Journal of Economic Entomology, 109(4), 2016, 1741–1749
doi: 10.1093/jee/tow100
Advance Access Publication Date: 31 May 2016
Research article

Forest Entomology

Optimizing Generic Cerambycid Pheromone Lures for Australian Biosecurity and Biodiversity Monitoring

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Received 11 February 2016; Accepted 19 April 2016

Abstract

The cerambycid beetles comprise a diverse family that includes many economically important pests of living and dead trees. Pheromone lures have been developed for cerambycids in many parts of the world, but to date, have not been tested in Australia. In this study, we tested the efficacy of several pheromones, identified from North American and European species, as attractants for cerambycids at three sites in southeast Queensland, Australia. Over two field seasons, we trapped 863 individuals from 47 cerambycid species. In the first season, racemic 3-hydroxyhexan-2-one was the most attractive compound among the eight pheromones tested. Subsequently, we aimed to optimize trapping success by combining this compound with other components. However, neither the addition of other pheromone components nor host plant volatiles improved the efficacy of 3-hydroxyhexan-2-one alone. We also tested a generic pheromone blend developed for North American cerambycids, and found that only the combination of this blend with host plant volatiles improved trapping success. The Australian cerambycid fauna is not well known, and effective lures for use in trapping beetles would greatly assist in the study of this important group. Effective semiochemical lures would also have implications for biosecurity through improved monitoring for invasive species.

Key words: Cerambycidae, pheromone, sampling, monitoring, host plant volatile

The Cerambycidae, or longhorned beetles, include ~33,000 described species worldwide, and new species are frequently discovered (Slipinski and Escalona 2013). Cerambycid beetles are among the most important insect pests, damaging and killing trees in natural and urban forests, plantations, and orchards, as well as degrading timber and wooden structures (e.g., Brockerhoff et al. 2006). The cryptic boring habits of cerambycid larvae make them high-risk candidates for accidental introduction into new areas of the world in wood products, nursery stock, and dunnage (Allen and Humble 2002, Haack 2006). Increases in global trade and more rapid transportation have led to increased movements of potential pest species and increased pressures on quarantine services worldwide. Broad-scale monitoring within forestry systems is often ineffective at detecting exotic species until they are already well established (Wardlaw et al. 2008). Instead, monitoring focused on likely points of entry and similar high risk or hazard sites (e.g., ports, airports, Quarantine Approved Premises) is more effective for early detection (Wylie et al. 2008, Brockerhoff et al. 2010). At these locations, population densities of colonizing exotic species are initially low, requiring highly sensitive and specific techniques for successful early detections. Traps baited with insect attractants are ideal candidates for such situations, and are increasingly important tools for biosecurity monitoring (Brockerhoff et al. 2010). Furthermore, attractant-baited traps are one of the primary tools used to demonstrate that areas are likely free of specific pest species, allowing produce and other goods to be shipped abroad without fumigation or other disinfestation treatments.

Over the past decade, a growing body of work has focused on developing pheromone-based trapping methods to detect and monitor various cerambycid species (Graham et al. 2010, 2012; Allison et al. 2011; Mitchell et al. 2011; Hanks and Millar 2013), including the testing of blends of pheromones to monitor multiple species simultaneously (Hanks et al. 2012, Hanks and Millar 2013). Although pheromones are quite species specific in the Insecta, there is often considerable conservation of pheromone structures in closely related groups within the Cerambycidae. Hence, a limited

number of structural motifs are shared by a diversity of species, with species specificity provided by variation in blend components (e.g., Hanks et al. 2007, Wickham et al. 2014), and by species which share one or more pheromone components having different daily or seasonal activity cycles (Mitchell et al. 2015). Generic lures prepared by the combination of components identified from individual species have proven quite successful in attracting multiple cerambycid species simultaneously in North America and Eurasia (Hanks et al. 2007, 2012; Wong et al. 2012; Hanks and Millar 2013; Sweeney et al. 2014; Wickham et al. 2014; Handley et al. 2015).

Generic semiochemical lures which attracted a number of species simultaneously would be of great value for the study of the Australian Cerambycidae. Despite being one of the most easily recognized beetle groups, and of considerable economic importance, little is known of their biology and ecology. To date, the literature on the Australian Cerambycidae has been fragmentary and largely limited to individual species or taxonomic descriptions of species groups. Thus, the primary objective of the work described here was to field test a panel of known cerambycid pheromones as attractants for the cerambycid fauna of southeast Queensland, Australia. These chemicals included *syn*- and *anti*-2,3-hexanediols, the homologous 2,3-octanediols, 3-hydroxyhexan-2-one, 3-hydroxyoctan-2-one, and 2-methylbutanol (common pheromone components from species of the subfamilies Cerambycinae and Prioninae), and (*E*)-6,10-dimethylundeca-5,9-dien-2-ol, or fuscumol, its corresponding acetate, and 2-(undecyloxy)ethanol, or monochamol (from species of the Lamiinae and Spondylidinae; Silk et al. 2007, Mitchell et al. 2011, Hanks and Millar 2013, Pajares et al. 2013, Wickham et al. 2014, Handley et al. 2015, and references therein).

