted with an isocratic flow (1 ml/min) of acetonitrile.

6,10,14-Trimethyl-2-pentadecanone was available from a previous project (Sasaerila et al. 2003), and octanal and nonanal were purchased (Sigma Aldrich, St Louis, MO, USA). The chemical purity of field-tested octanal, nonanal, 6,10,14-trimethyl-2-pentadecanone and 7Me7Me was 99%, 95%, 99% and 96%, respectively.

2.3.6. Field Trapping Experiment

The experiment was run in two adjacent fields (each 5.83 ha, 4.59 ha) near Pemberton, BC (50.429236, -122.907198) from which beetles had been collected for pheromone identification. The experiment followed a general protocol previously detailed (Gries et al. 2021), using a complete randomized block design with eight replicates situated in each field. Four additional replicates were placed in a grassy berm along a driveway leading up to one of the fields. Vernon pitfall traps (van Herk et al. 2018; available from Intko Supply Ltd., Chilliwack, BC, CA) were placed at ground level along the field's edge (Fig. 2.1 a), with 10-m and 20-m spacing between treatments and replicates, respectively. Traps were baited with synthetic CPCs (see below) dissolved in hexane of which 45-µL aliquots were pipetted onto 100% cotton pellets (size #0; Richmond Dental,

Charlotte, NC, USA). The cotton pellets were placed inside of 1-mL LDPE containers (diameter: 8 mm, height: 32 mm; wall thickness: 0.98 mm; product number: 00730; Kartell Labware, Noviglio, IT) which were open and suspended from the roof of traps. Each experimental replicate (N = 16 during weeks 1–3; N = 20 during weeks 4–7) consisted of five treatments: (1) an unbaited control; (2) 7-methyloctyl 7-methyloctanoate (7Me7Me) (10 mg); (3) a ternary blend of 7Me7Me (10 mg), octanal (1 mg) and nonanal (1 mg); (4) a binary blend of 7Me7Me (10 mg) and 6,10,14-trimethyl-2-pentadecanone (1 mg); and (5) a quaternary blend of 7Me7Me (10 mg), octanal (1 mg), nonanal (1 mg), and 6,10,14-trimethyl-2-pentadecanone (1 mg). As only 7Me7Me was female-specific (see Results) and thus deemed to be the major candidate pheromone component, it was field-tested at a dose 10-fold higher than that of the other EAD-active components even though the two aldehydes were as abundant as 7Me7Me in the headspace of females. The first 16 replicates of the experiment were installed on 12 April 2021, and the remaining four replicates on 3 May 2021. The experiment was terminated on 31 May 2021. Traps were checked and captured beetles were collected every seven days. Total counts of captured beetles were recorded and beetles in subsamples were identified to species and sex.

2.3.7. Identification of Captured Beetles

Click beetles were identified to species using taxonomic keys (Becker 1956). Specimens that were taxonomically ambiguous were identified solely based on genitalia characteristics, which are distinct for male *A. ferrugineipennis* (Becker 1956). Specimens with missing genitalia and otherwise badly damaged were excluded from analysis. A total of 88 (of 151) beetles captured in unbaited control traps and subsamples of >480 beetles captured in traps assigned to each of the four pheromone treatments (see above) were identified to species. Each of these subsamples consisted of five samples (up to 20 beetles each) taken from every collection week of the 7-week study (Table 1). Voucher specimens are retained at the Agassiz Research and Development Centre (Agassiz, BC, CA).

2.3.8. Statistical Analyses of Data

To determine whether the proportion of trap-captured *A. ferrugineipennis* males varied with treatment and collection week, beetle subsamples were selected randomly for each

treatment from five replicates per collection week, as mentioned above. Proportions were compared using generalized linear models with a binomial distribution and a logit link function (Proc GENMOD, SAS 9.2, SAS Institute, Cary, NC, USA), and mean proportions were calculated per treatment and collection week (Table 1). These mean proportions were then used to calculate the number of *A. ferrugineipennis* males collected per trap per week, and the interpolated number of beetles was summed over the 7-week collection period to calculate the total number of *A. ferrugineipennis* males collected per trap. Differences between treatments were analyzed using total counts with generalized linear models fitted with a negative binomial distribution and a log link function, and including factors for both treatment and replicate.

2.4. Results

2.4.1 Identification of Candidate Pheromone Components

GC-EAD analyses of headspace volatile extracts of female *A. ferrugineipennis* revealed five components (**1**, **2**, **3, 4** and **5** in Fig. 2.2) that elicited responses from male *A. ferrugineipennis* antennae. Whereas some other FID peaks also appeared to elicit antennal responses, these responses could not be repeated in recordings on other GC columns. Unlike components **1**–**4**, component **5** was female-specific and elicited the strongest antennal responses. The mass spectrum of **5** showed a base peak (*m*/*z* 159) and a molecular ion (*m*/*z* 284) indicative of a nonyl nonanoate. Yet, synthetic nonyl nonanoate – prepared according to Neises and Steglich (1978) – had retention indices significantly higher than those of **5** on all four GC columns (Table 2), indicating that **5** had at least one methyl branch. With 7-methyloctyl nonanoate (available from a previous project) still eluting too late (Table 2), we considered octanoates with methyl branches in both the acid and alcohol part of the ester. Reviewing the literature for previously reported di-methyl octanoates in click beetles, we found a study by Tolasch et al. (2007) that reported the presence of 7-methyloctyl 7-methyloctanoate (7Me7Me) in pheromone gland extracts of female *Elater ferrugineus*. We synthesized 7Me7Me and determined that its mass spectrum (Fig. 2.3) and retention indices (Table 2) were entirely consistent with those of beetle-produced **5**. Moreover, beetle-produced and synthetic 7Me7Me, each tested at 10 ng, elicited comparably strong responses from male antennae in GC-EAD recordings. To unequivocally prove that the methyl branches of **5** were indeed at C-

7, rather than at C-6 or C-5, we also synthesized 6-methyloctyl 6-methyloctanoate and 5-methyloctyl 5-methyloctanoate. As expected, neither the mass spectra nor the retention indices of these two esters matched those of beetle-produced **5** (Fig. 2.3, Table 2).

GC-MS analyses of beetle-produced 1 and 2 confirmed that they were octanal (1) and nonanal (2). Beetle-produced 3, with mass spectral fragmentation m/z 58 (indicative of a keto-group in C-2) and a molecular weight of 268 was identified as 6,10,14-trimethyl-2-pentadecanone by comparison with a synthetic standard at hand (Sasaerila et al., 2003). Beetle-produced 4 had the retention time of 6,10,14-trimethyl-2-pentadecanol (the corresponding alcohol of compound 3) but the amount present in extracts was not sufficient to obtain a mass spectrum to confirm this tentative assignment.

2.4.2. Field Experiment

The proportion of *A. ferrugineipennis* males among all click beetles captured varied with both treatment ($\chi 2 = 180.0$, df = 4,137, $P<0.0001$) and collection week ($\chi 2 = 324.6$, df = 6,137, $P < 0.0001$) (Table 1). Proportions of *A. ferrugineipennis* males were lowest in unbaited control traps, which captured mostly A. obscurus, A. lineatus (two invasive species recently found in the Pemberton area; van Herk et al. 2021c), and Limonius canus. There was no statistically significant difference ($P > 0.05$) in the proportion of *A. ferrugineipennis* males that were captured in traps baited with a 1-, 2-, 3- or 4-component blend of the CPCs (Fig. 2.4). The total number of *A. ferrugineipennis* males captured varied with both treatment ($\chi 2= 282.8$, df = 4,76, $P < 0.0001$) and replicate ($\chi 2 = 105.4$, df = 19,76, $P <0.0001$), with no statistically significant differences ($P > 0.05$) between CPC treatments, and with all captures in CPC-baited traps (range of means: 311.4–386.0) being significantly higher ($P < 0.0001$) than those in unbaited control traps (mean: 0.3) (Fig. 2.4). Small numbers of female *A. ferrugineipennis* (25), and of male and female *A. lineatus* (63, 19), *A. obscurus* (20, 2), *Limonius canus* (172, 0), and unidentified elaterids (11, 9) were collected in both baited and non-baited traps.

2.5. Discussion

Laboratory analyses and field trapping data indicate that 7-methyloctyl 7-methyloctanoate (7Me7Me) is the major sex pheromone component of female *A. ferrugineipennis.* The ester 7Me7Me elicited the strongest responses from male antennae in electrophysiological recordings, and all synthetic lures containing 7Me7Me in a field trapping experiment attracted large numbers of *A. ferrugineipennis* males. While octanal, nonanal and 6,10,14-trimethyl-2-pentadecanone are present in the headspace of female beetles, and are sensed by male antennae, these compounds do not enhance the attractiveness of 7Me7Me, at least not in the context as tested in our study.

Based on the abundance of 7Me7Me in the headspace of female *A. ferrugineipennis* and the strong responses it elicited from male antennae in GC-EAD recordings (Fig. 2.2), we hypothesized that 7Me7Me is the major sex pheromone component of female *A. ferrugineipennis.* We further hypothesized that its attractiveness may be enhanced by the minor candidate pheromone components that were less abundant and only modestly EAD-active. We designed our field experiment accordingly and baited traps with 7Me7Me alone, and in binary, ternary or quaternary combinations with the minor candidate pheromone components. All traps baited with 7Me7Me alone as a single lure constituent, or as part of a blend, captured – on average – nearly 1200-times more *A. ferrugineipennis* males than unbaited control traps (Fig. 2.4), supporting the conclusion that 7Me7Me is the major sex pheromone component of female *A. ferrugineipennis.*

Although octanal, nonanal and 6,10,14-trimethyl-2-pentadecanone are emitted by females and sensed by males (Fig. 2.2), they do not seem to play a role as synergistic sex attractant pheromone components (Fig. 2.4). Trap lures with or without these compounds were equally effective in attracting very large numbers of *A. ferrugineipennis* males. Conceivably, however, these compounds may express pheromonal activity when presented together with 7Me7Me at blend ratios wider, or narrower, than tested in our study. Alternatively, one or more of these compounds may have a pheromonal function in the context of species or mate recognition rather than mate attraction. If not, it would seem perplexing that *A. ferrugineipennis* females emit components which are both chemically diverse (ester, ketone, aldehydes) and recognized by males.

7-Methyloctyl 7-methyloctanoate, together with 7-methyloctyl 5-methylhexanoate, 7-methyloctyl octanoate and 7-methyloctyl (Z)-4-decenoate has previously been identified in pheromone gland extracts of female *Elater ferrugineus* (Tolasch et al. 2007), a rare predatory elaterid species inhabiting deciduous trees in Europe (Ranius et al. 2011). A synthetic blend of these four esters was field-tested and shown to attract *E. ferrugineus* males (Tolasch et al. 2007). A follow-up study (Svensson et al. 2012) then revealed that the pheromonal activity resides with 7-methyloctyl (Z)-4-decenoate as a single component. In electrophysiological recordings that tested the four esters, only 7-methyloctyl (Z)-4-decenoate elicited responses from male E. ferrugineus antennae, and in a field trapping experiment, only lures containing 7-methyloctyl (Z)-4-decenoate effectively attracted *E. ferrugineus* males, with the other three esters not contributing to the attractiveness of lures. While 7-methyloctyl 7-methyloctanoate (7Me7Me) has no pheromonal function in *E. ferrugineus*, it is the major sex attractant pheromone component of female *A. ferrugineipennis* (Figs. 2.2, 2.4) and is reported here as a new pheromone in the Insecta.

As 7Me7Me is produced by females of both *A. ferrugineipennis* (Elaterinae: Agriotini) and *E. ferrugineus* (Elaterninae: Elaterini), which represent two taxonomically distinct tribes with non-overlapping geographic distribution (Becker 1956; Tolasch et al. 2007; Nieto and Alexander 2010), it follows that the biosynthetic ability to produce 7Me7Me has evolved independently at least twice in the Elateridae, even though thus far it is a pheromone component only in A. ferrugineipennis.

The molecular structure of 7Me7Me differs from currently known *Agriotes* sex pheromones (Tóth 2013) which are commonly geranyl esters (Yatsynin and Rubnova 1983; Yatsynin et al., 1980, 1991; Tóth et al., 2002, 2003; Siirde et al. 1993), farnesyl esters (Yatsynin et al. 1980, 1991; Tóth et al. 2003; Tolasch et al. 2022) and – rarely – neryl esters (Tolasch et al. 2010; Tolasch and Steidle 2022). Within the *Agriotes* genus, *A. ferrugineipennis* is placed in the Limosus group (Becker 1956) for which no sex pheromone was known prior to our study. It is conceivable that other species in the *Limosus* group produce sex pheromones similar to 7Me7Me but more species in this group must be studied before any generalization is warranted.

In conclusion, trap lures containing 7Me7Me were exceedingly attractive to mate-seeking males, suggesting that 7Me7Me may be the major, and possibly the only, sex attractant pheromone component of female *A. ferrugineipennis*. Octanal, nonanal and

6,10,14-trimethyl-2-pentadecanone in the headspace of *A. ferrugineipennis* are all sensed by males but do not seem to serve as (synergistic) sex attractant pheromone components. Our prediction that they function in the context of species or mate recognition, rather other mate attraction, will need to be tested in another study.

2.6. References

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2.7. Tables

Table 2.1. Mean (SEM) proportions, and estimated total numbers, of *Agriotes ferrugineipennis* males (relative to all click beetles captured) collected in traps left unbaited (control) and in traps baited with 7-methyloctyl 7-methyloctanoate (7Me7Me) alone and in combinations with the aldehydes octanal and nonanal, the ketone 6,10,14-trimethyl-2-pentadecanone, and both the aldehydes and the ketone (Pemberton, British Columbia, 12 April to 31 May 2021; N = number of replicates).

Collection date	*N*	Stimuli tested				
		Unbaited	7Me7Me	7Me7Me + aldehydes	7Me7Me + ketone	7Me7Me + aldehydes + ketone
Mean (SEM) proportion of male *A. ferrugineipennis*						
19 April	5	0 (0)	0.03 (0.03)	0.50 (0.00)	0.52 (0.27)	0.13 (0.13)
26 April	5	0 (0)	0.38 (0.19)	0.38 (0.18)	0.38 (0.17)	0.42 (0.14)
03 May	5	0.21 (0.15)	0.87 (0.07)	0.96 (0.02)	0.99 (0.01)	0.99 (0.01)
10 May	5	0.18 (0.12)	0.94 (0.04)	0.97 (0.02)	0.98 (0.01)	0.97 (0.03)
17 May	5	0 (0)	0.99 (0.01)	0.97 (0.03)	0.98 (0.02)	0.99 (0.01)
24 May	5	0 (0)	0.93 (0.06)	0.91 (0.05)	0.88 (0.07)	0.89 (0.06)
31 May	5	0 (0)	0.83 (0.13)	0.89 (0.10)	0.78 (0.20)	0.81 (0.12)
total beetles collected		151	8246	6694	7708	8384
total beetles identified		88	506	480	488	490
Mean (SEM) number of male *A. ferrugineipennis*						
19 April	16	0 (0)	0.3 (0.1)	1.8 (0.7)	9.9 (3.6)	1.1 (0.4)
26 April	16	0 (0)	10.6 (4.1)	7.5 (2.7)	16.4 (5.6)	15.4 (6.0)
03 May	16	0.1 (0.1)	42.6 (19.6)	38.6 (18.6)	68.6 (25.2)	61.2 (29.4)
10 May	20	0.2 (0.1)	109.5 (21.9)	90.8 (16.9)	105.9 (16.3)	122.1 (19.7)
17 May	20	0 (0)	180.6 (25.0)	145.2 (20.4)	139.0 (17.2)	165.5 (19.2)
24 May	20	0 (0)	30.6 (6.3)	26.2 (5.1)	19.5 (4.4)	28.1 (4.5)
31 May	20	0 (0)	8.9 (3.2)	11.1 (3.4)	6.4 (2.0)	8.2 (1.6)
Sum		0.3 (0.1)	372.2 (59.5)	311.5 (47.9)	346.6 (48.8)	386.0 (54.5)

Table 2.2. Retention indices of straight-chain and methyl-branched esters on each of four GC columns. Note retention index matches between beetle-produced component 5 and synthetic 7-methyloctyl 7-methyloctanoate on each of four GC columns.

	Retention indices			
Compounds	DB-5	DB-210	DB-23	FFAP
Beetle-produced component 5 (in Fig. 2)	**1900**	**2184**	**2183**	**2128**
nonyl nonanoate	1975	2245	2278	2224
7-methyloctyl nonanoate	1937	2214	2230	2176
7-methyloctyl 7-methyl octanoate	**1900**	**2184**	**2183**	**2128**
6-methyloctyl 6-methyl octanoate	1917	2205	2215	2157
5-methyloctyl 5-methyl octanoate	1888	2149	2172	2116

2.8. Figures

Figure 2.1. (a, b) Photographs of a Vernon Pitfall Trap® placed in the field for testing candidate pheromone components (a) and in unobstructed view to reveal the trap bottom and lid (b); (c) a representative sample of *Agriotes ferrugineipennis* males captured in a single pheromone-baited trap over the course of seven days in Pemberton, British Columbia; (d) dorsal and ventral views of a single *A. ferrugineipennis* male, scale bar = 6 mm.

