Response of Invasive Longhorn Beetles (Coleoptera: Lamiinae) to Known Cerambycid Aggregation-Sex Pheromones in the Puna District of Hawaii Island

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Abstract. The Queensland longhorn borer (QLB; *Acalolepta aesthetica* [Olliff 1890]; Coleoptera: Cerambycidae: Lamiinae: Monochamini) and plumeria longhorn borer (PLB; *Lagocheirus obsoletus* [Thomson 1778] = *Lagocheirus undatus* [Voet 1778]; Coleoptera: Cerambycidae: Lamiinae: Acanthocini) are invasive longhorn beetle species that have become established on the island of Hawaii. Both QLB and PLB are polyphagous. Known hosts of QLB include cacao, citrus, kukui, and breadfruit in Hawaii, and QLB are known to attack live, healthy trees. Currently the beetle occurs in the Puna district of the island, but its range is expanding. PLB is a pest of plumeria and other ornamental plants throughout the state of Hawaii and elsewhere. As a first step towards developing a monitoring tool for these invasive beetles, we tested four known aggregation-sex pheromones of cerambycids in this subfamily—monochamol, fuscumol acetate, fuscumol, and geranylacetone—that have proven effective for attracting more than 30 lamiine species in different areas of the world. When tested in panel traps, these compounds individually and in a blend attracted 9 QLB total, which was not significantly different than the 5 QLB captured in solvent control traps. In contrast, traps baited with one of the tested compounds, fuscumol acetate, captured significantly more PLB than solvent blank control traps. We discuss future research directions for developing attractants using chemical ecology approaches to monitor QLB and PLB.

Key words: invasive species, longhorn beetles, pheromone trapping

Longhorn beetles (Coleoptera: Cerambycidae) are ecologically and economically important, both as beneficial native insects in forest systems (Haack and Byler 1993, Grove 2002, Teale and Castello 2011, Ulyshen 2014) and as invasive pests in recipient habitats (Haack et al. 2010, Parry and Teale 2011). Because larvae can persist cryptically within wooden commodities shipped internationally (e.g., lumber, wooden shipping materials, and finished wooden products), there is a high likelihood of cerambycid species being introduced into novel habitats (Haack 2006).

All known examples of volatile pheromones from cerambycids in the subfamily Lamiinae are male-produced aggregation-sex pheromones that attract both sexes, with multiple species often "sharing" pheromones. So far, four basic structures have been identified or implicated as aggregation-sex pheromones for ~30 lamiine species (Hanks and Millar

2016). These structures include fuscumol [(*E*)-6,10-dimethyl-5,9-undecadien-2-ol], the corresponding ester fuscumol acetate [(*E*)-6,10-dimethyl-5,9-undecadien-2-yl acetate], geranylacetone [(*E*)-6,10-dimethyl-5,9-undecadien-2-one], and monochamol [2-(undecyloxy)ethanol] (e.g., Fonseca et al. 2010, Pajares et al. 2010, Mitchell et al. 2011, Wickham et al. 2014, Ryall et al. 2015, Hughes et al. 2016, Meier et al. 2016, Bobadoye et al. 2018). Fuscumol, fuscumol acetate, and geranylacetone are also pheromone components for cerambycid species in the subfamily Spondylidinae (e.g., Silk et al. 2007, Halloran et al. 2018).

The Queensland longhorn borer (QLB) *Acalolepta aesthetica* (Olliff 1890) and the plumeria longhorn borer (PLB) *Lagocheirus obsoletus* (Thomson 1778) (= *Lagocheirus undatus* [Voet 1778]) are two invasive cerambycid species from the subfamily Lamiinae (tribes Monochamini and Ancanthocini, respectively) that have become established in Hawaii. QLB is native to Australia and was first collected from the Puna district of Hawaii island in 2009 (Matsunaga and Chun 2018). The large beetles (up to 4.5 cm body length) are polyphagous: confirmed hosts include ulu (breadfruit; *Artocarpus altilis*), kukui (candlenut; *Aleurites mouccanus*), queen sago palm (*Cycas circinalis*), *Citrus* spp., and cacao (*Theobroma cacao*). Gunpowder tree (*Trema orientalis*) and avocado (*Persea americana*) are two other possible hosts, but these require verification (Matsunaga and Chun 2018). Congeners of QLB have been shown to be attracted to monochamol in China (Wickham et al. 2014), and to pheromones from the subfamily Cerambycinae in Australia (Hayes et al. 2016). QLB can successfully attack live, apparently healthy trees (SG Chun pers. comm.; RMC pers. obs.), a behavior shared with the polyphagous Asian longhorn beetle and citrus longhorn beetle (*Anoplophora glabripennis* and *Anoplophora chinensis*, respectively; Cerambycidae: Lamiinae: Monochamini).