If effective in an Australian context, these lures could be utilized to investigate the under-studied Australian fauna. A better knowledge of our local fauna is critical in order to quickly recognize and rapidly respond to incursions of exotic species. Moreover, the lures have the potential to detect new incursions of exotic beetle pests before they have the chance to become established. Here, we used a bottom-up approach to test a multispecies lure for Australian cerambycid beetles across three sites, by identifying the most attractive individual compound from a group of known cerambycid pheromones, and then added additional components to investigate whether capture rates could be improved.

Materials and Methods

General Trapping Methods

Three field experiments were carried out during 2010–2012. First, we tested a number of known cerambycid pheromone components for their effectiveness in attracting local species in southeast Queensland (Experiment 1). Having identified the most effective individual compound, we then aimed to improve its efficacy by including additional pheromone compounds and host plant volatiles which have been used to attract cerambycid beetles in other areas of the world (e.g., Hanks et al. 2012, Wong et al. 2012; Experiment 2). In the second season, we tested the attractiveness of a cerambycid pheromone blend developed as a multispecies lure in the USA (see Hanks et al. 2012). This was tested with and without the addition of host plant volatiles (Experiment 3).

Experiments were conducted at three study sites in southeast Queensland (Supp. Table 1A [online only]): Boonah (−28.041, 152.632), Miva (−25.941, 152.512), and Oxley (−27.551, 152.967). The Boonah and Miva sites are remnant, selectively logged, dry sclerophyll eucalypt forests, dominated by spotted gum (*Corymbia citriodora* subspecies *variegata* [F.Muell.] Hill & Johnson). The understory is predominantly the invasive weed *Lantana camara* L. at Boonah, and both *L. camara* and *Acacia* species at Miva. The Oxley site is a smaller patch of remnant forest, with a mixture of wet and dry sclerophyll eucalypt species and a denser woody weed understory, principally *L. camara*.

Racemic 3-hydroxyhexan-2-one, racemic fuscumol and fuscumol acetate, and monochamol were obtained from Bedoukian Research (Danbury, CT). Racemic *syn*- and *anti*-2,3-hexanediols and 2,3-octanediols were prepared as previously described (Lacey et al. 2004). Racemic 2-methylbutanol was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Black flight-intercept panel traps (corrugated plastic, 1.2 m tall × 0.3 m wide, Alpha Scents Inc., West Linn, OR) treated with Fluon to improve trap catch rate (Graham et al. 2010) were used in all experiments. Trap buckets contained 50% aqueous propylene glycol as a killing agent and preservative. At each location, traps were suspended from tree branches so that the trap base was 1–1.5 m above ground level. Traps were positioned at least 20 m apart, and initial placement of lures was determined randomly. Pheromone emitters were clear polyethylene sachets (press-seal bags, 5.1 by 7.6 cm, 0.05 mm wall thickness; Fisher Scientific, Pittsburgh, PA). α-Pinene (Ultra High Release) and ethanol emitters were obtained from Alpha Scents Inc. The release rate of α-pinene was measured at 4.22 ± 0.18 g/d and ethanol at 1.12 ± 0.047 g/d (determined by mass loss under ambient conditions in southeast Queensland; M.W.G., R.A.H., and H.F.N., unpublished data).

Insects were collected from traps every 14 d, at which time pheromone lures were replaced, and treatments were rotated along the transect, ensuring that each lure was tested at each trap location to reduce positional bias. The ethanol emitters were replaced every 14 d and the α-pinene emitters were replaced after 6 wk. The sexes of trapped beetles were not recorded because the pheromones and attractants used generally attract adults of both sexes in similar numbers (e.g., Silk et al. 2007, Lacey et al. 2009, Teale et al. 2011, Wickham et al. 2014).