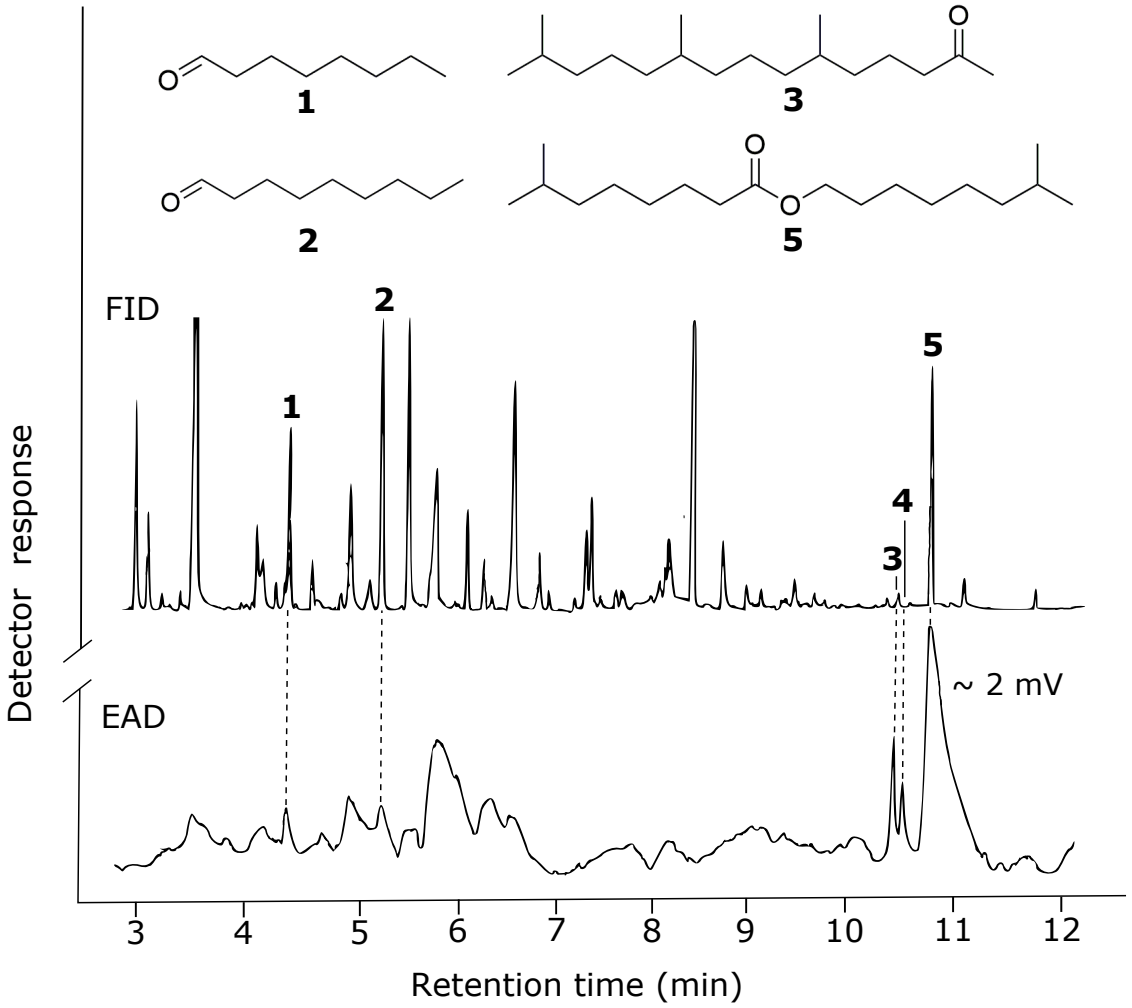

Figure 2.2. Representative responses of a gas chromatographic flame ionization detector (FID) and an electroantennographic detector (EAD: antenna of a male *Agriotes ferrugineipennis*) to aliquots of Porapak Q headspace volatile extract from conspecific females. Compounds 1, 2, 3, and 5 were identified as octanal (1), nonanal (2), 6,10,14-trimethyl-2-pentadecanone (3), and 7-methyloctyl 7-methyloctanoate (5). Compound 4 was tentatively identified as 6,10,14-trimethyl-2-pentadecanol, but the amount present in the extract was not sufficient to obtain a mass spectrum for confirmation. Other apparent antennal responses could not be repeated on various GC columns. Chromatography: DB-5 column; temperature program: 50 °C for 1 min, then 20 °C · min−1 to 280 °C.

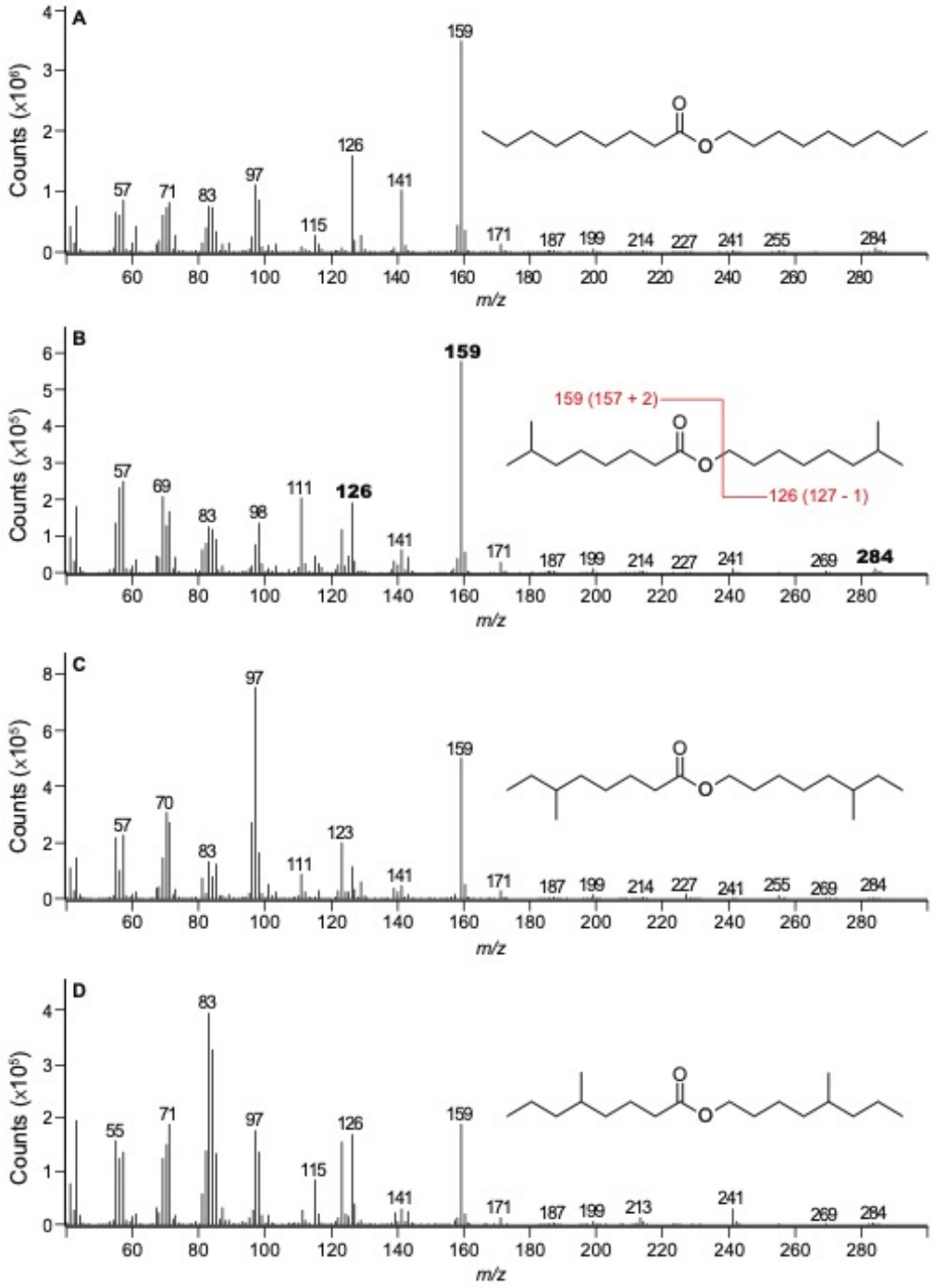

Figure 2.3. Mass spectra of synthetic nonyl nonanoate (A), 7-methyloctyl 7-methyloctanoate (7Me7Me) (B), 6-methyloctyl 6-methyloctanoate (C), and 5-methyloctyl 5-methyloctanoate (D) on a 5977A MSD (Agilent Technologies Inc.) coupled to a 7890B GC fitted with a DB-5MS column. The mass spectrum of 7Me7Me matched that of beetle-produced component 5 in Figure 2.2.

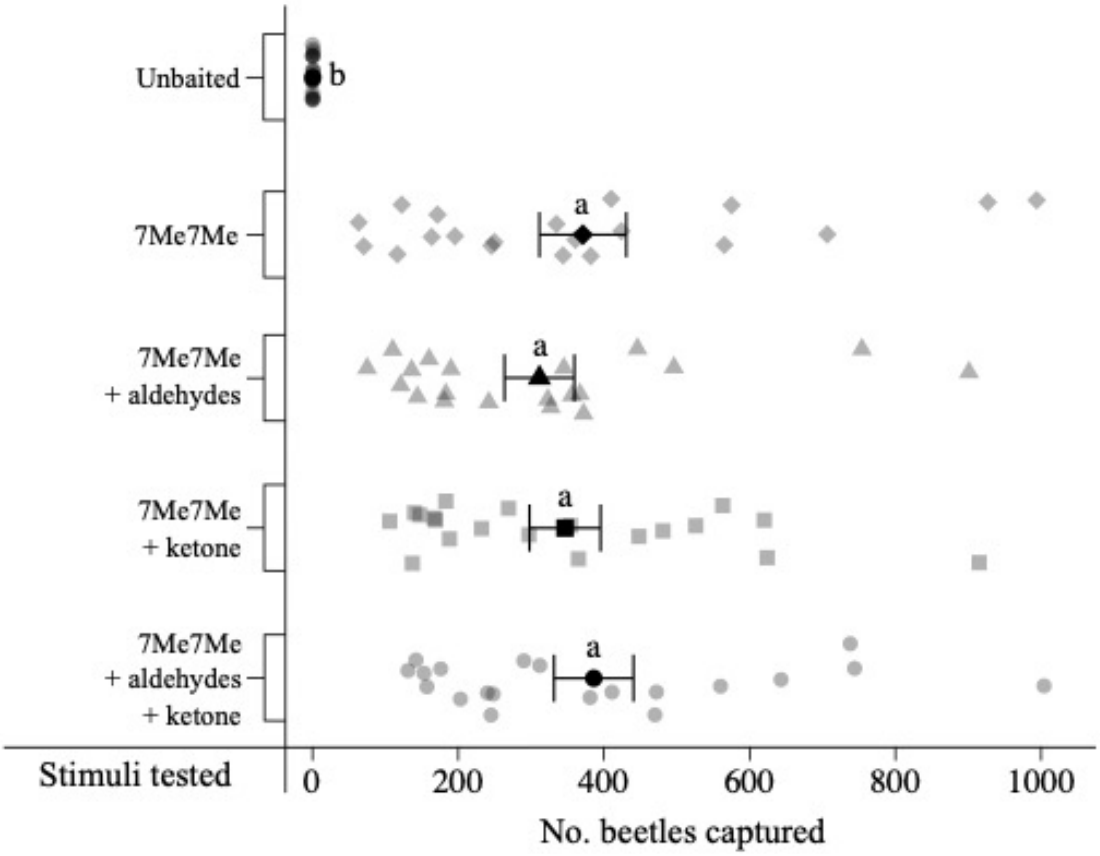

Figure 2.4. Captures of male click beetles, *Agriotes ferrugineipennis*, in a field experiment run near Pemberton (British Columbia) between 12 April and 03 May 2021 (N = 16 during weeks 1–3; N = 20 during weeks 4–7). Five treatments were tested: (1) unbaited (control); (2) 7-methyloctyl 7-methyloctanoate (7Me7Me) (10 mg); (3) 7Me7Me (10 mg) plus the aldehydes octanal (1 mg) and nonanal (1 mg); (4) 7Me7Me (10 mg) plus the ketone 6,10,14-trimethyl-2-pentadecanone (1 mg); and (5) 7Me7Me (10 mg) plus the two aldehydes (1 mg each) and the ketone (1 mg). Grey and black symbols show the number of beetles captured in each replicate and on average (mean ± standard error), respectively. Means with different letters indicate statistically significant differences in trap captures (generalized linear model fitted with a negative binomial distribution and a log link function; $P < 0.05$). Within each treatment, the data appear at slightly different heights due to a jitter function of the software program that is applied when the plot is produced.

Chapter 3.

Sex pheromone of Nearctic *Agriotes mancus* and its similarity to that of three Palearctic *Agriotes* invasive in North America[1]

[1]A near identical version of this chapter is published in Agricultural and Forest Entomology (2023, https://doi.org/10.1111/AFE.12568) with the following authors: Singleton, K, van Herk, W.G., Saguez, J., Scott, I., Gries, R., Gries, G.

KS, WvH & GG conceived the study; JS captured beetles for pheromone analyses and took the photograph in figure 3.1; KS & RG captured headspace volatiles; RG analyzed volatile extract as well as model compounds by GC-EAD and GC-MS; JS and IS ran field experiments; KS identified and determined the sex of beetles captured in traps; WvH analyzed capture data statistically; KS, WvH & GG wrote the first draft, and all authors reviewed and approved of the final draft.

3.1. Abstract

The wheat wireworm, *Agriotes mancus* (Coleoptera: Elateridae), is a predominant elaterid pest species in the Nearctic region, with a life history and morphology similar to those of *Agriotes obscurus*, *Agriotes lineatus* and *Agriotes sputator*, three Palearctic pest elaterids invasive in North America. Here, we report the identification and field testing of the sex pheromone of *A. mancus*. We collected headspace volatiles from female beetles on Porapak Q, and analyzed aliquots of Porapak extract by gas chromatographic - electroantennographic detection (GC-EAD) and by GC-mass spectrometry. In GC-EAD recordings, two esters – geranyl butanoate and geranyl hexanoate – elicited antennal responses from *A. mancus* males. In field experiments, trap lures containing both geranyl butanoate and geranyl hexanoate afforded large captures of *A. mancus* males, which were – on average – approximately 30-fold higher than captures in traps baited with a single ester. Traps baited with geranyl butanoate as a single-component lure captured a significant number of Palearctic *A. sputator*, indicating the establishment of A. sputator in its invaded Nearctic range. With the *A. mancus* sex pheromone now known, it can be included in the development of pheromone-based programs to monitor and manage native and invasive *Agriotes* pests in North America.

3.2. Introduction

Click beetles (Coleoptera: Elateridae) are a remarkably diverse family found worldwide. The larvae of agricultural pest species feed on economically valuable crops such as grains, forage, and potatoes (Traugott, 2015; Poggi et al., 2021; Vernon & van Herk, 2022). Worldwide, the genus *Agriotes* is of agricultural and economic importance, with several species considered significant pests (Becker, 1956; Ritter & Richter, 2014; Vernon & van Herk, 2022). Whereas the loss of revenue from physical damage that elaterid larvae inflict on crops in Canada is difficult to assess, on Prince Edward Island the annual potato crop damage due to wireworms, primarily the potato wireworm, *Agriotes sputator* (Linnaeus), and the associated costs for wireworm control were estimated at 10 million dollars in 2018 (King, 2018).

The wheat wireworm, *Agriotes mancus* (Say), is common in central and eastern Canada and in the north-eastern United States (Becker, 1956; van Herk & Vernon, 2014; Saguez et al., 2017; van Herk et al., 2021a), and is one of the most damaging Nearctic *Agriotes* species (Comstock & Slingerland, 1891; Rawlins, 1934; Glen et al., 1943). The life history of *A. mancus* resembles that of Palearctic *A. lineatus, A. obscurus,* and *A. sputator*, which have become pests in Canada since their establishment in the 1800s (Eidt, 1953; Becker, 1956; van Herk & Vernon, 2014; van Herk et al. 2021d; Vernon & van Herk, 2022). Larvae of *A. mancus* are known to feed on cereals, corn, potatoes, and field-grown vegetables (van Herk & Vernon, 2014).

The adult beetles of *A. mancus* (Fig. 3.1) are small (6.5–8.5 mm), often brown with gold-coloured pubescence, and with a pronotum that is slightly wider than long (Becker, 1956; Brooks, 1960). Typically, the beetles disperse by walking but – like *A. obscurus* – fly at higher temperatures (i.e., >20 ºC) (La France, 1967). According to life history studies, *A. mancus* completes a 4- to 6-year life cycle. After a 3- to 5-year larval stage, and overwintering in the adult stage, adults emerge between April and June, reproduce and lay eggs in May and June (Pettit, 1872; Comstock & Slingerland, 1891; Rawlins, 1934, 1940; LaFrance, 1967).

Synthetic sex pheromone lures of click beetles are useful tools to (*i*) monitor population trends of pest and endangered beetle species (Svensson et al., 2012; Vernon & van Herk, 2022), (*ii*) help predict crop damage by correlating beetle captures in pheromone-baited traps with larval abundance in the soil and with crop damage (Furlan

et al., 2020; Vernon et al., 2020), (*iii*) time seasonal abundance and insecticidal control measures (Ester & van Rozen, 2005; Vernon & van Herk, 2022), (*iv*) delineate the geographic distribution of species (Vernon et al., 2001; Subchev et al., 2006; Musa et al., 2013), (*v*) detect the presence and monitor the spread of invasive species (Singleton et al., 2022b), and (*vi*) track displacement of native species by invasive species (e.g., van Herk et al., 2021c). Also, in jurisdictions such as Canada where insecticides are being deregistered, or in organic production systems where insecticides must not be applied, synthetic sex pheromones are being developed for control of adult beetle populations through mass trapping, mating disruption, and attract & kill tactics (e.g., Vernon et al., 2014a,b; Reddy & Tangtrakulwanich, 2014; Kabaluk et al., 2015; van Herk et al., 2022a; Vernon & van Herk, 2022).

Sex pheromones of Palaeartic *Agriotes* spp. are commonly farnesyl esters (Yatsynin et al. 1980, 1991; Tóth et al. 2003; Tolasch et al. 2022), geranyl esters (Yatsynin and Rubnova 1983; Yatsynin et al., 1980, 1991; Tóth et al., 2002, 2003; Siirde et al. 1993), and – rarely – neryl esters (Tolasch et al. 2010; Tolasch and Steidle 2022). Prior to this study, the sex pheromone components of Neartic *A. mancus* were not known. Indeed, to date, sex pheromones have been identified for only nine click beetle species endemic to North America, including *Cardiophorus tenebrosus* and *Cardiophorus edwardsi* (Serrano et al., 2018), *Melanotus communis* (Williams et al., 2019), *Limonius canus* and *L. californicus* (Gries et al., 2021; van Herk et al., 2021b), *Selatosomus aeripennis destructor* (Gries et al., 2022), *Idolus californicus* (Serrano et al., 2022), *Agriotes ferrugineipennis* (Singleton et al., 2022a) and *Parallelostethus attenuatus* (Millar et al., 2022). Here, we report the identification of sex pheromone components of Nearctic *A. mancus* and reveal their structural and biosynthetic relatedness to those of Palearctic *Agriotes* congeners.