The plumeria longhorn borer (PLB) is known from the southern United States, the Caribbean, Central America, South America, and the South Pacific Islands (Mondaco 2008). It was first recorded in Hawaii in 1890 (Gressit and Davis 1972). The beetle is polyphagous, with recorded hosts in the genera *Plumeria*, *Manihot*, *Bursera*, *Euphorbia*, *Ficus*, *Forestiera*, *Jatropha*, *Spondias*, *Araucaria*, *Capsicum*, *Gossypium*, *Hibiscus*, and *Yucca* (Mondaco 2008). The species is a pest of concern on Oahu (MM Ramadan, pers. comm.). As a first step towards developing monitoring and possibly control tactics for these invasive species, we field tested the four generic lamiine pheromones described above—fuscumol, fuscumol acetate, geranylacetone, and monochamol—as possible attractants for these two invasive species. Here, we report the results of those tests.

Methods and Materials

Field bioassays testing fuscumol, fuscumol acetate, geranylacetone, and monochamol. At three sites in the upper Puna district of Hawaii island, we deployed black cross-vane, panel intercept traps coated with Fluon (Alpha Scents, Portland, Oregon, USA; Graham et al. 2010) hung from the lower branches of various tree species; beetles were live trapped for use in laboratory assays. All three sites were in a 4 km^2 area (center: 19°34'11.2"N 154°59'57.7"W). The first two sites were on properties in residential areas (i.e., surrounded on all sides by other residential properties), one with several kukui, citrus, avocado, and plumeria among other trees on the property (two spatial replicates), and the other with kukui, ulu, cacao, and plumeria among other trees on the property (one replicate).

Both residential sites had active infestations of QLB in about 20–30% of possible host trees on each property (RMC, pers. obs.); hosts were abundant in the neighborhoods as well. The third site was situated on a cacao farm with a few interplanted citrus trees and was surrounded by jungle (one replicate). This third site had active infestations of QLB in approximately 10–20% of the cacao trees at any given time (RMC, pers. obs.). Infestations were being actively managed via hand-removal by the farmer (the cacao trees were approximately 5 cm in diameter and thus had relatively thin bark, so beetle damage by late-instar larvae could be diagnosed by looking for soft spots along the bark).

At the two residential sites, due to the heterogeneous distribution of host and non-host trees, traps with different treatments were set up amid potential host trees, whereas the cacao farm site allowed for traps to be deployed in banana trees (not a known host for either species) ~20 m away from the citrus trees and ~50 m from the cacao trees (QLB-hosts), to limit positional effects. Traps were placed roughly 10–15 m apart in linear transects for each of the spatial replicates at the three sites, with a treatment randomly assigned to each trap. At the site in which there were two transects, the transects were 20 m apart. Traps were checked approximately weekly, and the order of treatments along a given transect was re-randomized after each check.

Two different subsets of candidate pheromone compounds were tested individually or in a blend in two consecutive field trapping experiments over the three sites. The first experiment ran from 17 April through 14 June 2018, and tested three treatments: racemic fuscumol acetate (50 mg/ml), monochamol (25 mg/ml), and blank solvent control. The second experiment ran from 14 June through 30 August 2018, and tested five treatments: racemic fuscumol (50 mg/ml), racemic fuscumol acetate (50 mg/ml), geranylacetone (25 mg/ml), a blend of these three compounds (at the same concentrations as the individual-compound treatments), and blank solvent control. The pheromones were dispensed from low-density polyethylene bags (0.05 mm wall thickness, 5 cm x 7.5 cm; Fisher Scientific, Milwaukee, Wisconsin, USA) with 2-propanol as the solvent; 1 ml of solution of a given treatment was loaded into a lure. Lures were changed weekly. Monochamol and geranylacetone were used at a concentration of 25 mg/ml rather than 50 mg/ml because they are single isomer compounds, in contrast to the two enantiomeric isomers present in racemic fuscumol and fuscumol acetate. All pheromones were purchased from Bedoukian Research (Danbury, Connecticut, USA).