Experiment 1

Traps were deployed from November 2010 to April 2011 with the following eight treatments (Table 1): *syn*-2,3-hexanediol (henceforth *syn*-C6-diol), *anti*-2,3-hexanediol (*anti*-C6-diol), *syn*-2,3-octanediol (*syn*-C8-diol), *anti*-2,3-octanediol (*anti*-C8-diol), 3-hydroxyhexan-2-one (C6-ketol), 3-hydroxyoctan-2-one (C8-ketol), fuscumol + fuscumol acetate, monochamol, and solvent control. Lures were loaded with 0.5 ml of a 100 mg/ml solution of each test compound in ethanol. Controls consisted of baggies loaded with 0.5 ml ethanol.

Experiment 2

Because we were interested in developing a generic lure that would attract a broad range of species, for this experiment, the most attractive individual compound from Experiment 1, C6-ketol, was tested alone, combined with the next most attractive pheromone compound (*syn*-C6-diol), combined with the plant volatiles α-pinene and ethanol, and combined with *syn*-C6-diol and the plant volatiles. Pheromone lures were constructed as already described, but using isopropanol as the solvent. The change in solvent as compared to Experiment 1 was to remove any possible effects of the attractiveness of the solvent (after Hanks and Millar 2013). Controls consisted of baggies loaded with 0.5 ml isopropanol. Traps were run

Table 1. Compounds and blends used in each of the three experiments as trap lures

Expt	Compound	Lure code
1	Control (ethanol)	A
	3-Hydroxyhexan-2-one (C6-ketol)[a]	B
	syn-2,3-Hexanediol (*syn*-C6-diol)[a]	C
	anti-2,3-Hexanediol (*anti*-C6-diol)	D
	3-Hydroxyoctan-2-one (C8-ketol)	E
	syn-2,3-Octanediol (*syn*-C8-diol)	F
	anti-2,3-Octanediol (*anti*-C8-diol)	G
	Fuscumol + fuscumol acetate[a]	H
	Monochamol[a]	I
2	Control (isopropanol)	J
	C6-Ketol[a]	K
	C6-Ketol + *syn*-C8-diol	L
	C6-Ketol + α-pinene + ethanol	M
	C6-Ketol + *syn*-C8-diol + α-pinene + ethanol	N
3	Control (isopropanol)	J
	Host	O
	Pheromone blend[b]	P
	Pheromone blend[b] + host	Q

[a]Components of the pheromone blend used in Experiment 3.

[b]Contains C6-ketol, *syn*-C8-diol, fuscumol, fuscumol acetate, monochamol, and 2-methylbutanol.

from October 2011 to March 2012 at the Miva and Oxley sites (Supp. Table 1B [online only]), with two replicates per site.

Experiment 3

From October 2011 to March 2012, we tested attraction of beetles to a cerambycid pheromone blend developed as a multispecies lure (Hanks et al. 2012), alone and in combination with host plant volatiles, at the Miva and Oxley sites (Supp. Table 1B [online only]). The pheromone blend contained C6-ketol, *syn*-C6-diol, fuscumol, fuscumol acetate, monochamol, and 2-methylbutanol, the first five of which had been tested individually in Experiment 1. The lure blend was formulated to contain 25 mg of each isomer per ml of isopropanol solution, and lures were loaded with 1 ml of the blend. Treatments were as follows: pheromone blend alone, plant volatiles alone, blend + plant volatiles, solvent control (isopropanol).

Across all three experiments, beetles were identified by comparison with voucher specimens in the DAF Insect Collection, or using keys for Australian lamiine species (Slipinski and Escalona 2013). Voucher specimens were deposited in the DAF Insect Collection, Brisbane, Australia.

Data Analysis

For each experiment, we tested treatment effects by comparing both the mean number of species, and the mean number of individuals captured (as measures of diversity) with the Kruskal–Wallis one-way nonparametric analysis of variance (GenStat v16.1.0.10916, VSN International Ltd). Pairs of means were compared by post hoc determination using pairwise Mann–Whitney U tests. Similar analyses were conducted separately for the dominant subfamilies (Cerambycinae and Lamiinae) to assess how treatment effects varied with taxonomy. In addition, we used a Bray-Curtis similarity matrix on the fourth root transformed data, and an Analysis of Similarity (ANOSIM) to examine multidimensional similarities among treatments (PRIMER, V 5.2.9, 2001). The ANOSIM tests are a range of Mantel-type permutations of randomization procedures, which make no distributional assumptions. These tests depend only upon rank similarities, and thus are appropriate for this type of data. In Experiment 1, where >10 individuals of a species were trapped, attraction to each of the lures compared to all other treatments was examined by a χ^2 goodness-of-fit analysis.

Results

In total, 863 individuals from 47 cerambycid species were trapped throughout the course of this study, all native Australian species (Table 2).