3.3. Materials and methods

3.3.1. Field collection of beetles

Beetles were collected using Vernon Pitfall Traps (van Herk et al., 2018; available from Intko Supply Ltd., Chilliwack, BC, CA), in May 2020 at the Centre de recherche sur les grains (CÉROM) near Saint-Mathieu-de-Beloeil, Quebec. Traps were placed along the perimeter of wheat fields and were baited with synthetic pheromone of *A. lineatus*

[geranyl butanoate and geranyl octanoate (1:1), 40 mg] or *A. obscurus* (geranyl hexanoate and geranyl octanoate (1:1), 40 mg], and captured live beetles were sent to Simon Fraser University, Burnaby, BC, for identification and sex determination. Beetles were identified to species using a taxonomic key (Becker, 1956), whereas the sex of beetles was determined based on their genitalia which were extruded by applying light pressure to the abdominal sternum. Field collections afforded only two females and 30 males which were kept, separated by sex, in plastic cups (140 mL; Fisher Scientific, Ottawa, ON, CA) with perforated lids to facilitate air exchange. Drawing on the protocol of a previous study (Singleton et al., 2022a), cups contained fresh grass and moist Kimwipes (delicate task wipers; Fisher Scientific, Ottawa, ON, CA) for both moisture and walk-on substrate, and small pieces (2 × 2 cm) of apple for food. Both cups were maintained at low temperature (~ 4 °C) to extend the beetles' longevity. Apple pieces were replaced once a week or when they had become soft and moldy, and Kim wipes (Fisher Scientific, Ottawa, ON, CA) were remoistened as needed. To reduce beetle mortality, cups were replaced every two weeks or when a beetle had died. Prior to collecting the beetles' headspace volatiles, cups were warmed to room temperature and the grass was replaced with a moist Kim wipe.

3.3.2. Collection of headspace volatiles

Headspace volatiles were acquired from two separate groups of two female and seven male *A. mancus* in two 24-h collections, following a protocol previously detailed (Gries et al., 2021). Briefly, 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 2 mL of pentane/diethyl ether (1:1; each >98% chemically pure; Fisher Scientific, Pittsburgh, PA, USA) and the extract was concentrated to 100 µL for analyses.

3.3.3. Gas chromatography with electroantennographic detection (GC-EAD) analyses

Aliquots of Porapak Q extracts, and of synthetic standards, were analyzed on a Hewlett-Packard 5890 gas chromatograph (GC) (Agilent Technologies Inc., Santa Clara, CA, USA) fitted with a flame ionization detector (FID) and one of four GC columns (DB-5, DB-210, DB-23, Free Fatty Acid Phase (FFAP); all 30 m × 0.32 mm ID; film thickness 0.25 µm; Agilent J & W column). For GC-EAD recordings (Gries et al., 2002), two GC columns (DB-5, FFAP) were used. were used. Helium served as the carrier gas (35 cm · s^{-1}) with the following temperature programs: 100 °C for 1 min, then 20 °C · min^{-1} to 220 °C (FFAP) or 280 °C (DB-5). The injector port and FID were set to 260 °C and 280 °C, respectively. For each GC-EAD recording, an antenna with its tip cut off 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 & Everton, 1980). Each antenna (n = 5) was utilized in only one GC-EAD recording. Antennal responses to compounds in the column effluvium – that was directly released into a stream of medical non-humidified 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.).

3.3.4. GC-mass spectrometric (MS) analyses

Headspace volatiles that elicited antennal responses were deemed candidate pheromone components (CPCs) and were analyzed by GC-MS, using both a Varian Saturn 2000 Ion Trap GC-MS and an Agilent 7890B GC coupled to a 5977A MSD (both Agilent Technologies Inc.). Both instruments were operated 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 · s^{-1}). The injector port, ion trap and transfer line of the Saturn Ion Trap were set to 250 °C, 200 °C and 280° C, respectively, and the temperature program was as follows: 50 °C for 5 min, 10 °C · min^{-1} to 280 °C (held for 10 min). The injector port of the Agilent MS was set to 250 °C, the MS source to 230 °C, and the MS quadrupole to 150 °C, using the same temperature program as for the Saturn Ion Trap. To identify CPCs in Porapak-Q headspace volatile extract, their

retention indices (Van den Dool & Kratz, 1963) and mass spectra (70 eV) were compared with those of authentic standards that were purchased.

3.3.5. Chemicals

Geranyl butanoate (> 95% chemically pure) and geranyl hexanoate (> 98%) were purchased from Penta Manufacturing (Fairfield, NJ, USA). Neryl butanoate and neryl hexanoate were synthesized by esterification of butyric acid and hexanoic acid (both Sigma-Aldrich; 99%), respectively, with nerol (Fluka; >90%).

3.3.6. Field trapping experiments

Candidate pheromone components were tested at CEROM (Saint-Mathieu-de-Beloeil, Quebec) in field experiments 1 (2020) and 2 (2021), near the location (45.5826° N, 73.2375° W) where beetles were collected for pheromone identification. Both experiments used a complete randomized block design. In experiment 1 (n = 12), Vernon pitfall traps were placed at ground level along a field's edge, with 10-m spacing between treatments in each replicate, and with >100-m spacing between replicates. Traps were placed on 26 June 2020 (towards the end of the beetles' swarming period), and captured beetles were collected every 2–3 days until 24 July. In experiment 2 (n = 24), traps were placed with 10-m and 100-m spacing between treatments and replicates, respectively. Traps were placed on 05 May 2021, and captured beetles were collected every 2–4 days until 18 June. For both experiments, traps were baited with synthetic CPCs that 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. Each of experiments 1 and 2 had four treatments: (1) an unbaited control; (2) geranyl butanoate (40 mg); (3) geranyl hexanoate (40 mg); and (4) geranyl butanoate (40 mg) and geranyl hexanoate (40 mg). The 40-mg dose for each ester was chosen because it is proven effective in commercial lures for *A. lineatus* and *A. obscurus,* and it made lure replacement unnecessary during the trapping season. Captured beetles were identified and their sex was determined per treatment (trap captures of the same treatment combined) in 2020, and per individual trap in 2021.

3.3.7. Identification of captured beetles

Click beetles were identified to species using a taxonomic key (Becker, 1956). Specimens that were taxonomically ambiguous were identified solely based on genitalia characteristics. Specimens with missing genitalia or otherwise badly damaged were excluded from analysis. All beetles collected were identified to species. Hume Douglas (Agriculture and Agri-Food Canada, Ottawa) confirmed the identity of voucher specimens which are retained at the Agassiz Research and Development Centre (Agassiz, BC, CA).

3.3.8. 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 in SAS Enterprise Guide v.7.1 (SAS Institute, Cary, NC, USA). Analyses were performed on the total number of *Agriotes* spp. captured in 2020 (collection dates, species, and sexes combined), and on *A. mancus* or *A. sputator* males (collection dates combined) in 2021.

3.4. Results

3.4.1. Identification of candidate pheromone components

GC-EAD analyses of headspace volatile extracts of female *A. mancus* revealed two components (1, 2 in Fig. 3.2) that elicited responses from male *A. mancus* antennae. Both compounds were unique to females according to total ion chromatograms (GC-MS) of male and female headspace volatiles. We focused identification efforts exclusively on EAD-active 1 and 2, because only compounds that are sensed by olfactory receptors can possibly be sex attractant pheromone components and elicit behavioural responses. The mass spectra of 1 and 2, with diagnostic fragmentation ions *m*/*z* 69, 93, 121 and 136, revealed the isoprene-characteristic backbone of geraniol or nerol, indicating that both compounds were esters of geraniol or nerol. Compound 1 was tentatively identified as geranyl butanoate based on the diagnostic acyl fragment *m*/*z* 71 and the molecular ion *m*/*z* 224. Similarly, compound 2 was tentatively identified as geranyl hexanoate

based on the acyl fragment *m*/*z* 99 and the molecular ion *m*/*z* 252. Also, the retention indices (Van den Dool & Kratz, 1963) of compounds 1 (DB-5: 1556; DB-210: 1796; DB-23: 1918; FFAP: 1911) and 2 (DB-5: 1749; DB-210: 2000; DB-23: 2124; FFAP: 2104) differed by about 200 units on each of the four GC columns, further substantiating the tentative molecular assignments. GC-MS analyses of beetle-produced 1 and 2 (1:1 ratio), and of authentic esters, then confirmed that 1 and 2 were geranyl butanoate (1) and geranyl hexanoate (2). Synthetic neryl butanoate and neryl hexanoate eluted substantially earlier than 1 and 2, respectively.

3.4.2. Field experiments

In experiment 1, captures of *Agriotes* beetles varied between treatments (χ^2 = 20.3, df = 3,33, P = 0.0001). Over the entire trapping period, traps baited with geranyl butanoate or with a blend of geranyl butanoate and geranyl hexanoate captured, on average, 3.5 (± 2.6) and 2.8 (± 0.9) beetles, respectively, whereas traps baited with geranyl hexanoate captured, on average, only 0.3 (± 0.2) beetles. Of the 42 *Agriotes* males captured in traps baited with geranyl butanoate, 32 were identified as *A. sputator* and one as *A. mancus.* In contrast, of the 33 male beetles captured in traps baited with a blend of geranyl butanoate and geranyl hexanoate, 31 were identified as *A. mancus*. The remaining two male beetles were identified as *Agriotes pubescens*.

In experiment 2, large captures of both *A. mancus* (752 males) and *A. sputator* (217 males), the latter species not found prior to 2020 in central Canada, warranted separate data analyses for both species. The number of *A. mancus* males captured varied with treatment (χ^2 = 135.1, df = 3,85, $P < 0.0001$) and replicate (χ^2 = 40.2, df = 7,85, $P < 0.0001$) (Fig. 3.3A). Most beetles [season-long mean across replicates (henceforth 'mean'): 29.2 (± 4.6)] were captured in traps baited with a blend of geranyl butanoate and geranyl hexanoate, and significantly fewer beetles were captured in traps baited with geranyl hexanoate [mean: 1.3 (± 0.3)], geranyl butanoate [mean: 0.8 (± 0.4)], or left unbaited [mean: 0.2 (± 0.1)] (Fig. 3.3A). Trap lures containing both esters afforded captures of *A. mancus* males which were – on average – approximately 150-fold higher than those in unbaited control traps.

The number of *A. sputator* males captured also varied with treatment (χ^2 = 94.5, df = 3,85, $P < 0.0001$) and replicate (χ^2 = 27.9, df = 7,85, P =0.0002) (Fig. 3.3B). Most beetles [mean: 8.2 (± 2.2)] were captured in traps baited with geranyl butanoate, and

significantly fewer beetles were captured in traps baited with the blend of geranyl butanoate and geranyl hexanoate [mean: 0.8 (± 0.2)], or left unbaited [mean: 0.04 (± 0.04)].

3.5. Discussion

Geranyl butanoate and geranyl hexanoate are synergistic sex pheromone components of *A. mancus*. Both esters were emitted by female beetles and captured in headspace volatile collections, elicited antennal responses from conspecific males in electrophysiological recordings, and synthetic lures disseminating both esters in field experiments attracted significantly more *A. mancus* males than single-esters lures. Lower captures of beetles in experiment 1 (26 June to 24 July 2020) than in experiment 2 (05 May to 18 June 2021) could possibly be attributed to late-season trapping in experiment 1 when beetle activity was already declining.

The 2-component sex pheromone of Nearctic *A. mancus* (geranyl butanoate & geranyl hexanoate) reported here resembles the 2-component sex pheromone of Palearctic *A. obscurus* (geranyl hexanoate & geranyl octanoate; Tóth et al., 2003) and *A. lineatus* (geranyl butanoate & geranyl octanoate; Tóth et al., 2003) but differs from the single-component sex pheromone of Palearctic *A. sputator* (geranyl butanoate; Tóth, 2003). All four congeners belong to the *Mancus* complex within the genus *Agriotes* (Becker, 1956). They could maintain specificity of their sexual communication systems through at least three mechanisms: (1) the pheromone blends of *A. mancus*, *A. obscurus* and *A. lineatus* all differ in one of their two major components (Tóth et al., 2003; Tóth, 2013); (2) both pheromone components need to be present in the blend to attract male *A. mancus*, *A. obscurus* and *A. lineatus*, whereas a single component (geranyl butanoate) is sufficient to strongly attract male *A. sputator* (Tóth et al., 2003; Tóth, 2013); and (3) attraction of male *A. sputator* to conspecific female-produced geranyl butanoate is suppressed in the presence of geranyl hexanoate (this study), which is a pheromone component of both female *A. mancus* (this study) and female *A. obscurus* (Tóth et al., 2003). Whether seasonal or diel activity periods of *A. mancus* and of the three Palaearctic invaders are sufficiently different to contribute to reproductive isolation is not yet known. As the distribution range of Nearctic *A. mancus* did not overlap with that of Palearctic *A. obscurus, A. lineatus* and *A. sputator* prior to their invasion of the Nearctic range in the 1800s (Brown 1940; Eidt, 1953; Becker, 1956;

Vernon & van Herk, 2022), there was no selection pressure on *A. mancus* to produce sex pheromone components dissimilar to those of the three Palearctic invaders.

With esters identified as sex pheromone components in the Nearctic elaterids *A. mancus* (this study) and *A. ferrugineipennis* (Singleton et al., 2022a), and with esters commonly reported as sex pheromones in Palearctic *Agriotes* congeners (Tóth et al., 2003; Tóth, 2013), there is emerging evidence that the pathway for pheromone biosynthesis is conserved among new- and old-world *Agriotes* congeners. However, pheromones of further *Agriotes* species in North America would need to be identified to substantiate the emerging evidence, and genus assignment of current species would need to be confirmed by DNA barcoding. Considerable overlap in pheromone components produced by *Agriotes mancus* (geranyl butanoate & geranyl hexanoate), *A. obscurus* (geranyl hexanoate & geranyl octanoate), *A. lineatus* (geranyl butanoate & geranyl octanoate) and *A. sputator* (geranyl butanoate) supports the placement of all four species in the genus *Agriotes*. Conversely, the major sex pheromone component of *Agriotes ferrugineipennis*, 7-methyloctyl 7-methyloctanoate (Singleton et al., 2022a), more closely resembles the sex pheromone of *Elater ferrugineus* (Tolasch et al., 2007), casting doubt whether *Agriotes ferrugineipennis* is indeed placed in the correct genus. Rigorous DNA barcoding of Palaearctic and Neartic *Agriotes* spp. would help definitively resolve phylogenetic relationships and confirm, or refine, current taxonomic placements.

As some of the key elaterid pest species in North America (e.g., *Limonius* spp., *Agriotes* species mentioned herein) have similar life histories and their larval wireworms inflict comparable types of crop damage (Vernon & van Herk, 2022), it may be more efficient to assess the combined population densities of all elaterids present, and their combined pest impact, rather than the abundance and impact of individual species. Combining the sex pheromones of multiple elaterid species in a single trap lure to monitor or mass trap populations of co-occurring elaterids is of interest to farmers. This strategy has been tested in integrated pest management programs for other insect taxa, such as cerambycid beetles (e.g. Sweeney et al., 2014; Hanks et al. 2018; Fan et al., 2019; Rice et al. 2020), moths (e.g. Brockerhoff et al. 2013; Knight et al., 2014; Preti et al. 2020), true bugs (Yasuda et al. 2010; Kim et al. 2015), and mealybugs (Waterworth et al. 2011; Sullivan et al., 2023). As a key feature of such a lure, the pheromone for each species must express the same level of attractiveness as it would in

a single pheromone lure. Thus, compatibility of all pheromones in that same lure must be carefully assessed. For example, limoniic acid – the major sex pheromone component of *L. canus* and *L. californicus* (Gries et al., 2021) – does not reduce attraction and captures of other click beetle taxa such as *S. a. destructor* and *A. lineatus* that co-occur with *Limonius* spp. (van Herk et al., 2021b). In contrast, sex pheromones of *Agriotes* spp. suppress cross-attraction of some sympatric congeners. For example, combining the sex pheromone of *A. obscurus* and *A. lineatus* in a single trap lure, with the intention to capture males of both species, reduced lure attractiveness to *A. obscurus* males 5-fold relative to the *A. obscurus* specific lure (Vernon et al., 2014b, van Herk et al., 2022b). Similarly, the pheromone for *A. mancus* comprising geranyl butanoate and geranyl hexanoate, with the former ester being the single-component sex pheromone of *A. sputator* (Tóth, 2003; van Herk et al., 2021d), strongly suppressed attraction of *A. sputator* males relative to the *A. sputator* specific pheromone (this study). These data indicate that the presence of particular *Agriotes* species in a given location can be missed unless (*i*) there is prior knowledge about the click beetle biodiversity, (*ii*) traps are deployed with single, species-specific *Agriotes* pheromone lures, or (*iii*) specific blends have been tested, and components have been demonstrated not to interfere with optimal attraction of all target species. Based on data currently available, it seems that pheromones of distantly related taxa, but not of sympatric congeners with contrasting pheromones, can be combined in a single lure without compromising optimal attractiveness of each pheromone to the target species. This inference, however, would need to be proven correct prior to the development of commercial lures that contain sex pheromones of multiple species.

3.6. Conclusion

We report the identification of the *A. mancus* sex pheromone as a blend of two synergistically active pheromone components: geranyl butanoate and geranyl hexanoate. The pheromone blend was identified by capturing headspace volatiles of females, analysing headspace volatile extract through a combination of electrophysiological recordings and mass spectrometric analyses, and testing synthetic trap lures in field experiments. The sex pheromone of Nearctic *A. mancus* resembles that of Palearctic *A. lineatus*, *A. obscurus* and *A. sputator,* indicative of a shared biosynthetic pathway. Significant captures of *A. sputator* males in traps baited with

geranyl butanoate in two consecutive years indicate establishment of *A. sputator* in its invaded North American range (Singleton et al., 2022b).

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3.8. Figures

Figure 3.1. Dorsal view of a male *Agriotes mancus*. The composed image was obtained by stacking and stitching, using a VHX digital microscope (150× magnification; Keyence, Mississauga, ON, CA).