Collected beetles were sexed based on antennal length and abdomen size for QLB (males have antennae ~2–2.5x body length and less robust abdomens, whereas females have antennae ~1.5x body length and much rounder and more robust abdomens), and based on presence (male) or absence (female) of antennal spurs on the tip of the 4th antennal flagellomere for PLB (IP Swift, pers. comm.). We did not have the sexing characteristics available for PLB for the first experiment and beginning of the second experiment, thus some beetles are listed as 'unsexed' in Table 1. Voucher specimens of both species have been archived at U.S. Pacific Basin Agricultural Research Center (Hilo, Hawaii), and are available upon request.

Statistical analyses. The field trapping experiments were spatially and temporally replicated, with temporal replicates representing the number of times trap catches were counted. Temporal replicates where no beetles of a given species were trapped in any treatment were removed from analyses, as zero-catch days were typically associated with inclement weather.

Table 1. Total number of QLB (*Acalolepta aesthetica*) and PLB (*Lagocheirus obsoletus*) captured in traps baited with generic aggregation-sex pheromones (fuscumol, fuscumol acetate, monochamol, and geranylacetone) known for the cerambycid subfamily Lamiinae. Solvent blank was 2-propanol. The blend consisted of fuscumol, fuscumol acetate, and geranylacetone.

		QLB		PLB		
	Treatment	**Female**	**Male**	**Female**	**Male**	**Unsexed**
Experiment 1	Solvent blank	3	1	0	0	0
(17 Apr–	Fuscumol acetate	1	2	0	0	7
14 Jun 2018)	Monochamol	0	1	0	0	1
Experiment 2	Solvent blank	1	0	1	0	0
(14 Jun–	Fuscumol	1	0	0	0	0
30 Aug 2018)	Fuscumol acetate	1	1	1	2	6
	Geranylacetone	1	0	0	0	0
	Blend	0	1	1	1	2

Because of low trap catches, species were analyzed by the total number of beetles caught to increase statistical power (Table 1). Data were analyzed with a max-*t* test (Herberich et al. 2010). This test is robust to departures from assumptions of normality and homogeneity of variances, a common occurrence with trap catch data. These analyses were conducted in R (3.4.2; The R Foundation; R Core Team, 2017).

Results

Field assays testing fuscumol, fuscumol acetate, geranylacetone, and monochamol. A total of 4 female and 4 male QLB were trapped in the first experiment (5 replicates, once zero-catch days were removed from the analysis), and 4 and 2 respectively in the second experiment (3 replicates without zero-catch days; Table 1). There was no significant difference in trap catches between the solvent controls (5 QLB total) and any of the pheromone treatments (5 total QLB in fuscumol acetate traps, and 1 each in the other pheromone treatments; Table 1) (exp. 1: $p \geq 0.15$; exp. 2: $p \geq 0.99$; Figs. 1 and 2).

A total of 8 unsexed PLB were trapped in the first experiment (4 replicates without zero-catch days), and 3 females, 3 males, and 8 unsexed individuals in the second experiment (10 replicates without zero-catch days; Table 1). In both experiments, fuscumol acetate was significantly more attractive (16 PLB total) than the solvent controls (1 PLB total) (exp. 1: $p = 0.02$; exp. 2: $p = 0.037$; Figs. 3 and 4), whereas no other treatments were significantly different from the solvent control.

Discussion

None of the traps baited with the four known cerambycid pheromones captured more QLB than control traps. This was somewhat surprising, in part based on the generic nature of these compounds in the attraction of other species of lamiines (see Introduction), but also because *Acalolepta formosana* (Breuning 1935), a species closely related to QLB, was found to be attracted to monochamol in China (Wickham et al. 2014), using trapping methods similar to those used in this research.