Experiment 1

The three trapping locations differed in the number and species composition of beetles trapped, despite identical trapping effort (ANOSIM: Global $R = 0.011$, $P = 0.002$; Table 3a). Of 31 species trapped in this experiment, 21 were caught at only one site, nine were caught at two sites, and only one species (*Ancita marginicollis* [Boisduval]) was trapped at all three sites. Data were combined across study sites to determine which lures caught the greatest diversity of species.

Beetles from three cerambycid subfamilies were trapped in Experiment 1. A single beetle from the subfamily Prioninae was trapped in a fuscumol + fuscumol acetate trap; all others were from the two principal subfamilies found in Australia, the Cerambycinae and Lamiinae. Considering these two subfamilies separately, 69% of cerambycine individuals were caught in traps baited with C6-ketol, and another 18.4% were captured in traps baited with *syn*-C8-diol. The lamiine captures were more evenly spread across lure types, and there were no differences among treatments, including the control.

There was no significant difference among treatments in the number of species or number of individuals caught per date (Table 3b; Kruskal–Wallis: species $H = 12.0$, df = 8, $P = 0.15$; individuals $H = 12.2$, df = 8, $P = 0.14$). More than half of the individuals (54%) and species (58%) collected came to traps baited with C6-ketol, and there were three times as many unique species caught at traps baited with this lure than with any other compound (Table 3b). This lure, C6-ketol, was the only treatment which differed significantly from the control, with more individuals ($P < 0.001$) and more species ($P < 0.001$) caught in traps baited with this compound than in control traps (Fig. 1). For species with >10 individuals trapped, there were three species trapped significantly more often in traps baited with the C6-ketol lure than all other treatments. Traps baited with this compound caught 92% of *Adrium* species 1 ($n = 12$, $\chi^2 = 4.17$, $P = 0.004$), 97% of *Bethelium tillides* (Pascoe) ($n = 32$, $\chi^2 = 14.1$, $P < 0.001$), and all *Xylotrechus australis* (Laporte & Gory) ($n = 14$, $\chi^2 = 7.0$, $P < 0.001$). Four other species were caught in sufficient numbers to allow statistical analyses (*Amphirhoe decora* Newman, *A. marginicollis*, *Ancita niphonoides* [Pascoe], and *Demonax chrysodores* [White]), but none of these were significantly attracted to the C6-ketol treatment. The next most attractive compound (although not significantly so), in terms of individuals (18% of total captures) and species (26% of total captures) was *syn*-C8-diol. In addition, there was a very specific attraction of one species (*D. chrysodores*) to this compound compared to all others ($\chi^2 = 8.5$, $P < 0.001$). As stated above, these compounds were therefore used as the basis for subsequent experiments aimed at developing more attractive lures.

Multivariate, nonparametric analyses also showed that there were differences in the diversity and abundance of beetles attracted to the nine treatments in Experiment 1 (ANOSIM: Global $R = 0.027$, $P = 0.001$). Post hoc pairwise tests showed that the mean for C6-ketol

Table 2. Subfamily, tribe, species and total numbers of cerambycid beetles captured, and list of lures in traps in which beetles were captured, for the three lure development experiments