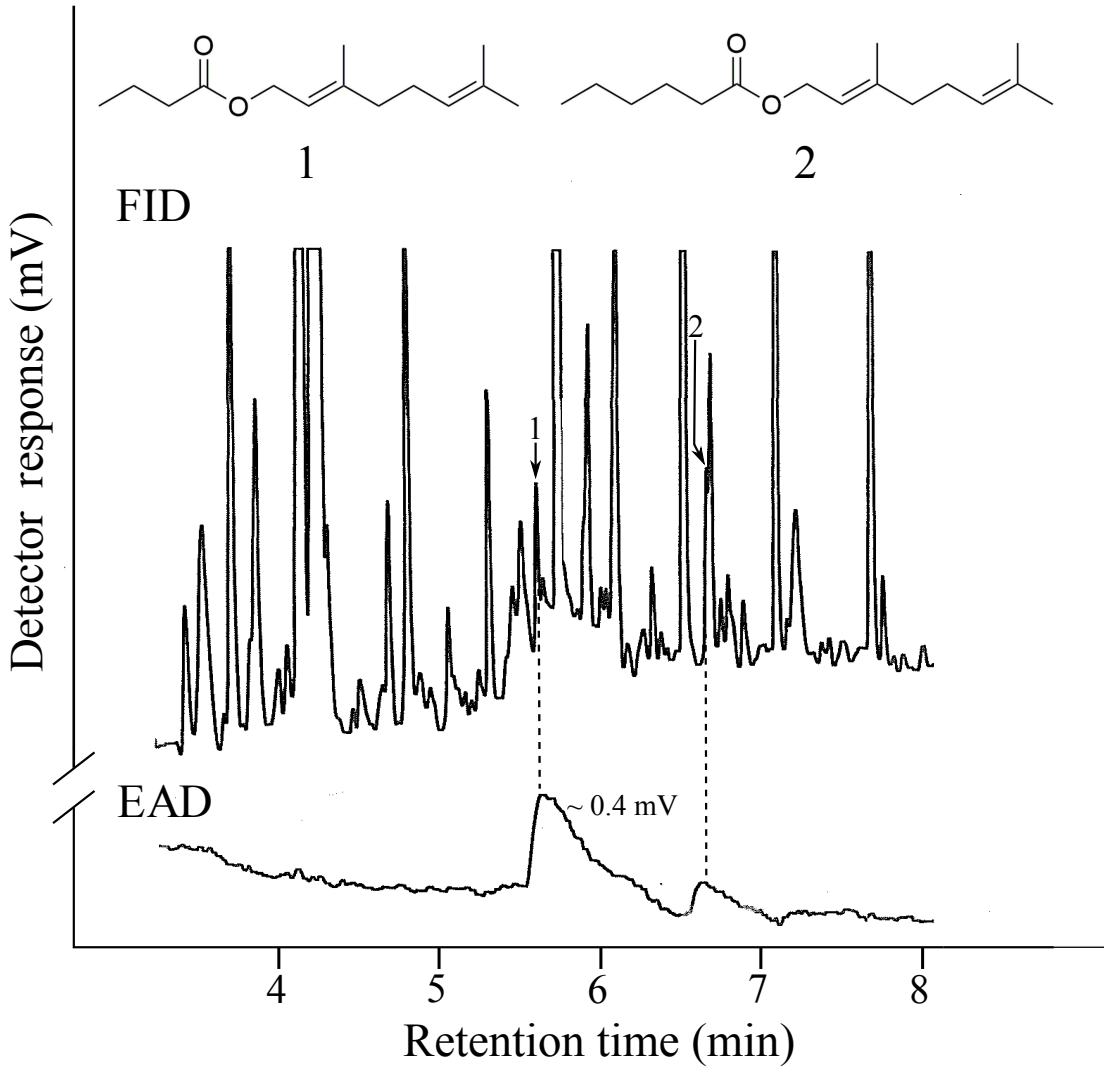
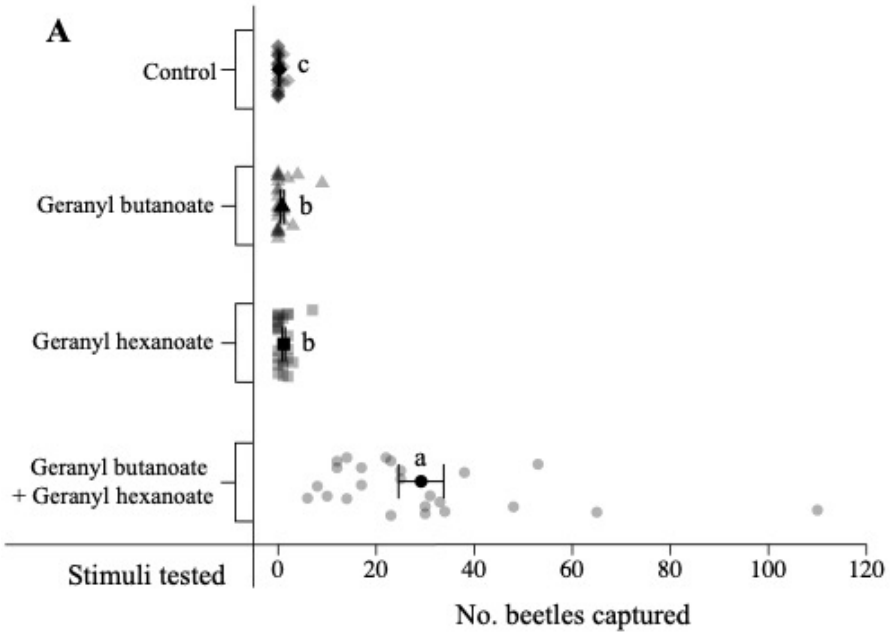


Figure 3.2. Representative responses of a gas chromatographic flame ionization detector (FID) and an electroantennographic detector (EAD: antenna of a male *Agriotes mancus*) to an aliquot of Porapak Q headspace volatile extract from conspecific females. Compounds 1 and 2 were identified as geranyl butanoate (1) and geranyl hexanoate (2). Chromatography: DB-5 column; temperature program: 50 °C for 1 min, then 20 °C · min^{-1} to 280 °C.


A
Control
Geranyl butanoate
Geranyl hexanoate
Geranyl butanoate
+ Geranyl hexanoate
c
b
b
a
Stimuli tested
0
20
40
60
80
100
120
No. beetles captured

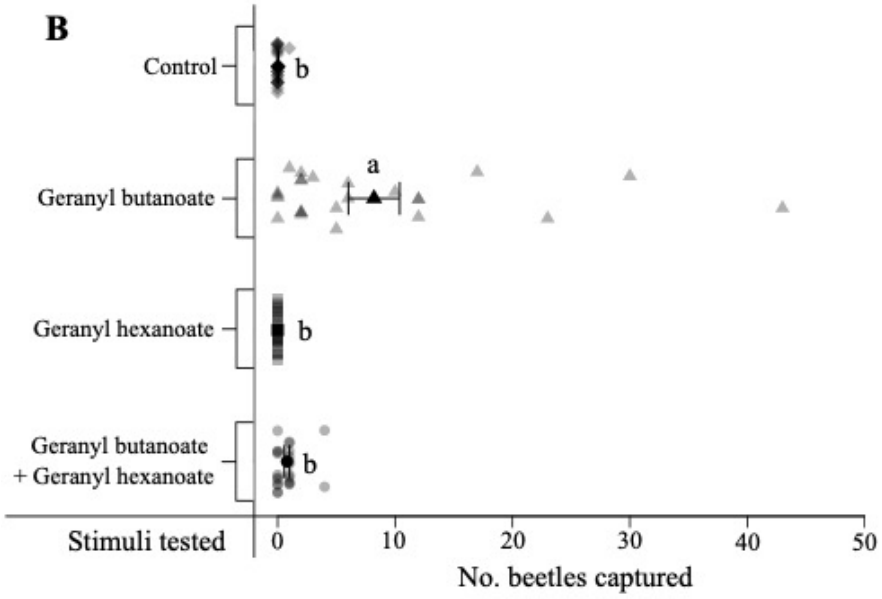

B
Control
Geranyl butanoate
Geranyl hexanoate
Geranyl butanoate
+ Geranyl hexanoate
b
a
b
b
Stimuli tested
0
10
20
30
40
50
No. beetles captured

Figure 3.3. Captures of male *Agriotes mancus* (A) and *A. sputator* (B) in field experiment 2 (n = 24) run near Saint Mathieu-de-Beloeil (Quebec, Canada) between 05 May and 18 June 2021. Four treatments were tested: (1) unbaited (control); (2) geranyl butanoate (40 mg); (3) geranyl hexanoate (40 mg); (4) geranyl butanoate (40 mg) and geranyl hexanoate (40 mg). Grey and black symbols show the number of beetles captured in each replicate over the entire season and on average across replicates (mean ± standard error), respectively. Means with different letters indicate statistically significant differences in trap captures (generalized linear model fitted with a negative binomial distribution and a log link function; $P < 0.05$). Within each treatment, the data appear at slightly different heights due to a jitter function of the software program that is applied when the plot is produced.

Chapter 4.

Spectral sensitivity of click beetles (Coleoptera: Elateridae) and their responses to light stimuli in laboratory and field experiments

Singleton, K, van Herk, W.G., Pickett, C., Blake, A., Asad, S., Furtado, K., Saguez, J., Gries, G.

KS, WvH & GG conceived the study; AB designed methodology for electroretinograms and measuring light intensity; KS, CP, WVH, SA, KF & JS ran experiments, KS, WvH, & JS captured beetles for electroretinogram and light studies; KS, WVH and JS ran field experiments; KS identified and determined the sex of beetles captured in traps; KS, WvH & AB analyzed data statistically; KS wrote the first draft, and all authors reviewed and approved of the final draft.

4.1. Abstract

With increasingly fewer insecticides registered to control the larvae of pest click beetles (Coleoptera: Elateridae), integrative beetle management, including pheromone- and light-based trapping of adult beetles, must be explored as an alternative strategy. Here, we analyzed the spectral sensitivity and color preference of nine elaterids across six genera in electrophysiological recordings and in behavioural bioassays. In electroretinogram recordings (ERGs), dark-adapted beetles were exposed to narrow wavebands of light in 10-nm increments from 330 nm to 650 nm. All beetles proved most sensitive to green (515–538 nm) and ultra-violet (UV) light (~360 nm). In four-choice bioassay arenas with three light emitting diodes [LEDs; green (525 nm), blue (470 nm), red (655 nm)] and a dark control as test stimuli, beetles discriminated between test stimuli, being preferentially attracted to green and blue LEDs. In field experiments, Vernon pitfall traps® fitted with a green, blue or white LED captured significantly more male and female *Agriotes lineatus* and *A. obscurus* than dark control traps. When traps were baited with green or blue LEDs at light intensities that differed by 10-fold, the traps baited with higher light intensity lures captured numerically more beetles but trap catch data in accordance with light intensity did not differ statistically. Light-based trapping may be a viable tool for monitoring elaterid species known not to have pheromones.

4.2. Introduction

Wireworms, the larvae of pestiferous elaterid beetles, impact both the yield and quality of economically important crops, such as potatoes, grains, and cereals (Traugott et al., 2015; Nikoukar & Rashed, 2022). Crop damage will likely increase over time because invasive elaterids continue to expand their range (Traugott, 2015; Poggi et al., 2021; Vernon & van Herk, 2022; Singleton et al., 2022), necessitating the development of novel monitoring and management tools (Poggi et al. 2021; Vernon & van Herk 2022).

Light-based trapping is one potential tactic to study the behaviour, activity periods, and population trends of agricultural, stored-product, and forest beetle pests. Light-based trapping or other light-based technologies have been deployed to determine the flight periods of adult beetles (Amorós et al. 2022), monitor population dynamics of agricultural pest beetles (Al-Deeb et al. 2012; Amorós et al. 2022), assess beetle diversity (Hebert et al., 2000; Pablo-Cea et al. 2022), measure vertical stratification of beetles in their inhabited ecosystem (Stork et al., 2016), repel stored-produce pest beetles (Kim et al., 2013; Miyatake et al. 2016), curtail population size (Arakaki et al., 2015; Santi et al. 2022), capture and identify invasive beetle species (Cruz-López et al. 2022), and serve as a push-pull system in timber processing plants (Pawson et al., 2009). Because sex pheromones are typically species-specific communication signals (Regnier and Law, 1968) and are effective at long range (Phillips, 1997), synthetic sex pheromone lures are routinely deployed to manipulate the behaviour of target pest beetles. However, as synthetic sex pheromone lures attract the signal-receiving but not the signal-emitting sex, the signalling sex – commonly females – is inadvertently excluded from estimates of population trends or beetle control measures. This exclusion may result in inaccurate population estimates, may miss movements of female populations, and ultimately may misinform pest management decisions.

Many beetle taxa rely on vision to locate mates (Szentesi et al. 2003; Yang et al. 2017; Wang et al. 2022), forage for hosts (Szentesi et al. 2003) or other resources (Harmon et al. 1998; Szentesi et al. 2003; Wang et al. 2022), and to initiate or continue flight (Boiteau, 2012). Surprisingly, vision research on elaterid beetles is lagging behind. To date, the spectral sensitivity is not known even for the most severe elaterid pests in the Holarctic, and only a few reports have documented spectral sensitivity or captures of elaterid pests in light-based traps. In the earliest study (Genung, 1972), aerial black light

traps were deployed in Southern Florida and captured moderate numbers of the corn wireworm, *Melanotus communis*, the southern potato wireworm, *Conoderus falli*, and the elaterid *Glyphonyx bimarginatus*. In 2010, Lall et al. reported the spectral sensitivity of four neotropical, bioluminescent click beetles, documenting that all four species show a strong preference for green light and a moderate preference to light in the near ultra-violet (UV) range. Presumably, this sensitivity is fine-tuned for their specific bioluminescent signals, but it remains unknown whether this fine-tuned sensitivity is representative for all elaterids. In a 2015 field study in Atlantic Canada, white-light solar powered “Noronha Elaterid Light Traps” (NELTs) were effective at capturing both male and female *Agriotes sputator* and *Hypnoidus abbreviatus* (Noronha, unpubl. data).

Electroretinogram and intracellular recordings inform the selection and design of light stimuli for light traps, with wavelength and intensity of these stimuli being adjustable to capture target species (Cohnstaedt et al., 2008). Our objectives in this study were to (1) determine the spectral sensitivity of nine elaterid species, (2) record attraction of elaterids to light stimuli in laboratory experiments, and (3) determine field captures of elaterids in light traps in three locations in Canada.

4.3. Materials and methods

4.3.1. Field collection of beetles

In May and June of 2020 and 2022, nine elaterid species were collected in several locations in Canada (Table 1) for electroretinogram recordings and behavioral bioassays. Upon arrival of beetles in the laboratory (Simon Fraser University (SFU), Burnaby, BC, CA), they were sorted by sex and species (in groups of ~20) (Becker, 1956), if they had not been identified prior to shipment. Beetles were maintained in separate plastic cups (140 mL; Fisher Scientific, Ottawa, ON, CA) with perforated lids to facilitate air exchange. Cups contained fresh grass for both moisture and walk-on substrate for beetles, and small pieces (2 × 2 cm) of apple for food. All cups were kept at a low temperature (~ 4 °C) to extend the beetles’ longevity. Apple pieces were replaced once a week or when they had become soft and moldy, and Kimwipes (delicate task wipers; Fisher Scientific, Ottawa, ON, CA) were remoistened as needed. To reduce beetle mortality, cups were replaced every two weeks or when a beetle had died.

4.3.2. Electroretinograms

4.3.2.1 Preparation of beetles and electrodes

In preparation for electroretinogram recordings, a single beetle was taken from cold storage and immediately immobilized ventral side down on a glass microscope slide covered in playdough (The Michaels Companies Inc., TX, USA). A small ball of wax (SpofaDental a.s., 506 46 Jicin, CZ) was placed under the beetle's head, angling it upward to gain better access to its eyes. Finally, the beetle's abdomen and thorax were covered in a thin layer of playdough, leaving the right dorsal prothorax exposed. Electrodes (1.2 × 0.69 × 100 mm; Sutter Instrument Novato, CA, USA) were formed with a micropipette puller (Model P-1000, Sutter Instrument Co., CA, USA), fitted with a silver wire (0.084 mm outer diameter) and filled with Ringer's solution (Staddon & Everton, 1980), yielding a resistance of 1–10 MΩ.

4.3.2.2. Electroretinograms recording

The spectral sensitivity of compound eye photoreceptors was determined through electroretinogram (ERG) recordings in a Faraday cage, drawing on a protocol previously detailed (Peach et al., 2019; Peach & Blake, 2023). To this end, the microscope slide carrying the immobilized beetle was secured on a platform below a binocular microscope (Wild M10, Leica Microsystems, ON, CA). A micromanipulator (Leitz, Vienna, AT) was used to insert the recording electrode into the dorsal anterior region of the left eye, and the reference electrode into the upper right region of the prothorax. The light source was positioned ~0.5 cm above, and perpendicular to, the exposed eye. The Faraday cage was then closed, and the beetle was left in darkness for 30 min to dark-adapt.

Light stimuli of narrow wavebands were generated using a 35-Watt Xenon Arc light source (HPX-2000, Ocean Optics, Dunedin, FL, USA), and a fibre optic scanning monochromator (MonoScan 2000, Mikropak GmbH, Ostfildern, DE). A 600-µm optical fibre (QP600-1-SR-B X, Ocean Optics) fitted with a collimator (LC-4U-THD, Multimode Fiber Optics, Hackettstown, NJ, USA) transmitted the light from the monochromator through a 0–2 log circular variable neutral density wheel (fused silica, 200–2500 nm; Reynard Corp., San Clemente, CA, USA) which was positioned directly in front of a 20:80 beam splitter ("polka dot" 4-2001; Optometrics, Ayer, MA, USA). The smaller portion of the light was directed to a calibrated cosine-corrector-fitted (CC-3-UV-S, Ocean Optics) spectrophotometer (HR-4000, Ocean Optics), and the absolute irradiance

was determined using SpectraSuite software (Ocean Optics). The remaining portion of the light was directed towards the beetle's eye via a second collimator attached to a 1000-µm single fibre optic cable (PCU-1000-2-SS, Multimode Fiber Optics), with a Sub-Miniature-A (SMA) terminus. A custom-built programmable shutter (R. Holland, Science Technical Centre, SFU) located between the beam splitter and the collimator was manually opened for 0.5 s every 10 s to expose the eye to a test stimulus at an intensity of 1.0×10^{13} photons/cm^2/s.

In one continuous series, each eye was exposed to 66 narrow wavebands, ascending in 10-nm increments from 330 nm to 650 nm, and then descending back to 330 nm. For wavelengths above 600 nm, a Lexan filter was placed in front of the light, but before the neutral density wheel, to avoid the production of a secondary UV peak in these ranges.

The recorded voltages were low-pass filtered at 1 kHz and amplified 10× by the electrometer (Duo 773 electrometer, World Precision Instruments, Sarasota, FL, USA) before being digitized by the data acquisition system (Lab-Trax-4/16, World Precision Instruments, Sarasota, FL, USA) at 1 kHz and recorded in LabScribe (v4, iWorx Systems Inc, Dover, NH). Sensitivity at each wavelength was then determined by comparing the response voltages to an intensity–response function determined for each beetle. The sensitivities across the spectral range (330–650 nm) were then normalized by their 97.5% quantile value (giving a range roughly between 0–1), and then averaged between the down-sweep (650–330 nm) and up-sweep (330–650 nm) to give final sensitivity values for each beetle. The sensitivities of multiple beetles were averaged across recordings to give a mean and standard error for sensitivity across the spectral range.

ERGs were recorded for nine species (with replicates reported in parentheses): *Agriotes ferrugineipennis* males and females (5 each); *Corymbitodes moerens* males and females; *Selatosomus aeripennis destructor* males and females (5 each); *Aeolus mellilus* females (5); *Hypnoidus abbreviatus* females (5); *Agriotes obscurus* males and females (5 each); *Agriotes lineatus* males and females (5 each); *Agriotes pubescens* males (5); and *Limonius canus* males (5) and females (4).