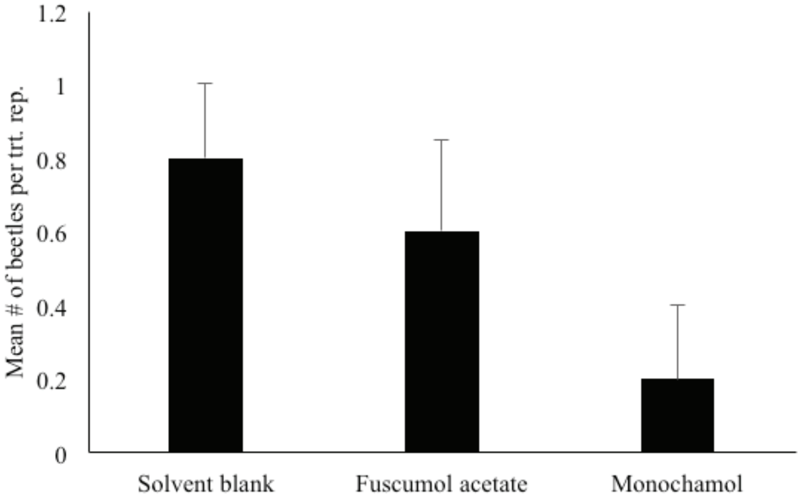

Figure 1. Mean (± SE) numbers of QLB (*Acalolepta aesthetica*) caught in traps baited with solvent control, fuscumol acetate, or monochamol (experiment 1). There were no significant differences between the treatments and the solvent control (max-t test, $p > 0.05$).

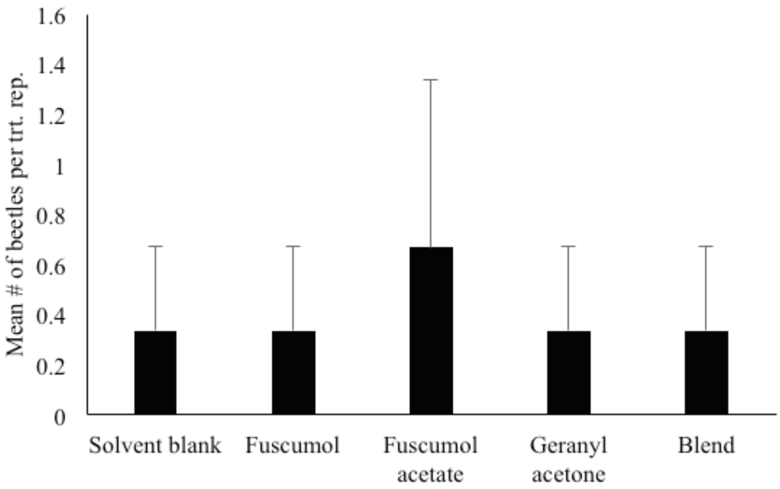

Figure 2. Mean (± SE) numbers of QLB (*Acalolepta aesthetica*) caught in traps baited with solvent control, fuscumol, fuscumol acetate, geranylacetone, or a blend of the three compounds (experiment 2). There were no significant differences between the treatments and the solvent control (max-t test, $p > 0.05$).

In contrast, the bioassay data indicated that fuscumol acetate may be an attractant pheromone candidate for PLB, with fuscumol acetate baited traps catching significantly more beetles than controls. Based on the lower trap catches in the blend of fuscumol+fuscumol acetate+geranylacetone versus the fus-

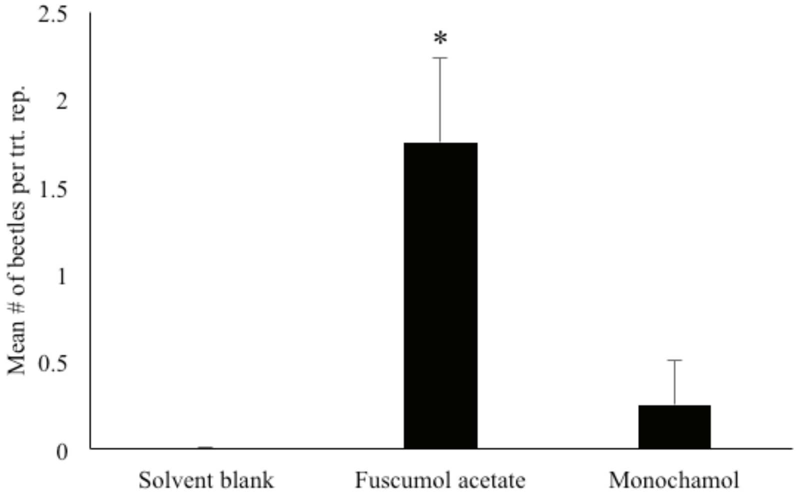

Figure 3. Mean (± SE) numbers of PLB (*Lagocheirus obsoletus*) caught in traps baited with solvent control, fuscumol acetate, or monochamol (experiment 1). Means with an asterisk are significantly different than the solvent control (max-t test, $p < 0.05$).