Subfamily, tribe, species[a]	Expt 1	Expt 2	Expt 3	Total	Lure[b]	Site
Cerambycinae						
Callidiopini						
Adrium species 1	12	307	12	330	B, C, J, K, L, M, N, Q	Miva, Oxley
Adrium species 2	1	2	0	3	B, L, N	Miva
Adrium species 3	0	1	0	1	K	Miva
Bethelium signiferum (Newman)	3	15	2	20	B, K, L, M, N, P, Q	Miva, Oxley
Bethelium species 1	1	0	0	1	B	Miva
Bethelium spinicorne Blackburn	2	0	0	2	B	Boonah
Bethelium tillides (Pascoe)	32	234	17	283	B, D, K, L, M, N, O, P, Q	Boonah, Miva, Oxley
Ceresium species 1	1	14	2	17	B, D, G, K, L, M, N, O, Q	Oxley
Ceresium species 2	1	0	0	1	B	Oxley
Ceresium species 3	0	1	0	1	L	Miva
Didymocantha oblique Newman	2	1	2	4	A, F, J, P	Boonah, Miva
Cerambycini						
Pachydissus sericus Newman	0	1	0	1	K	Oxley
Clytini						
Chlorophorus curtisi (Laporte & Gory)	0	1	1	2	K, Q	Miva, Oxley
Demonax chrysoderes (White)	17	6	0	23	F, K, L, M, N	Boonah, Miva, Oxley
Xylotrechus australis (Laporte & Gory)	14	9	2	25	B, K, L, M, N, P, Q	Boonah, Miva, Oxley
Xylotrechus reginae Aurivillinus	3	1	1	5	B, N, P	Miva, Oxley
Xylotrechus species 1	0	1	1	2	N, Q	Oxley
Heteropsini						
Aridaeus thoracicus (Donovan)	0	4	2	6	M, N, O, P	Miva, Oxley
Cremys diopthalmus (Pascoe)	2	5	0	7	B, K, M, N	Miva, Oxley
Phoracanthini						
Coptocerus species 1	1	0	0	1	F	Boonah
Pytheini						
Brachytria centralis Pascoe	0	4	0	4	K, L, N	Miva, Oxley
Obrida comate Pascoe	3	4	5	12	B, D, L, M, N, O, Q	Oxley
Pempsamaera species 1	1	0	0	1	F	Oxley
Rhopalophorini						
Amphirhoe decora Newman	13	3	14	27	A, B, D, E, F, G, J, O, P, Q	Miva, Oxley
Tessarommatini						
Tessaromma sericans (Erichson)	1	0	0	1	H	Boonah
Tillomorphini						
Tilloforma species 1	0	5	0	5	N	Oxley
Uracanthini						
Rhinopthalmus nr *modestus*	0	1	0	1	L	Oxley
Lamiinae						
Acanthocinini						
Didymocentrotus denticollis (Pascoe)	3	4	2	8	B, D, H, J, M, N, O	Oxley
Apomecynini						
Ropica exocentrioides Pascoe	1	0	0	1	C	Oxley
Sybra species 1	1	0	0	1	E	Oxley
Zorilispe robustum (Oke)	1	0	0	1	A	Oxley
Monochamini						
Acalolepta mixta (Hope)	0	6	4	10	K, L, M, N, O, Q	Oxley
Pteropliini						
Rhytiphora bankii (F.)	3	4	8	15	B, E, F, J, K, M, P, Q	Boonah, Oxley
Rhytiphora basalis (Aurivillinus)	0	0	1	1	Q	Miva
Rhytiphora pedicornis (F.)	1	0	0	1	B	Miva
Rhytiphora piperitia Hope	0	2	0	2	K, N	Miva
Rhytiphora pulverulens (Boisduval)	1	0	0	1	E	Boonah
Rhodopinini						
Ancita antennata (Pascoe)	8	4	4	16	B, C, G, H, K, M, O, P, Q	Miva, Oxley
Ancita marginicollis (Boisduval)	10	0	0	10	A, B, D, F, G, H	Boonah, Miva, Oxley
Ancita niphonoides (Pascoe)	14	2	0	16	A, C, D, F, I, N	Miva
Ancita varicornis (Germar)	0	1	0	1	M	Miva
Bucynthia spiloptera (Pascoe)	0	0	1	1	Q	Oxley
Zygocerini						
Zygocera pruinosa (Boisduval)	1	1	1	3	G, L, P	Miva
Zygocera species 1	1	0	0	1	E	Miva

(continued)

Table 2. continued

Subfamily, tribe, species[a]	Expt 1	Expt 2	Expt 3	Total	Lure[b]	Site
Prioninae						
Anacolini						
Sceleocantha glabricollis Newman	1	0	0	1	H	Boonah
Tragosomini						
Phaolus metallicum (Newman)	0	0	2	2	Q	Miva
Total	156	644	70	863		

[a]Ten individuals from six morphospecies that could not be identified were excluded from analyses.
[b]Lure code as per Table 3.

Table 3. Total number of species and individuals trapped in Experiment 1, sorted with respect to: a) sampling site, and b) test lure

a	Boonah	Miva	Oxley	Total
Total no. of species	12	15	15	31
Total no. of individuals	28	87	45	160
Total no. of tribes	8	7	10	15
Total no. of cerambycine species (individuals)	7 (23)	9 (61)	8 (30)	18 (114)
Total no. of lamiine species (individuals)	4 (4)	5 (26)	7 (15)	12 (45)
Total no. of unique species	5	6	6	

b	Control	C6-ketol	*syn*-C6-diol	*anti*-C6-diol	C8-ketol	*syn*-C8-diol	*anti*-C8-diol	Fucumol + fuscumol acetate	Monochamol	Total
Total no. of species	5	18	4	7	5	8	5	5	2	31
Total no. of individuals	5	86	7	10	5	28	6	6	7	160
Total no. of tribes	4	8	3	5	3	7	4	4	2	15
Total no. of cerambycine species (individuals)	2 (2)	12 (79)	1 (1)	4 (6)	1 (1)	5 (21)	2 (2)	1 (1)	1 (1)	18 (114)
Total no. of lamiine species (individuals)	3 (3)	6 (7)	3 (6)	3 (4)	4 (4)	3 (7)	3 (4)	3 (4)	1 (6)	12 (45)
Total no. of unique species	1	9	1	0	3	3	1	2	0	

was significantly greater than the mean for the remaining treatments, none of which differed from each other or the control.