4.3.3. Laboratory experiments

4.3.3.1. Experimental design

To test attraction of beetles to light stimuli, we designed and built 4-choice bioassay arenas (Figure 4.1), with all parts purchased from Southern Irrigation (Chilliwack, BC, CA). Arenas consisted of a central 4-way polyvinyl chloride (PVC) cross connector (**1**; Figure 4.1 a,b) into which four PVC pipes (**2**; 21 × 4.2 cm) were inserted, each with a sanded interior to facilitate beetle movement. Pipes were further connected to a T-shaped PVC tube (**3**; 10.2 × 5 cm). A PVC adaptor (**4**; 4 cm × 4.2 cm diameter) at the distal end of **3** accommodated a PVC cap (**5**; 4.2 cm × 5 cm diameter) housing an inward-facing light emitting diode (LED) (**6**), which was secured with hot glue and silicone in the lid opening (~0.5 cm) of a 50-mL Falcon® (**7**) (Fischer Scientific, Ottawa, ON, CA) hot-glued to the PVC cap. LED wires (**8**) were funneled through a small hole (2 cm diameter) in PVC caps, and were connected to a 12-V battery (MK Powered, Toronto, ON, CA) in an adjacent room, with one battery assigned to each of two bioassay arenas. The third arm of **3** pointed downwards into a clear cup (**9**; 140 mL; Fischer Scientific) which served as a 'pitfall trap' for responding beetles.

All experiments were run in the Research Annex on the Burnaby Campus of SFU during May and June of 2021, and during June to August of 2022. For all experiments, the room lights were turned off, and the room temperature was maintained at 23–25 °C. To initiate a bioassay, a single beetle was placed in an open vial (**10**; 2.5 cm × 2.3 cm diameter) which was then inserted into a hole (2.2 cm diameter) of the PVC cross (**1**), allowing the beetle to enter the bioassay arena and to respond to light stimuli. Circular layers (6 cm) of cheesecloth (eight in Exp. 2; 16 in Exps. 3–7) (Western Family, Save on Foods, Burnaby, BC, CA) were placed between **4** and **7** to dim light intensities in arenas. All beetles ending up in a pitfall trap (**9**) within 40 min of entering the arena were considered responders. All other beetles were considered non-responders and were excluded from statistical analyses. Between replicates, bioassay arenas were dismantled and all parts (**1-4**, **9**, **10**) that beetles could have contacted during bioassays were cleaned using Sparkleen (Fischer Scientific).

4.3.3.2. Spectroscopy and LED calibrations

Prior to placing LEDs in the laboratory or field (see section below), they were calibrated in the laboratory using a spectrophotometer (HR-4000, Ocean Optics). The intensity

(photons/cm^2/s) of LEDs was measured 5 cm from the tip of the spectrophotometer through a black heat shrink tube (0.8 cm diam). Intensity output was recorded using SpectraSuite software (Ocean Optics). Measurements were taken from single LEDs (green, blue, red), two LEDs (UV), and three LEDs (2 UV and 1 green). The reflectance of the cheesecloth and the Styrofoam half domes (Fig. 4.8) were measured with a JAZ spectrometer (Ocean Optics) calibrated with a 99% Spectralon reflectance standard (SRS-99-010, Labsphere, NH) and were found to reflect evenly in the near UV and human visible range.

4.3.3.3. Side bias experiments

In 2021, two experiments were run to test for potential side-bias, using *A. ferrugineipennis* males which were abundantly available. In experiment 1 (n = 35), each arena arm (**2**; Figure 4.1 a,b) was fitted with a small piece of black velvet fabric secured with twist ties to the outside of the PVC cap (**5**) to ensure that no light could enter the arena and that its interior was completely dark. In four replicates at a time, a choice was recorded when a beetle fell into the cup (**9**) that was immediately below the T-tube (**3**). In experiment 2 (n = 12), a single green LED (525 nm, LED INL-5AG30; Digikey, Thief River Falls, MN, USA) was mounted in each arena arm and set to an intensity of 1.40 E+16 photons/cm^2/s. In each PVC cap (**5**), 16 circular layers of cheesecloth (6 cm diam) were placed in front of each LED to dim the light in the arena. Two replicates were run at a time, and similar to experiment 1, a choice was recorded when a beetle fell into a pitfall trap cup (**9**).

4.3.3.4. Colour preference testing (Exps. 3-7)

In bioassay arena (see above) experiments 3–7, four treatments were randomly assigned (Random.org) to one of the four PVC pipes: (1) no LED (dark control), (2) a red LED (655 nm, MCL053RHC; Newark, Chicago, IL, USA), (3) a blue LED (470 nm, C503B-BCN-CV0z; Digikey), and (4) a green LED (520 nm, Digi Key). All LEDs were set to the same intensity (2.2×10^{15} photons/cm^2/s), with eight circular layers of cheesecloth (6 cm diam) secured in front of LEDs to help diffuse light and dim brightness. A response was scored, when a beetle fell into a pitfall trap (**9**). Experiments tested the response of *S. destructor* males (Exp. 3, n = 21) and females (Exp. 4, n = 42), *A. pubescens* males (Exp. 5, n = 54)*, L. canus* males (Exp. 6, n = 15), and *A. lineatus* males (Exp. 7, n = 18).

4.3.4. Field experiments

4.3.4.1. Trap design and trapping protocol

A modified Vernon pitfall trap® (van Herk et al. 2018; available from Intko Supply Ltd., Chilliwack, BC, CA) (Figure 4.1 c,d) was deployed in all experiments. Two wooden dowels (**11**; 0.8 × 22 cm; Home Depot, Toronto, ON, CA) were inserted into holes in the trap bottom rim (**12**) to support a black corrugated plastic board trap lid (**13**; 0.43 × 24 × 24 cm; Michaels, Mississauga, ON, CA) hot-glued to the dowels 10 cm above the trap bottom rim. A 50-mL plastic Falcon® tube (**14**), with an LED (**15**) hot-glued in a hole (~ 0.5 cm) of the Falcon® tube's lid, was suspended with a 19-gauge galvanized steel wire (**16**; Home Depot, Toronto, ON, CA) between the dowels. Each LED was connected to a circuit board that was fitted with both a potentiometer (CT-94W-103, DigiKey) and a trimmer resistor (PV36W203C01B00; DigiKey) to allow for fine-tune calibration of light intensity. The LED's wire (**17**) was connected via a double male-female non-insulated adapter (210613, Ancor; Menomonee Falls, WI, USA) which, in turn, hooked onto a central battery (Figure 4.1e). A Styrofoam half sphere (**18**; 1.8 cm diam; Michaels, Mississauga, ON, CA), glued with its flat side to the trap's lid, served as a reflecting surface to evenly distribute the LED light. The following LEDs (all 5 mm in size) were used in field experiments: green (525 nm, LED INL-5AG30), blue (470 nm, C503B-BCN-CV0z), white (C513A-WSN-CW0Z0152) (all from Digikey), red (655 nm, MCL053RHC) and UV (365 nm, LED EOLD-365-525) (both from Newark).

All field experiments were run using a complete randomized block design, with approximately 5-m spacing between traps in each replicate and 10–14 m between replicates. All four (or five) treatments in each experimental replicate were laid out in a square (Figure 4.1e), with a rechargeable 12-V battery (**19**; Exps. 8–10: MK 12-Volt 18AH SLA Battery, Canadian Tire; Panasonic LC-R127R2P; Exps. 11,12: 12V 7Ah, DigiKey) inside a Rubbermaid bin (**20**) in the centre, 4.5 m from each trap. Battery size determined the time interval between charges. In experiments 8–10 and 11–12, batteries were replaced every five and two days, respectively, to ensure sustained optimal LED outputs. Once every week, all beetles captured in each trap were collected. All beetles were identified to the species level based on a taxonomic guide (Becker, 1956), and their sex was determined.

4.3.4.3. Colour preference testing (Exp. 8)

In experiment 8 (26 April to 31 May 2021), traps were placed along the edge of a crop-rotated potato field in Pemberton, British Columbia (50.429236 ° N, -122.907198° W), known to have high populations of several elaterid species. In each replicate (n = 7), four treatments were tested: (1) dark control, (2) one green LED (1.40 E+16 photons/cm2/s), (3) two UV LEDs (each 7.10E+15 photons/cm2/s), and (4) one green LED (1.40 E+16 photons/cm2/s) together with two UV LEDs (each 7.10E+15 photons/cm2/s).

4.3.4.4. Colour preference testing (Exps. 9–11)

Experiments 9–11 were set up in three locations: Exp. 9 – Pemberton, British Columbia (50.429236° N, -122.907198° W); Exp. 10 – Saint Mathieu-de-Beloeil, Quebec (45.5826° N, 73.2375° W), and Exp. 11 – Agassiz, British Columbia; (49.242003° N, -121.76571° W). In experiment 9 (n = 7; 11 May to 09 June 2022), traps were placed within a crop-rotated potato field. In experiment 10 (n = 7; 17 May to 21 June 2022), traps were placed along the side of a wheat field, and in experiment 11 (n = 6; 22 April to 29 June 2022), traps were placed in a grassy field immediately behind a highbush blueberry planting. In experiments 9 and 10, four treatments were tested: (1) dark control, (2) one blue LED, (3) one green LED, and (4) one red LED (all LEDs calibrated to 1.40E+16 photons/cm^2/s). Experiment 11 tested the same four treatments and one white LED (1.40E+16 photons/cm^2/s) as a fifth treatment.

4.3.4.5. Light intensity preference testing (Exp. 12)

Experiment 12 (n = 6; 22 April to 29 June 2022) was set up in a grassy field at the Agassiz Research and Development Centre (Agassiz, British Columbia; 49.242003° N, -121.76571° W). Five treatments were tested: (1) dark control, (2) one blue LED at high light intensity (2.7E+16 photons/cm^2/s), (3) one blue LED at low light intensity (2.7E+15 photons/cm^2/s), (4) one green LED at high light intensity (2.7E+16 photons/cm^2/s), and (5) one green LED at low light intensity (2.7E+15 photons/cm^2/s).

4.3.5. Statistical analyses

4.3.5.1. Electroretinograms

Green opsin templates, based on peak absorbance wavelength of the alpha band (λ_{max} α), were generated, using parameters from Stavenga (2010) based on a template from Govardovskii et al. (2000). A green opsin template was used for all species and both

sexes tested. Whereas sensitivity in the UV region was observed, a UV template was not applied, because most species lacked a UV peak that was also explained by the green template. Moreover, the possible presence of a screening pigment in the eye (Lall et al., 2010) may significantly shift the spectral sensitivity of UV receptors from the underlying UV template. Analyses were run and graphs were prepared using R statistical software (v4.1.2; R Core Team 2021).

4.3.5.2. Laboratory and field experiments

Laboratory and field data were analyzed with, and graphs were prepared using R statistical software (v4.1.2; R Core Team 2021). Laboratory colour preference data were analyzed by χ^2-tests, and pairwise comparisons between treatments used the 'Ryans test' (Ryan, 1960). Field data were analyzed with a generalized linear mixed models using a negative binomial distribution and a log link function to account for overdispersion in our count data (Brooks et al. 2017). All models incorporated block into the models as series of random intercepts. To test for a treatment effect for each species and sex within an experiment, a model including the color and/or intensity treatment factor was compared to a null model that included only random effects using a likelihood ratio test. To test for sex specific differences, we compared models with and without an interaction between sex and treatment, again with a likelihood ratio test. Pairwise comparisons between treatments in were analyzed using a general linear hypothesis statement with Tukey's adjustment (Hothorn et al. 2008). Analyses were run on the total number of *Agriotes* spp. captured in each experiment (collection dates combined). Captures in experiment 10 were too low to warrant statistical analyses.

4.4. Results

4.4.1. Electroretinograms

Following 30 minutes of dark adaptation, eight of the nine species studied exhibited two spectral sensitivity peaks: a primary peak in the green range and a secondary (smaller) peak in the UV range. In contrast, male and female *A. ferrugineipennis* had peak spectral sensitivity in the UV range (~360 nm) and secondary sensitivity in the green range, at 520 nm for males and 526 nm for females (Figure 4.2a).

Female *C. moerens* showed near equal sensitivity to UV (~360 nm) and green (521 nm) light, whereas male *C. moerens* were most sensitive to green (527 nm) and less sensitive to UV light (Figure 4.2b).

Male *S. destructor* responded most strongly to a 515-nm wavelength, the lowest peak sensitivity wavelength across all recordings (Figure 4.2c). The peak green sensitivity wavelength of female *S. destructor*, *A. mellillus* and *H. abbreviatus* was similarly low, ranging between 517 nm and 518 nm (Figure 4.2d,e). Conversely, the primary spectral sensitivity wavelength of *A. obscurus* and *A. lineatus* was about 20 nm higher. For male and female *A. obscurus,* green sensitivity peaked at 536 and 538 nm, respectively (Figure 4.2f), and for male and female *A. lineatus* it peaked at 536 and 534 nm, respectively (Figure 4.2g). Male *A. pubescens*, and male and female *L. canus*, were similarly sensitive in the green range (531–533 nm), with males exhibiting peak sensitivity at 532 and 533 nm, and females at 531 nm (Figure 4.2h,i).

4.4.2. Laboratory experiments

With no side bias detectable in experimental arenas, we proceeded with colour preference experiments. When offered the four treatment stimuli of no light (dark), red (655 nm), blue (470 nm) and green (525 nm) light, male and female *S. destructor* discriminated between the four treatments (males: $\chi^2 = 19.25$, $df = 3$, $p < 0.0001$; females: $\chi^2 = 9.2$, $df = 3$, $p < 0.05$; Figure 4.3a,b), but male *A. pubescens* did not ($\chi^2 = 7.14$, $df = 3$, $p < 0.06$; Figure 4.3c). Trend-wise, male and female *S. destructor* and male *A. pubescens* favored blue and green. The response rates of both male *L. canus* and *A. lineatus* were too low to warrant statistical analyses, but male *A. lineatus* seem to prefer green (Figure 4.3e).

4.4.3. Field experiments

4.4.3.1. Colour preference testing (Exp. 8)

In experiment 8, captures of *A. lineatus* in dark control traps and in traps baited with green (525 nm), UV (365 nm), and both green and UV light varied with treatment (Figure 4.4a), both for males ($\chi^2 = 7.76$, $df = 3$, $p = 0.051$) and females ($\chi^2 = 8.89$, $df = 3$, $p = 0.031$), but the effect of treatment differed between the sexes ($\chi^2 = 10.78$, $df = 3$, $p = 0.013$). Males, but not females, were captured significantly more often in UV-lit traps

than in dark control traps. While females, were caught significantly more in green-lit traps than UV-lit traps. When the model was fit to *A. lineatus* males poor model fit was observed. When the outlier in the UV treatment was removed, the fit was resolved however the treatment effect disappeared. As it is likely that this trap was in a beetle dense area in the field, the outlier was kept as the data point is still relevant to our study. Trap captures of *A. obscurus* also varied with treatment, both for males (χ^2 = 18.25, *df* = 3, $p < 0.001$; Figure 4.4b) and females (χ^2 = 16.37, *df* = 3, $p < 0.001$; Figure 4.4b), revealing no sex-specific treatment responses (χ^2 = 2.77, *df* = 3, $p = 0.43$). More males and females were captured in green-lit traps than in dark control traps.

4.4.3.2. Colour preference testing (Exps. 9-11)

In experiment 9, captures of *A. lineatus* in dark control traps and in blue-lit (470 nm), green-lit (525 nm), or red-lit (655 nm) traps varied with treatment (Figure 4.5a) for females (χ^2 = 11.59, *df* = 3, $p = 0.009$) but not for males (χ^2 = 4.99, *df* = 3, $p = 0.17$), with 3.7-times more females captured in blue-lit traps than in dark control traps and no sex-specific treatment responses (χ^2 = 1.99, *df* = 3, $p = 0.57$). Low captures of *A. obscurus* (Figure 4.5b) did not reveal discrimination between treatments for males (χ^2 = 2.81, *df* = 3, $p = 0.42$) and captures of females in experiment 9 and beetles in experiment 10 (Saint Mathieu-de-Beloeil, Quebec) were too low to be analyzed.

In experiment 11, captures of *A. lineatus* in dark control traps and in blue-lit (470 nm), green-lit (525 nm), and white- or red-lit (655 nm) traps (Figure 4.6a) varied with treatment for males (χ^2 = 13.70, *df* = 4, $p = 0.008$; Figure 4.6a) but not for females (χ^2 = 6.90, *df* = 4, $p = 0.14$; Figure 4.6a), with nearly 3-times more males captured in blue-, green-, or white-lit traps than in dark control traps. Similarly, captures of *A. obscurus* varied with treatment (Figure 4.6b), both for males (χ^2 = 27.037, *df* = 4, $p < 0.0001$) and for females (χ^2 = 48.27, *df* = 4, $p < 0.0001$). Blue-, green- and white-lit traps all captured – on average – 2.8- and 4.6-times more males and females, respectively, than dark control traps. Male and female *A. lineatus* responded similarly to treatment stimuli (χ^2 = 1.51, *df* = 4, $p = 0.83$), as did male and female *A. obscurus* (χ^2 = 6.034, *df* = 4, $p = 0.20$).

4.4.3.3. Light intensity preference testing (Exp. 12)

In experiment 12, captures of *A. lineatus* in dark control traps and in traps baited with green (525 nm) or blue (470 nm) lights at low or high intensity (2.7E+15 photons/cm^2/s

and 2.7E+16 photons/cm^2/s, respectively) varied with treatment for males (χ^2 = 16.54, *df* = 4, *p* = 0.002) but not for females (χ^2 = 4.85, *df* = 4, *p* < 0.0001) (Figure 4.7a), with significantly more males captured in high blue-lit traps than in dark control traps. Captures of *A. obscurus* also varied with treatment (Figure 4.7b), both for males (χ^2 = 33.47, *df* = 4, *p* < 0.0001) and for females (χ^2 = 28.371, *df* = 4, *p* < 0.0001), with significantly more males and more females captured in blue- or green-lit traps irrespective of light intensity. Male and female *A. lineatus* responded similarly to treatment stimuli (χ^2 = 3.40, *df* = 4, *p* = 0.55), as did male and female *A. obscurus* (χ^2 = 3.04, *df* = 4, *p* = 0.55).