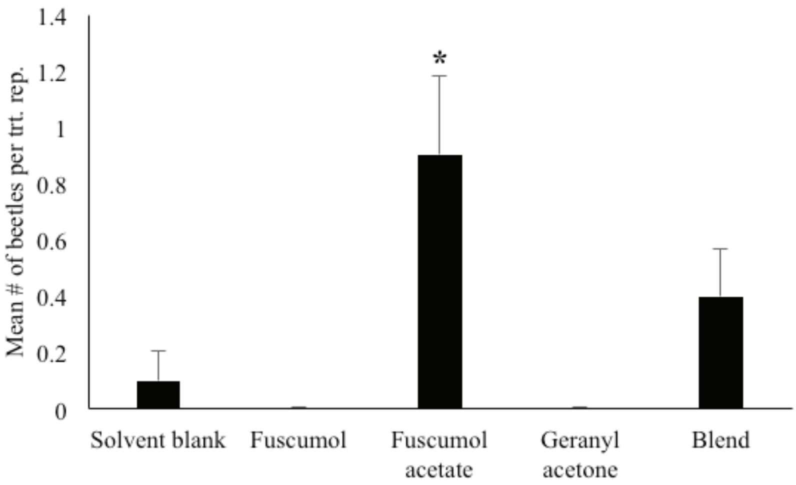

Figure 4. Mean (± SE) numbers of PLB (*Lagocheirus obsoletus*) caught in traps baited with solvent control, fuscumol, fuscumol acetate, geranylacetone, or a blend of the three compounds (experiment 2). Means with an asterisk are significantly different than the solvent control (max-t test, $p < 0.05$).

cumol acetate alone, fuscumol and/or geranylacetone may be antagonistic. From a management standpoint, this is a useful discovery for monitoring or control of PLB, because fuscumol acetate is commercially available and relatively inexpensive to deploy. Furthermore, based on recent results with other lamiine species showing increased lure efficacy to naturally produced enantiomeric ratios

(Hughes et al. 2016, Meier et al. 2016, Silva et al. 2019), it is possible that PLB produces only one enantiomer of fuscumol acetate, or a nonracemic mixture of the enantiomers. Thus, future research will evaluate whether correct enantiomeric blend could improve lure efficacy.

Because QLB were not attracted to any of the four compounds tested here, even though we knew they were present due to continued new infestations of host trees in the vicinity and the continuous trapping of QLB adults in low numbers in the various treatments (including solvent blank treatment traps), this suggests that (1) QLB does not use attractant pheromones at all, (2) its pheromone consists of compounds other than those tested, or (3) one of the enantiomers in the racemic blends or the ratio tested was antagonistic. The first possibility seems unlikely because, of the several hundred cerambycid species that have been examined for pheromone use, species in only one genus (*Phoracantha* Newman 1840, in the subfamily Cerambycinae) have shown no evidence for using attractant pheromones (Millar and Hanks 2017).

To our knowledge, population dynamics and phenological data of QLB and PLB in Hawaii are still unknown and thus it is possible that the low trap catches of beetles in this study were constrained by the low population levels of QLB and PLB at the time of trapping. Also, low trap catches could have resulted from sub-optimal lures tested in terms of release rate, ratio, and stereochemistry. We are not aware of any effective control methods for these two species of cerambycid, and due to the cryptic nature of cerambycids, future research will focus on using a combination of chemical ecology and electrophysiology techniques, such as coupled gas chromatography-electroantennographic detection (GC-EAD), for the isolation and identification of potential pheromone compounds released by QLB and PLB, identification of attractive kairomone compounds released from host plants, food and symbiotic microbes, and optimization of ratio and release rates of those chemicals to develop effective monitoring and potential behavioral control methods to manage QLB and PLB in Hawaii.

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