Experiment 2

Capture rates were not improved by addition of *syn*-C8-diol, host volatiles, or both to traps baited with C6-ketol (Table 4). All treatments were significantly different from controls in terms of both the numbers of species (Kruskal–Wallis $P < 0.001$) and the numbers of individuals ($P < 0.001$) caught, but did not vary otherwise (Fig. 2).

Captures were dominated by two species: *Adrium* species 1 and *B. tillides* (541 of a total of 644; 84%). Again, for these species there were no significant differences in the mean numbers of beetles attracted to the different treatments, except that all were different from the means for the control (*Adrium* species 1: $H = 31.5$, $df = 4$, $P < 0.001$; *B. tillides*: $H = 28.3$, $df = 4$, $P < 0.001$). Similarly, for lamiine species, there were no differences among treatments, with the exception that all treatments were more attractive than the control.

Multivariate nonparametric analyses showed that there were differences in the diversity and abundance of beetles attracted to the five treatments (ANOSIM: Global $R = 0.064$, $P = 0.003$). Pairwise tests showed that all the lures were different from the control, but there were no differences among any treatment type.

Experiment 3

The combination of the blend of pheromones with host plant volatiles was significantly more attractive than the control or either attractant alone, in terms of both numbers of species (Kruskal–Wallis $P < 0.001$) and individuals ($P < 0.001$) trapped (Fig. 3), whereas neither the host volatiles nor the pheromone blend alone differed significantly from the control. Six times as many individuals, three times as many species, and six unique species were trapped with the combined lures compared with the control (Table 5). There was only one species that was common to all treatments in Experiment 3, *A. decora*. Neither the control, nor plant volatiles alone treatments attracted any unique species, whereas three species only came to the pheromone blend alone. The combined lure trapped the greatest numbers of individuals ($n = 42$), species ($n = 15$), and unique species ($n = 6$; Fig. 4, Table 5). Sixty-seven percent of cerambycine individuals were caught in traps baited with the combined lure, whereas the numbers of lamiine beetles were not affected by lure type (Table 5).

Multivariate nonparametric analyses also showed that there were differences in the diversity and abundance of beetles attracted to the four treatments (ANOSIM: Global $R = 0.62$, $P = 0.046$). Pairwise tests showed that the combined lure was different from the control.

Discussion

Our study showed C6-ketol to be an effective attractant across a broad range of cerambycid taxa native to Australia, with traps baited with this compound capturing 18 different cerambycid species from nine tribes within the subfamilies Cerambycinae and

Lamiinae. It was highly attractive to a number of individual species. C6-ketol is well known as a pheromone component for many species in the subfamily Cerambycinae in various regions of the northern hemisphere (e.g., Hanks et al. 2007; Ray et al. 2009; Hanks and Millar 2013; Imrei et al. 2013; Mitchell et al. 2013, 2015), and is considered to be a highly conserved pheromone component within the Cerambycidae (Wickham et al. 2014, Mitchell et al. 2015). Our results support this finding and demonstrate its attractiveness to members of the Cerambycinae in Australia, further evidence that it is truly ubiquitous on a global scale.