4.5. Discussion

Four main results were obtained in our study of elaterid spectral sensitivity and attraction to light stimuli: (1) all nine species studied exhibited spectral sensitivity in the green and UV range; (2) blue, green and white LEDs (the latter containing blue and green light) were attractive in field settings to male and female *Agriotes* spp.; (3) UV light elicited responses from photoreceptors but on its own was not attractive to beetles nor did it enhance the attractiveness of green LEDs; (4) green and blue LEDs as trap lures set to low and high light intensity afforded similar captures of male and female *A. obscurus*, suggesting that light intensity is not a critical factor for beetle captures, at least not within the intensity range (2.70 E+15 photons/cm^2/s to 2.70 E+16 photons/cm^2/s) tested here. Below, we shall elaborate on these results.

In electroretinogram recordings, eight of the nine elaterid species studied exhibited primary spectral sensitivity in the green range and secondary sensitivity in the UV range. Only male and female *A. ferrugineipennis* had peak spectral sensitivity in the UV range, and female *C. moerens* were equally sensitive to both UV and green light. Overall, these results are consistent with electroretinogram data for most insects under dark adaptation (Briscoe and Chitka, 2001), and for four bioluminescent elaterids (*Fulgeochlizus bruchii*, *Pyrearinus termitilluminans*, *Pyrophorus punctiatissimus*, *P. divergeus*) native to Brazil (Lall et al. 2010). All four species showed broad sensitivity peaks in the green range with a smaller secondary peak ('shoulder') in the near UV range, suggesting the presence of short- and long-wavelength tuning between visual receptors and bioluminescence emissions, as shown in lampyrid fireflies (Lall et al. 1980, 1982; Eguchi et al. 1984). In contrast, none of the elaterids investigated here is

bioluminescent and none is known to use visual signals for communication in the context of mate finding or recognition. Drawing on findings that many elaterids, including most of our study species (Tóth et al., 2003; Tóth, 2013; Gries et al., 2021, 2022; Singleton et al., 2022) use pheromone signals for sexual communication and that *Limonius* click beetles are active during the daytime (Lemke et al. 2022), it follows that the green sensitivity of our study species may play a role in a context other than mate location or recognition. Conceivably, green-sensitive photoreceptors may guide males and females to rendezvous sites, or gravid females to oviposition sites such as grassy areas and pastures (Gough & Evans, 1942; Milosavljević *et al., 2016)*. Alternatively, the spectral sensitivity of our study species stems from symplesiomorphy between Elateridae and Lampyridae. Phylogenomics supports the concept that lampyroids, including fireflies (Lampyridae), may instead be classified as Elateridae, suggesting a common ancestor of the Lampyridae and Elateridae (Douglas et al. 2021). If proven correct, this common ancestor may have been bioluminescent, with spectral sensitivity to green/yellow bioluminescence signals being retained in elaterids.

The spectral sensitivity of our study species to green (515–538 nm) and UV (360 nm) light in electroretinograms guided the selection of green (520 nm) and UV (365 nm) LEDs as lures in laboratory and field experiments. Because a pre-screening field study revealed surprising attraction of elaterids also to blue-lit traps, we included blue (470 nm) LEDs in further experiments, which substantiated the results of the pre-screening study. Attraction of elaterids to blue light was not previously known and could not have been predicted because electroretinograms with neotropical elaterids (Lall et al. 2010), and with nearctic elaterids (this study), did not reveal any sensitivity to the blue wavelength range. However, as the blue LED emission spectrum overlapped with the green spectral sensitivity recorded for each of the nine species (Figure 4.2), it is conceivable that the blue LED trap lures may have stimulated a green photoreceptor, and thus prompted captures of beetles in the blue-light baited traps. Furthermore, the common ancestor of modern beetles lacked the opsin class that provides sensitivity to blue-light wavelengths (Sharkey et al. 2017). However, in some coleopteran taxonomic families, opsin duplication has restored blue-wavelength sensitivity (Lord et al. 2016; Sharkey et al. 2017).

Laboratory and field experiment data combined revealed that green and blue LEDs are attractive to elaterids representing three genera: *Selatosomus*, *Limonius* and

Agriotes. Due to limited availability of some beetles in laboratory bioassays or due to low captures of beetles in some field experiments, not all data sets are statistically significant but similar trends emerged in all behavioral studies. Furthermore, whenever both males and females were captured in significant numbers, like male and female *A. obscurus* in experiments 8 (Figure 4.4 c,d) and 9 (Figure 4.6 c,d), their capture rates in response to LED trap lures proved consistent. The failure of UV LEDs to attract beetles, or to enhance the attractiveness of green LEDs, is surprising given the beetles' spectral sensitivity to UV-wavelengths. However, UV-wavelengths may play a navigational role during the beetles' foraging activities, as insects can use color patterns of the sky to derive compass information (Wehner 1984). For example, in the absence of other cues ball-rolling dung beetles, are capable of using celestial chromatic gradients to maintain their bearing (el Jundi et al. 2015). Alternatively, beetles may instead actively seek green stimuli representing host plants which commonly have high long-wave reflection (green) and low UV reflection (Prokopy and Owens 1983).

Our findings provide impetus for further click beetle management studies. With the proven applicability of LEDs for attracting *Agriotes lineatus* and *A. obscurus*, it would be of interest to run similar studies with populations of parthenogenic species, such as *Aeolus mellillus* and *Hypnoidus bicolor*, which do not produce sex pheromones (Stirrett, 1936; Zacharuk 1958) and cannot be monitored through pheromone-based trapping. If trap catch data of adult beetles were to correlate with larval population densities, then trapping data could be used to monitor population trends and to inform pest management decisions. Furthermore, as females of sexually reproducing elaterids are typically not attracted to their own sex pheromone (for exception see Tóth et al., 2015; Vuts et al., 2018; van Herk et al., 2022), or are even modestly deterred by it (Gries et al., 2022), LEDs as trap lures afford the opportunity of capturing female and male beetles and tracking their sex ratio over time. As LED-baited traps capture significantly fewer beetles than pheromone-baited traps, light traps on their own are not sufficiently effective to curtail elaterid populations through mass trapping. Instead, they may be useful as monitoring tools, and in combination with other control tactics (e.g., application of entomopathogens) may contribute to integrated monitoring and management of elaterid pest species.

4.6. References

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4.7. Tables

Table 4.1. List of species collected by sweep netting or trapping at various locations in Canada. Beetles were used to determine their spectral sensitivity in electroretinogram recordings and to test their behavioural responses to light stimuli in laboratory bioassays.

Year	Species collected	Collection type	Location	GPS coordinates
2020	*Corymbitodes moerens*	Sweep netting	Pemberton, BC	50.4292, -122.9072
	Limonius canus	Sweep netting	Pemberton, BC	50.4292, -122.9072
	Selatosomus destructor	Sweep netting	Vulcan, AB	50.4038, -113.2522
	Agriotes lineatus	Trapping[1,5]	Agassiz, BC	49.2420, -121.7658
	Agriotes obscurus	Trapping[2,5]	Agassiz, BC	49.2420, -121.7658
	Agriotes pubescens	Trapping[1,5]	Saint-Mathieu-de-Beloeil, QC	45.5826, -73.2375
2022	*Limonius canus*	Sweep netting	Pemberton, BC	50.4292, -122.9072
	Corymbitodes moerens	Sweep netting	Pemberton, BC	50.4292, -122.9072
	Aeolus mellillus	Sweep netting	Saint-Mathieu-de-Beloeil, QC	45.5826, -73.2375
	Hypnoidus abbreviatus	Sweep netting	Saint-Mathieu-de-Beloeil, QC	45.5826, -73.2375
	Agriotes ferrugineipennis	Trapping[4,5]	Pemberton, BC	50.4292, -122.9072
	Agriotes lineatus	Trapping[1,5]	Agassiz, BC	49.2420, -121.7658
	Agriotes obscurus	Trapping[2,5]	Agassiz, BC	49.2420, -121.7658
	Agriotes pubescens	Trapping[1,5]	Saint-Mathieu-de-Beloeil, QC	45.5826, -73.2375
	Selatosomus destructor	Trapping[4,5]	Vulcan, AB	50.4038, -113.2522

[1]Lure for *Agriotes lineatus*: *g*eranyl butanoate & geranyl octanoate (1:1) (Tóth et al., 2003); 40 mg, Penta Manufacturing, Fairfield, NJ, USA
[2]Lure for *Agriotes obscurus:* geranyl hexanoate & geranyl octanoate (1:1) (Tóth et al., 2003); 40 mg, Penta Manufacturing
[3]Lure for *Agriotes ferrugineipennis:* 7-methyloctyl 7-methyloctanoate (Singleton et al., 2022); 10 mg, available from Gries-lab
[4]Lure for *Selatosomus destructor*: (*Z*,*E*)-α-farnesene (Gries et al., 2022); 4 mg, available from Gries-lab
[5]Vernon pitfall traps® (van Herk et al. 2018); available from Intko Supply Ltd., Chilliwack, BC

4.8. Figures

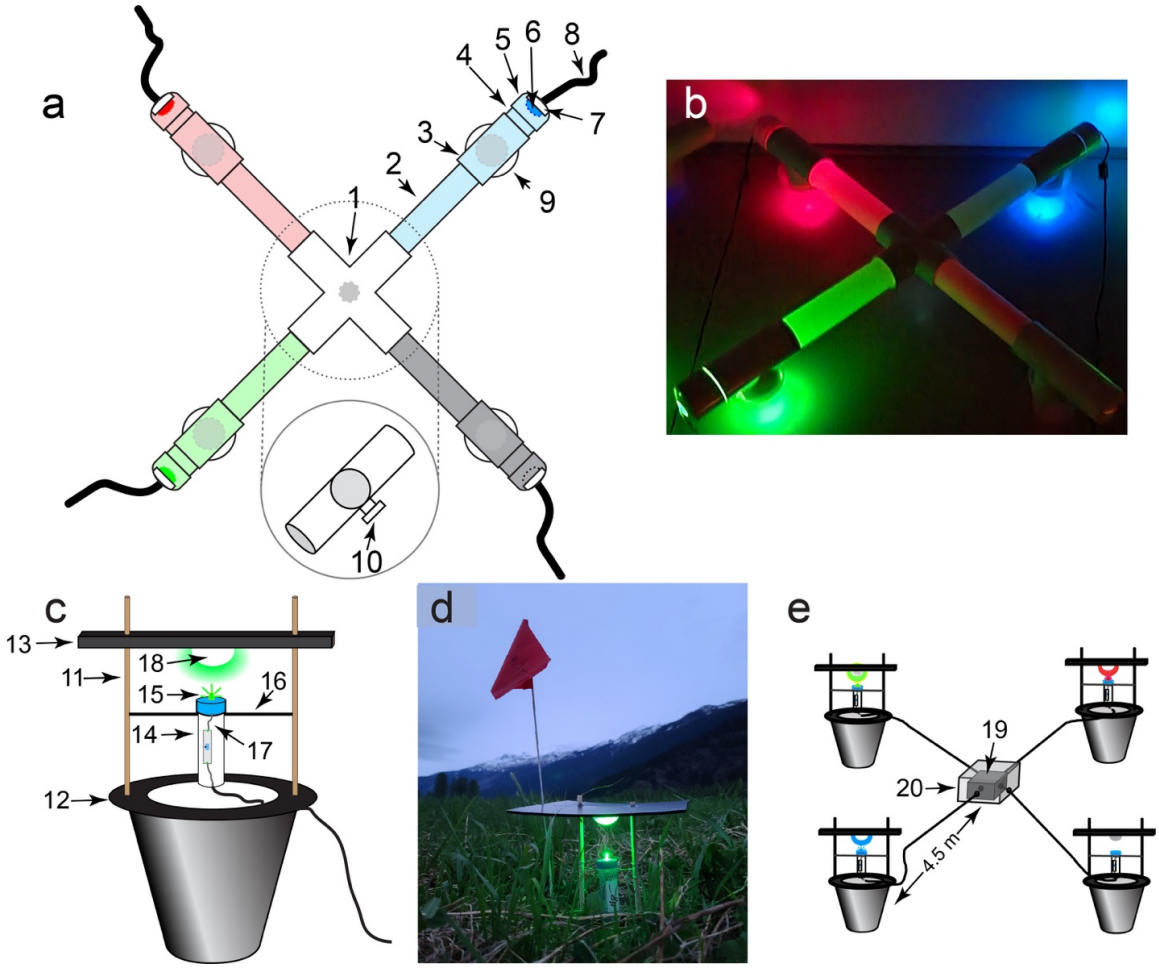


Figure 4.1. Graphic and photographic illustrations of (a, b) a bioassay arena built for laboratory experiments 1–7, (c, d) a Vernon pitfall trap® baited with an LED light and tested in field experiments 8–12, and (e) the layout of an experimental replicate. (a) A polyvinyl chloride (PVC) cross connector (1) was connected to four PVC pipes (2), each with sanded interior to facilitate beetle movement, and further connected to a T-shaped PVC tube (3); a PVC adaptor (4) at the distal end of 3 accommodated a PVC cap (5) housing an inward-facing light emitting diode (LED) (6), which was secured with hot glue and silicone in a lid opening (~0.5 cm) of a 50-mL falcon tube hot-glued to the PVC cap (7). LED wires (8) were funneled through a small hole (2 cm diameter) in PVC caps, and were connected to a 12-V battery. The third arm of 3 pointed downwards into a clear cup (9) which served as a 'pitfall trap' for responding beetles. To initiate a bioassay, a single beetle was placed in an open vial (10) which was then inserted into a hole (2.2 cm diameter) of the PVC cross (1), allowing the beetle to enter the bioassay arena and to respond to light stimuli. (c, d) Two wooden dowels (11) were inserted in holes in the trap bottom rim (12) to support a black corrugated plastic board trap lid (13) hot-glued to the dowels 10 cm above the trap bottom rim; a 50-mL plastic falcon tube (14), with an LED (15) hot-glued in a hole (~ 0.5 cm) of the falcon tube's lid, was suspended with steel wire (16) between the dowels. Each LED was connected to a circuit board that was fitted with both a potentiometer and a trimmer resistor to allow for fine-tune calibration of light intensity. The LED's wire (17) was connected to a battery via a double male-female non-insulated adapter which, in turn, hooked onto a central battery (see e); a Styrofoam half sphere (18) glued with its flat side to the trap's lid, served as a reflecting surface to evenly distribute the LED light. (e) Design of an experimental replicate, with a rechargeable 12-V battery (19) inside a Rubbermaid bin (20) in the centre, and traps positioned in a square 4.5 m from the central battery.

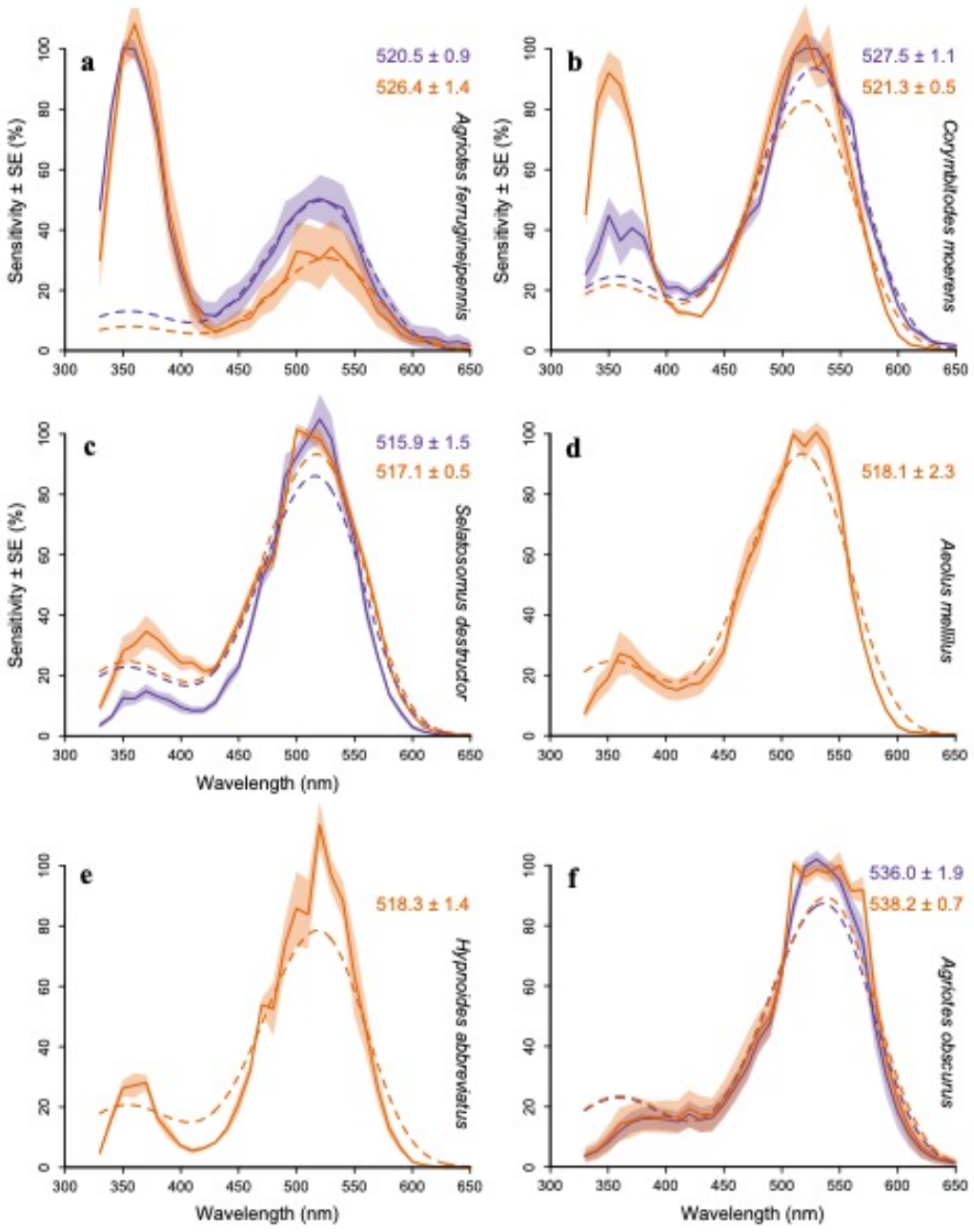

a
520.5 ± 0.9
526.4 ± 1.4
Agriotes ferrugineipennis
b
527.5 ± 1.1
521.3 ± 0.5
Corymbitodes moerens
c
515.9 ± 1.5
517.1 ± 0.5
Selatosomus destructor
d
518.1 ± 2.3
Aeolus mellillus
e
518.3 ± 1.4
Hypnoides abbreviatus
f
536.0 ± 1.9
538.2 ± 0.7
Agriotes obscurus
Sensitivity ± SE (%)
Wavelength (nm)

Figure 4.2. Mean spectral sensitivities (solid lines) and standard errors (shaded areas) of compound eyes obtained in electroretinogram (ERG) recordings with nine elaterid beetle species. Purple and orange lines represent males and females, respectively, and numbers of specimens used in recordings are reported in parentheses: *Agriotes ferrugineipennis* males (5) and females (5); *Corymbitodes moerens* males (5) and females (5); *Selatosous destructor* males (5) and females (5); *Aeolus mellillus* females (5); *Hypnoidus abbreviautus* females (5); *A. obscurus* males (5) and females (5); *A. lineatus* males (5) and females (5); *A. pubescens* males (5), and *L. canus* males (5) and females (4). Mean and standard error of maximal spectral sensitivity are reported in each subpanel. Dotted lines represent green opsin templates of males and females based on peak absorbance wavelength of the alpha band (λmax_α), using parameters from Stavenga (2010) based on a template from Govardovskii et al. (2000).