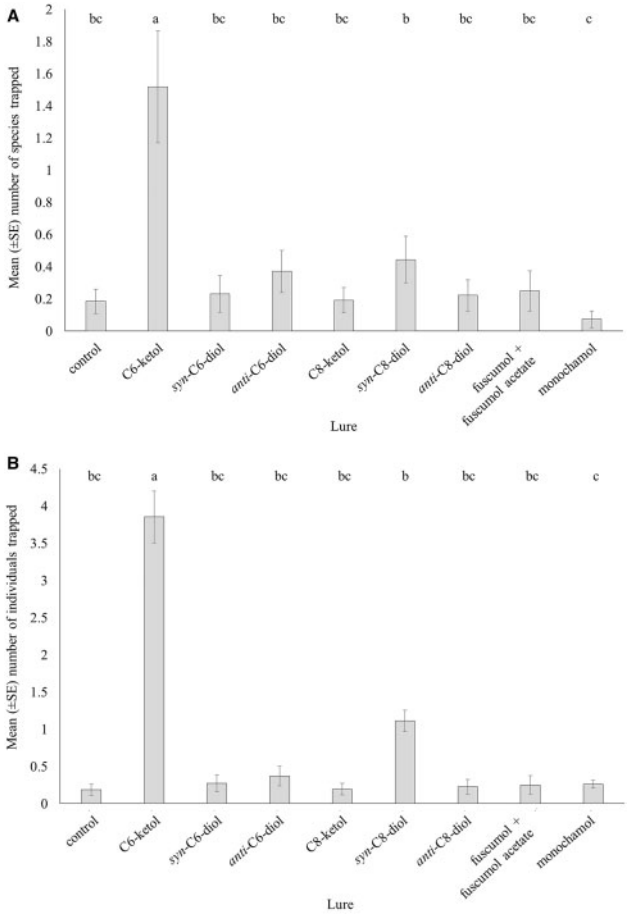

Fig. 1. Mean (± 1 SE) number per trap in Experiment 1 of: a) cerambycid species (Kruskal–Wallis $H = 3.49$, df = 8, $P < 0.001$), and b) cerambycid beetle individuals ($H = 32.0$, df = 8, $P < 0.001$). Bars surmounted by the same letter are not significantly different.

The overall effectiveness of this individual compound was not significantly changed by blending it with an additional known cerambycid pheromone compound, *syn*-C8-diol, with the host volatiles α-pinene and ethanol, or both. Although a number of different species were trapped in the combination blends, there was no overall change in the species diversity. Northern hemisphere species of two of the common genera captured in our study, *Demonax* and *Xylotrechus*, are known to be attracted to compounds with 2,3-alkanediol (e.g., *syn*-C8-diol) or hydroxyketone (e.g., C6-ketol) motifs (e.g., Hanks and Millar 2013, Hanks et al. 2012, Wickham et al. 2014).

Thus, our data suggest that C6-ketol may be a useful lure for a diversity of Australian cerambycid taxa. An effective generic lure will aid in the targeted collection of local Australian fauna, supporting studies on biodiversity and population dynamics. Despite the economic importance of this beetle family, our understanding of Australian species is fragmentary, and often limited to isolated species or generic descriptions (Slipinski and Escalona 2013). Of the 31 species collected in our study, almost half (45%) were identified only to genus because they do not appear to have been formally described and named. An expanded knowledge of our local fauna is critical from a biosecurity perspective, because rapid and effective detection of incursions of exotic species is predicated on a thorough knowledge of the endemic fauna.

We also tested the effectiveness of a pheromone blend developed for cerambycids in North America (e.g., Hanks et al. 2012, Handley et al. 2015). This blend was not attractive to the broad range of Australian cerambycid species. However, the addition of the plant volatiles α-pinene and ethanol significantly increased capture rates in terms of both total number of species and number of individuals trapped. Adding host volatiles to the pheromone blend led to the capture of nine species not trapped by pheromone blend alone, nine species not trapped by the host volatiles alone, and six species not trapped by either. However, four species (three cerambycines and one lamiine) attracted by the pheromone blend alone apparently were inhibited by the addition of host volatiles. *Adrium* species 1, the most frequently trapped species across all three experiments, was only trapped by the combination treatment in Experiment 3. This is in contrast to *B. tillides*, *A. decora*, and *A. antennata*, which were trapped by host volatiles, the pheromone blend, and the combination of the two.

In fact, there was some suggestion of a possible inhibitory effect of the blend. In particular, *Adrium* species 1 was attracted to C6-ketol as a single component (Experiment 1), and to the combination of this compound with *syn*-C8-diol, with host volatiles, or both (Experiment 2), but not to the blend (Experiment 3). Individuals from the genus *Ancita* were caught in traps baited with eight of the nine single component lures tested in Experiment 1, but few were

Table 4. Total number of species and individuals trapped in Experiment 2, sorted with respect to lure treatment

	Control	C6-ketol	C6-ketol + *syn*-C8-diol	C6-ketol + host	C6-ketol + *syn*-C8-diol + host	Total
Total no. of individuals	7	127	106	202	202	644
Total no. of species	5	15	13	14	18	29
Total no. of tribes	2	9	5	7	9	14
Total no. of cerambycine species (individuals)	3 (5)	11 (122)	11 (103)	9 (194)	14 (196)	21 (620)
Total no. of lamiine species (individuals)	2 (2)	4 (5)	2 (3)	5 (8)	4 (6)	8 (24)
Total no. of unique species	2	3	3	1	3	