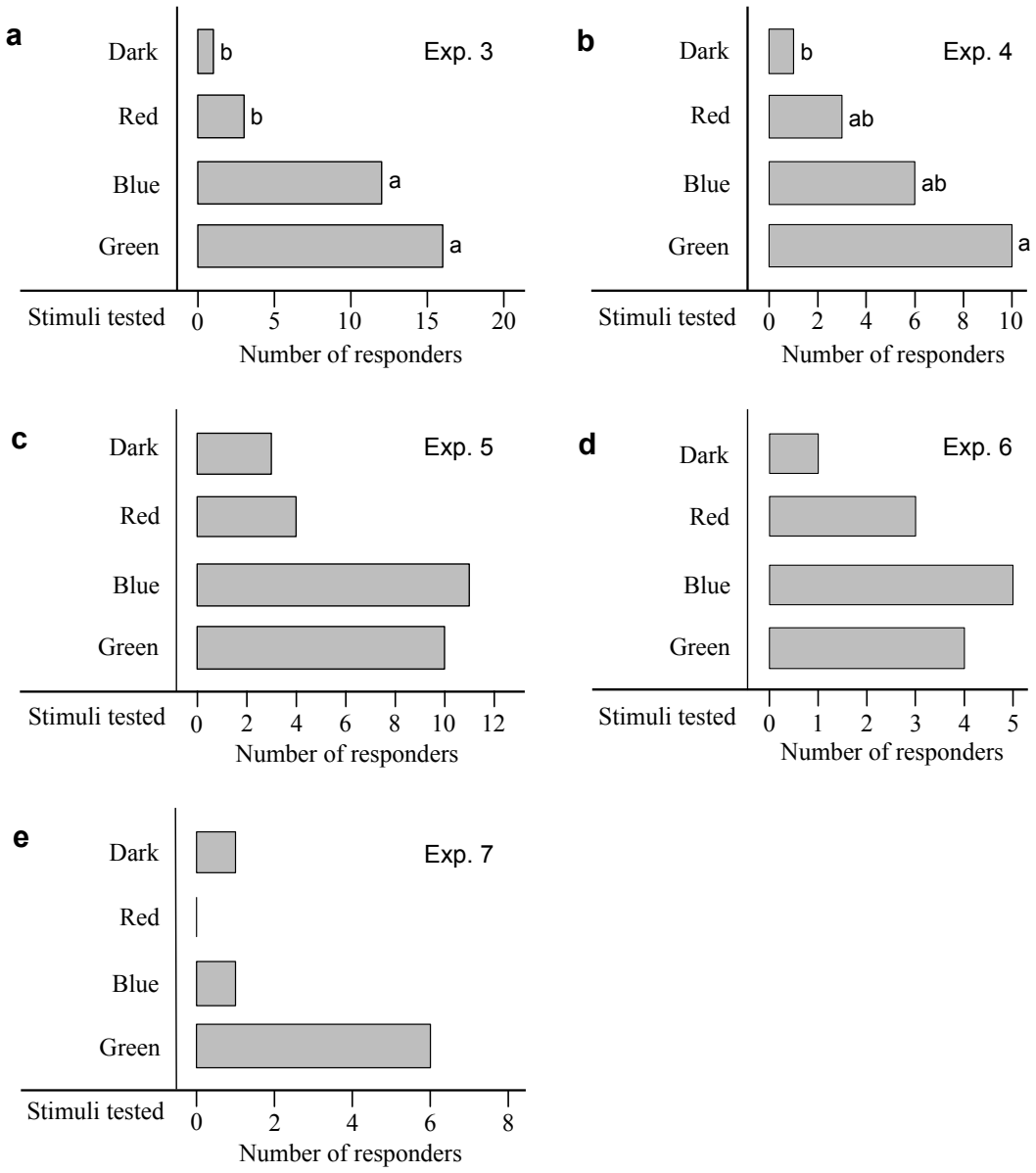
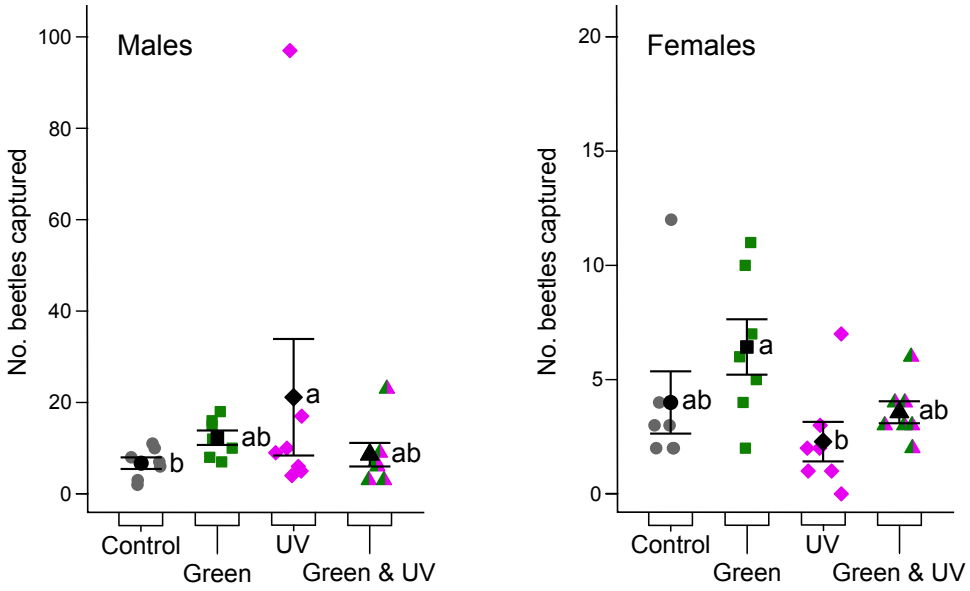


Figure 4.3. First-choice responses of *Selatosomus destructor* males (a) and females (b), *Agriotes pubescens* males (c), *Limonius canus males* (d), and *A. lineatus* males (e) to light emitting diodes (LEDs) in laboratory arena (Figure 4.1a,b) experiments 3–7. Four light stimuli were tested: (1) a dark control; (2) one red LED (655 nm); (3) one blue LED (470 nm); and (4) one green LED (525 nm). The light intensity of each LED was set to 2.2 × 10^{15} photons/cm^2/s. Bars with different letters in experiment 3 and 4 indicate statistically significant different preferential responses by beetles (Ryans test, $P < 0.05$).

a


Agriotes lineatus
Males
Females
No. beetles captured
100
80
60
40
20
0
20
15
10
5
0
b
ab
a
ab
ab
a
b
ab
Control
Green
UV
Green & UV

b

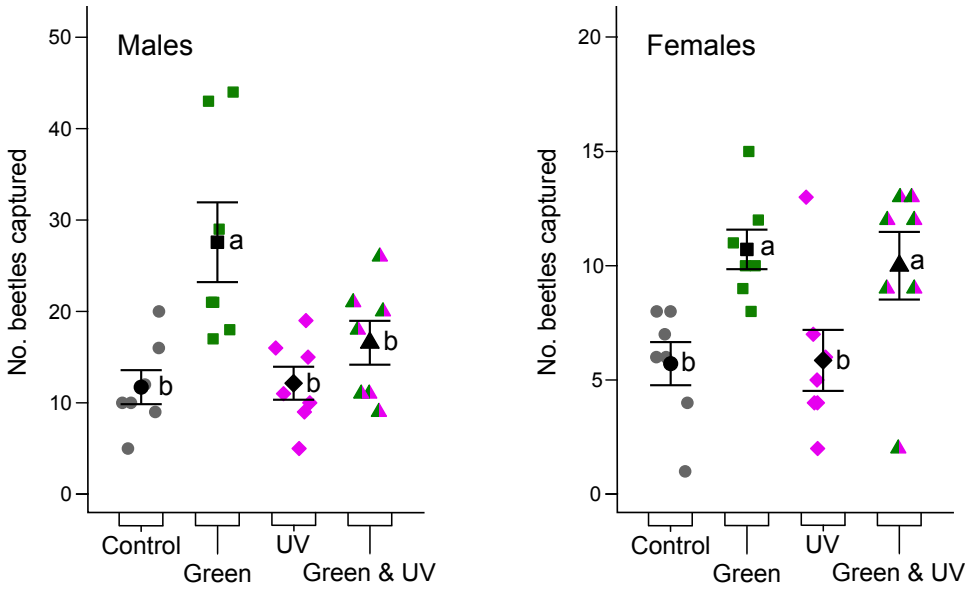
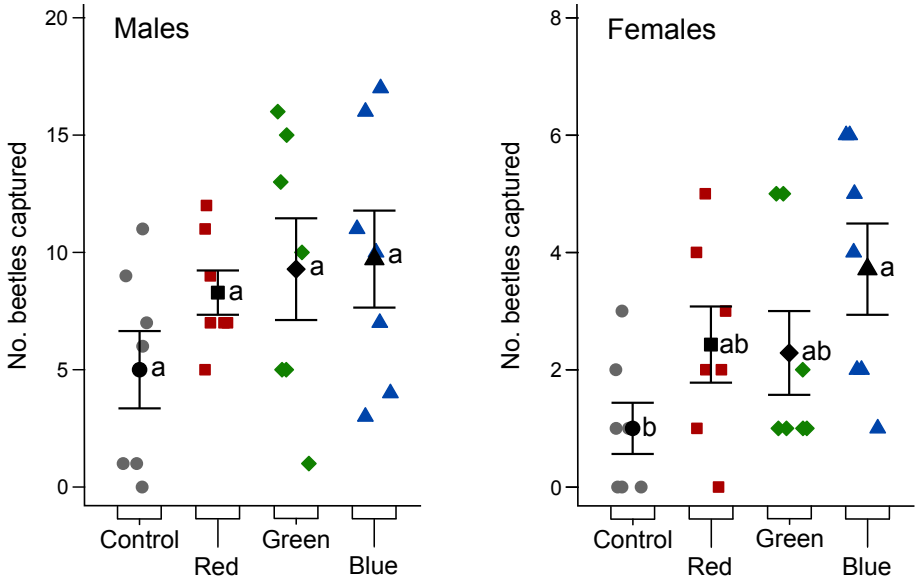

Agriotes obscurus
Males
Females
No. beetles captured
50
40
30
20
10
0
20
15
10
5
0
b
a
b
b
b
a
b
a
Control
Green
UV
Green & UV

Figure 4.4. Captures of male and female *Agriotes lineatus* (a) and *A. obscurus* (b) in experiment 8 (n = 7) in Vernon pitfall traps® fitted with light emitting diodes (LEDs) or kept dark. The experiment was run between 26 April and 31 May 2021 near Pemberton (British Columbia, Canada) testing four treatments: (1) a dark control; (2) one green LED (525 nm; 1.40 E+16 photons/cm^2/s); (3) two ultra-violet (UV) LEDs (365 nm; each 7.10E+15 photons/cm^2/s), and (4) one green LED (1.40 E+16 photons/cm^2/s) and two UV LEDs (each 7.10E+15 photons/cm^2/s). Grey and black symbols show the number of beetles captured in each replicate and on average (mean ± standard error), respectively. Means with different letters indicate statistically significant differences in trap captures (generalized linear mixed model fitted with a negative binomial distribution and a log link function; P < 0.05). Within each treatment, the data appear at slightly different width due to a jitter function of the software program that is applied when the plot is produced.

a

Agriotes lineatus



b

Agriotes obscurus

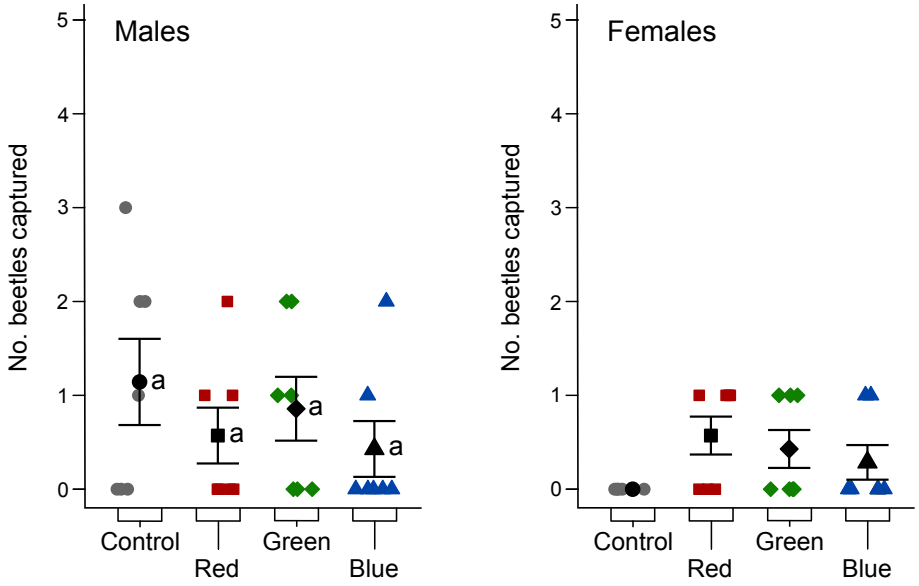


Figure 4.5. Captures of male and female *Agriotes lineatus* (a) and *A. obscurus* (b) in experiment 9 (n = 7) in Vernon pitfall traps® fitted with light emitting diodes (LEDs) or kept dark. The experiment was run between 11 May and 9 June 2022 near Pemberton (British Columbia, Canada), testing four treatments: (1) dark control; (2) one red LED (655 nm; 1.40 E+16 photons/cm^2/s); (3) one green LED (525 nm; 1.40 E+16 photons/cm^2/s); and (4) one blue LED (470 nm; 1.40 E+16 photons/cm^2/s); grey and black symbols show the number of beetles captured in each replicate and on average (mean ± standard error), respectively. Means with different letters indicate statistically significant differences in trap captures (generalized linear mixed model fitted with a negative binomial distribution and a log link function; P < 0.05). Within each treatment, the data appear at slightly different width due to a jitter function of the software program that is applied when the plot is produced.

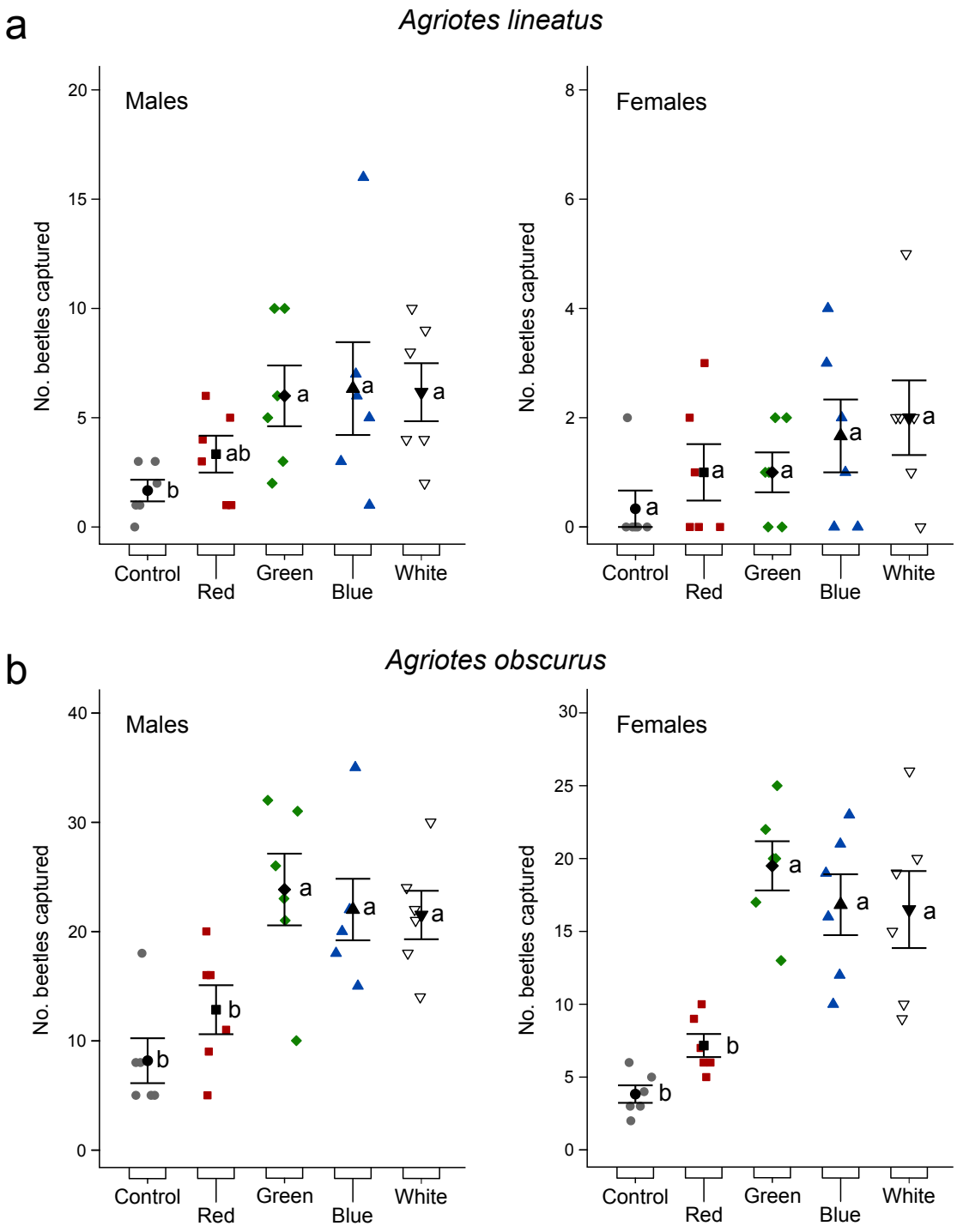

a
Agriotes lineatus
Males
Females
No. beetles captured
Control
Red
Green
Blue
White
b
Agriotes obscurus
Males
Females
No. beetles captured
Control
Red
Green
Blue
White

Figure 4.6. Captures of male and female *Agriotes lineatus* (a) and *A. obscurus* (b) in experiment 11 (n = 6) in Vernon pitfall traps® fitted with light emitting diodes (LEDs) or kept dark. The experiment was run between 22 April and 29 June 2022 near Agassiz (British Columbia, Canada), testing five treatments: (1) a dark control; (2) one red LED (655 nm; 1.40 E+16 photons/cm^2/s; (3) one green LED (525 nm; 1.40 E+16 photons/cm^2/s); (4) one blue LED (470 nm; 1.40 E+16 photons/cm^2/s); and (5) one white LED (1.40 E+16 photons/cm^2/s). Grey and black symbols show the number of beetles captured in each replicate and on average (mean ± standard error), respectively. Means with different letters indicate statistically significant differences in trap captures (generalized linear mixed model fitted with a negative binomial distribution and a log link function; P < 0.05). Within each treatment, the data appear at slightly different width due to a jitter function of the software program that is applied when the plot is produced. Captures of female *A. obscurus* were too low to warrant statistical analyses.

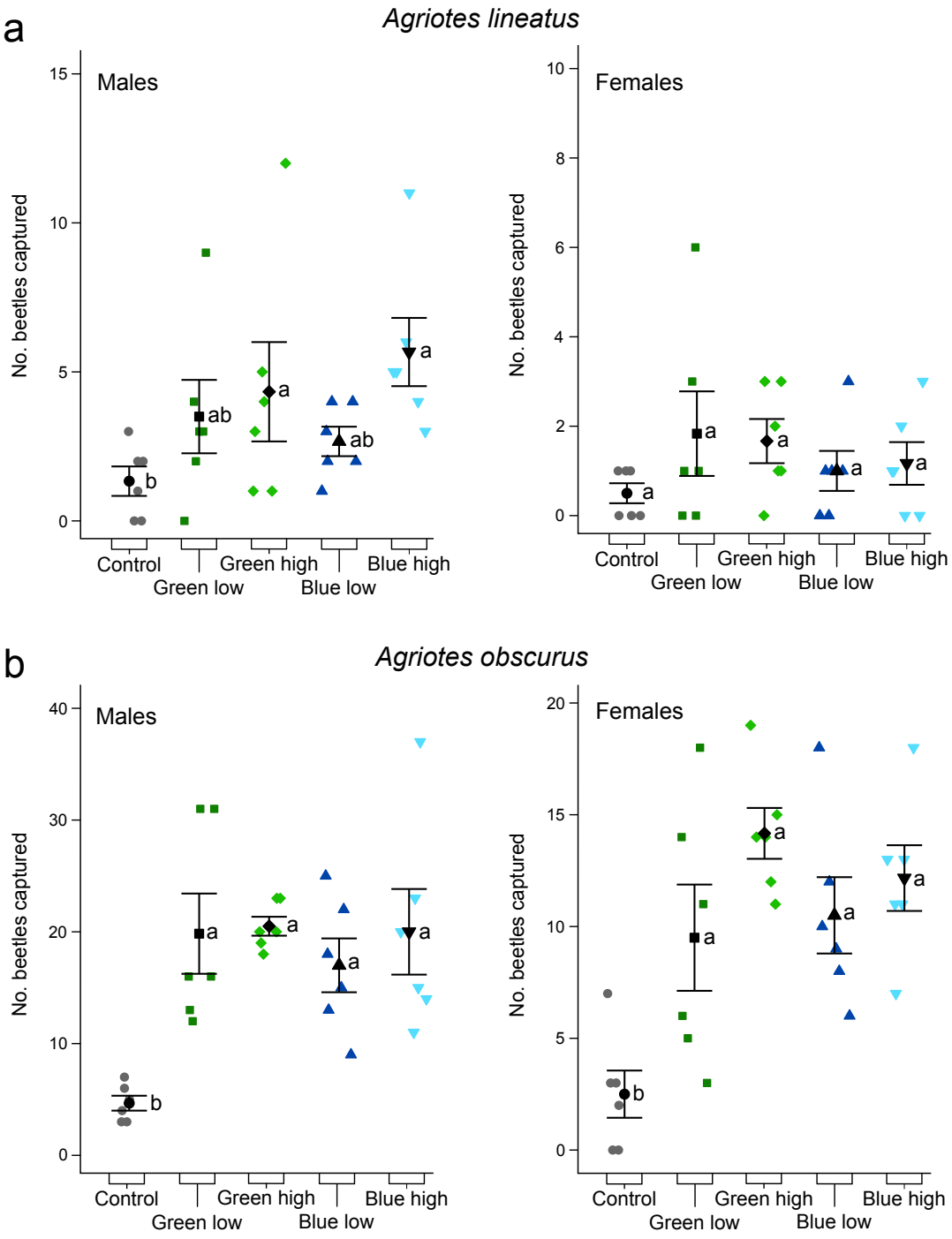

a
Agriotes lineatus
Males
Females
No. beetles captured
Control
Green low
Green high
Blue low
Blue high
b
Agriotes obscurus
Males
Females
No. beetles captured
Control
Green low
Green high
Blue low
Blue high

Figure 4.7. Captures of male and female *Agriotes lineatus* (A) and *A. obscurus* (B) in experiment 12 (n = 6) in Vernon pitfall traps® fitted with light emitting diodes (LEDs) set to low or high intensity, or kept dark. The experiment was run between 22 April and 29 June 2022 near Agassiz (British Columbia, Canada), testing five treatments: (1) a dark control; (2) one green LED (525 nm; 2.70 E+15 photons/cm^2/s = low intensity); (3) one green LED (2.70 E+16 photons/cm^2/s = high intensity); (4) one blue LED (2.70 E+15 photons/cm^2/s = low intensity); and (6) one blue LED (2.70 E+16 photons/cm^2/s = high intensity). Grey and black symbols show the number of beetles captured in each replicate and on average (mean ± standard error), respectively. Means with different letters indicate statistically significant differences in trap captures (generalized linear mixed model fitted with a negative binomial distribution and a log link function; P < 0.05). Within each treatment, the data appear at slightly different width due to a jitter function of the software program that is applied when the plot is produced.

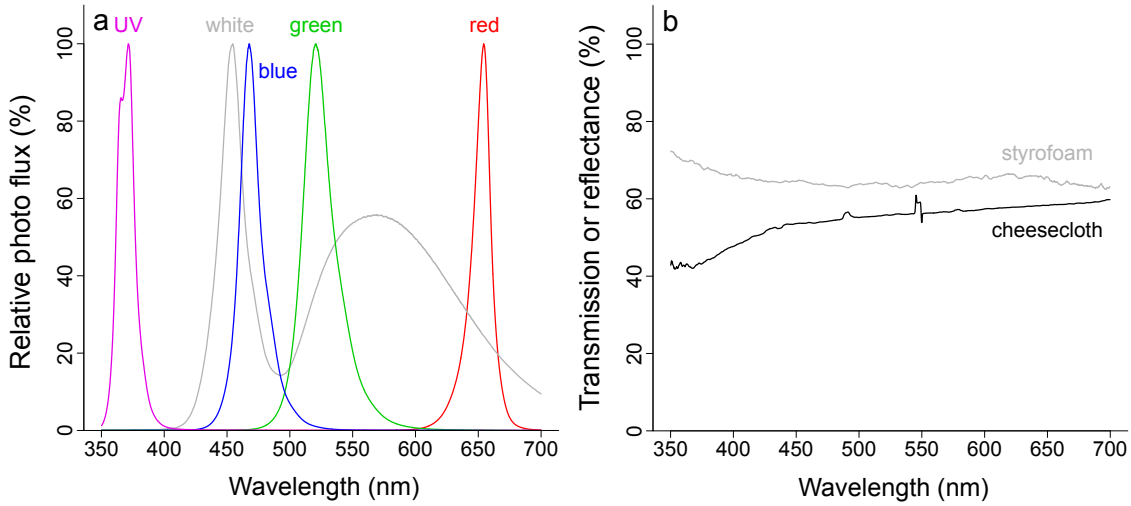


Figure 4.8. (a) Relative photon flux of the five LEDs tested in experiments 1-12. (b) Reflectance and transmission spectra of Styrofoam and cheesecloth, respectively, used in field and laboratory experiments.

Chapter 5.

First record of the invasive wireworm *Agriotes sputator* Linnaeus, 1758 (Coleoptera: Elateridae) in Quebec, Canada, and implications of its arrival[1]

[1]A near identical version of this chapter is published in the Pan Pacific Entomology (2022, https://doi.org/10.3956/2022-98.3.184) with the following authors: Singleton, K, van Herk, W.G., Saguez, J., Douglas, H.B. Gries, G.

KS, WvH & GG conceived the study, JS & HBD ran field experiments; KS identified and determined the sex of beetles captured in traps; KS, WvH & GG wrote the first draft, and all authors reviewed and approved of the final draft.

5.1. Scientific Note

The potato wireworm, *Agriotes sputator* Linnaeus, 1758 (Coleoptera: Elateridae), is one of three European *Agriotes* species that have become significant agricultural pests in North America. The adult beetles are small (6.0–8.0 mm in length) with dark brown coloration, sparse punctures on the prosternum, and curved lateral lobes on the male aedeagus (Fig. 5.1; Becker 1956), but, unlike the larvae, they do not cause crop damage. The larvae live in the soil for 4–5 years and progress through a variable number of instars (e.g., 10–12 in Russia, 7–9 in England; Zacharuk 1962) feeding on plant roots, seeds, and stems, including harvestable belowground plant parts (Parker & Howard 2001). Pupation occurs in late summer, and beetles emerge the following year and are active from April to August, with females typically laying eggs in May and June (Parker & Howard 2001). These general life history traits are shared with the other two European *Agriotes* pest species now present in Canada (*A. lineatus* Linnaeus*,*1767 and *A. obscurus* Linnaeus, 1758), and also *Agriotes* pests native to North America (e.g., *A. mancus* Say, 1923; LaFrance 1967).

Agriotes sputator is now widespread across North and Central Europe and temperate Asia, but its original native range is not known (Furlan & Tóth 2007, Vernon & van Herk 2018). In Canada, specimens of *A. sputator* were first found on 'beach drift' in Tabusintac, New Brunswick in 1939 (Brown 1940), but it is not clear when *A. sputator* was first inadvertently introduced to North America (Eidt 1953). Since it has established in eastern Canada, *A. sputator* has become the predominant wireworm pest of high value crops, such as potatoes in Prince Edward Island (PEI) and Nova Scotia (Fox 1961, Noronha 2011, Vernon & van Herk 2018, van Herk et al. 2021c). Prior to 2020, *A. sputator* had not been found in Quebec or further west in North America.

In May through July of 2020 and 2021, 32 and 217 *A. sputator* males, respectively, were captured in Vernon Pitfall Traps (van Herk et al. 2018) baited with geranyl butanoate (4.0 mg), the sex pheromone of *A. sputator* (Tóth 2013). Traps were deployed in the edge of agricultural fields (corn, cereals and soybean) in Saint-Mathieu-de-Beloeil, Quebec (45.58220, -73.236740) as part of a field experiment to test candidate sex pheromone components for *A. mancus* (Singleton et al., unpublished data). Captured beetles were identified using a taxonomic guide (Becker 1956) and by comparing them with reference specimens at the Canadian National Collection of

Insects, Arachnids and Nematodes (CNC) (Agriculture and Agri-Food Canada), where voucher specimens were deposited.

This is the first record of *A. sputator* in Quebec, documenting its range expansion into Central North America. Captures of 32 and 217 *A. sputator* males in two consecutive years is evidence that the population is already well established and is increasing in size. These field trapping data are of interest for several reasons. First, *A. sputator* is the predominant wireworm pest attacking potato in PEI. In some fields in PEI, trap captures of >100 beetles per day are common (van Herk et al. 2018). Such high populations of *A. sputator* cause significant yield loss (20–30%) due to non-marketable tubers and prompted some growers to discontinue potato production (Vernon & van Herk 2018). Once established in other parts of Canada, *A. sputator* may inflict similar damage to high-value vegetable crops (e.g., potato) grown there, as well as to cereals and other crops.

Second, now that *A. sputator* is established in agricultural areas of Quebec it may displace the currently predominant native pest wireworms *Hypnoidus abbreviatus* Say, 1823 and *A. mancus* that are considered less damaging to crops (Saguez et al. 2017). This type of species displacement has previously been noted in other parts of Canada. In PEI, e.g., *A. sputator* appears to have largely displaced the native *H. abbreviatus* and is now the predominant pest wireworm here (Vernon & van Herk 2018). In Nova Scotia, *A. obscurus*, *A. lineatus* and *A. sputator* have partly displaced *A. mancus* and *H. abbreviatus* (Eidt 1953, Fox 1961). In parts of southern British Columbia, the also invasive and highly damaging *A. obscurus* and *A. lineatus*, following their introduction to Western Canada in the 1800s, have largely displaced the once predominant pest wireworms *A. sparsus* LeConte, 1884 and *Limonius canus* LeConte, 1853 (Vernon & Tóth 2007, van Herk et al. 2021b). Of note is that *A. sputator* has not yet been detected in western Canada (van Herk et al. 2021b), though it would likely establish if introduced. Monitoring for *A. sputator* in western Canada and Ontario and in other regions in Quebec should be considered, as further range expansion is likely.

Third, the Europe-native *A. sputator* and North America-native *A. mancus* appear to share pheromone components (Singleton et al., unpublished data), a phenomenon also reported for *Agriotes* congeners in Europe (Tóth 2013) and in the Pacific Northwest. In southwestern British Columbia, *A. oregonensis* Becker, 1956, *A. sparsus* and *A. ferrugineipennis* LeConte, 1861 are occassionally captured in traps baited with the

pheromone of *A. lineatus* and/or *A. obscurus* (van Herk et al. 2021b), and in Oregon, traps baited with the pheromone of *A. sputator* captured modest numbers of *A. sparsus* (Kamm et al. 1983). Captures of multiple *Agriotes* species in traps baited with synthetic *Agriotes* pheromone components may potentially facilitate the development of generic pheromone-based monitoring tools, provided that the taxonomic identity of captured beetles is not critical for pest management decisions.

Agriotes sputator is the first of three non-native *Agriotes* pests in North America (*A. sputator, A. lineatus, A. obscurus*) to arrive in Quebec and the central lowlands of North America, which are among the world's largest agricultural growing areas. The arrival of *A. sputator* poses a substantial threat to crop yields and market values. Moreover, it implies that there is a pathway of introduction that other exotic wireworm species may follow, spreading from Atlantic Canada and/or the Pacific Coast to other growing areas in North America. Such spread is thought to have occurred, and to still occur, through wireworm-infested soil that is moved together with plants for landscaping (Douglas 2011, van Herk et al. 2021b). As these *Agriotes* species are not considered regulated plant pests in North America, there are few practical options to slow their spread to new growing regions. With most currently registered insecticides enabling crop stand establishment but not significantly reducing wireworm populations in the field (van Herk et al. 2021a), work is needed to develop effective monitoring and pest control tools for *Agriotes* pest species.

5.2. References

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5.3. Figures

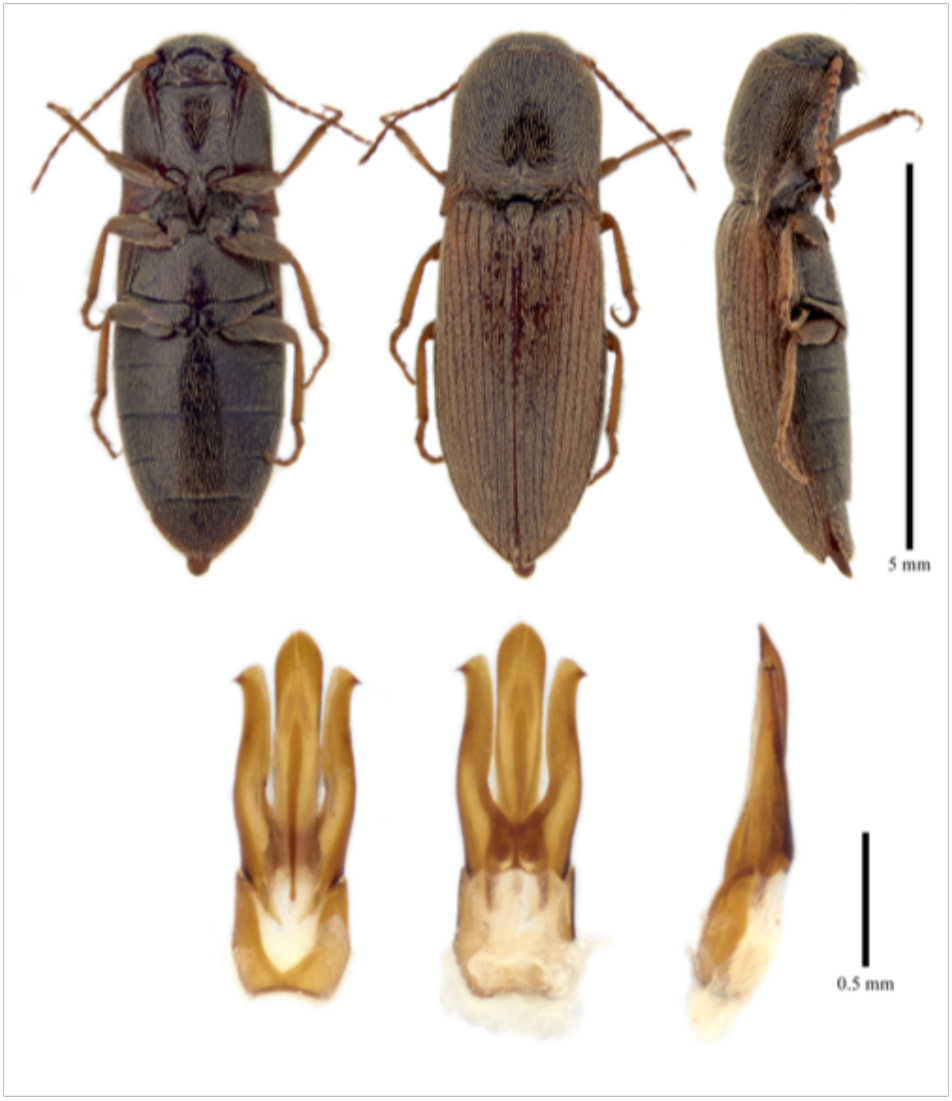

Figure 5.1. Ventral, dorsal and lateral views of *Agriotes sputator* males (top row, left to right), and their aedeagi (bottom row), captured in traps in Saint-Mathieu-de-Beloeil, Quebec, during June and July 2020. Photo: K. Savard, AAFC.