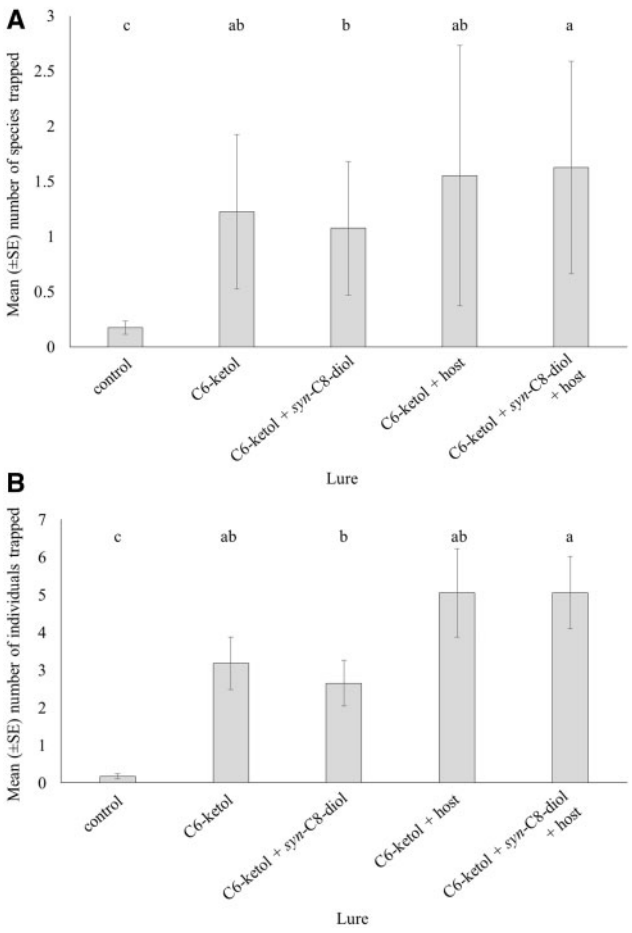

Fig. 2. Mean capture rates per trap to each of the lure treatments in Experiment 2 of a) cerambycid species ($H = 45.4$, df = 4, $P < 0.001$), and b) cerambycid beetle individuals (Kruskal–Wallis $H = 48.8$, df = 4, $P < 0.001$). Bars surmounted by the same letter are not significantly different.

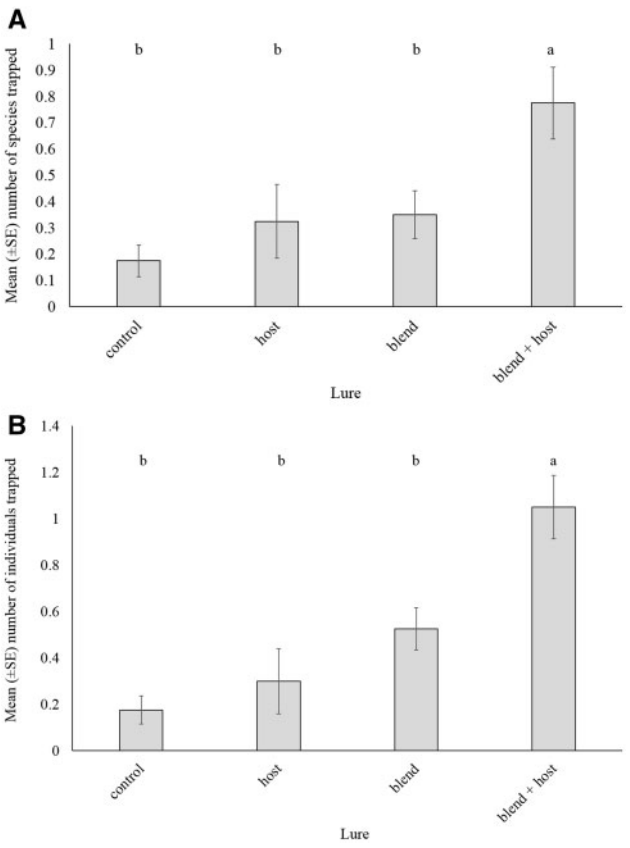

Fig. 3. Mean capture rates per trap to each of the lure treatments in Experiment 3 of a) cerambycid species (Kruskal–Wallis $H = 16.9$, df = 3, $P < 0.001$), and b) cerambycid beetle individuals ($H = 18.4$, df = 3, $P < 0.001$). Bars surmounted by the same letter are not significantly different.

captured in traps baited with the pheromone blend in Experiment 3. The low numbers of the otherwise common *Adrium* species 1 and *Ancita* species attracted to the blend lure suggests that one or more components of this combination lure (possibly 2-methylbutanol, the only blend component not included in Experiment 1) may be inhibitory. Inhibitory effects caused by minor pheromone components have been shown in a number of cerambycid species (e.g., Wong et al. 2012, Mitchell et al. 2015).

The plant volatiles α- and β-pinene and ethanol have been reported as attractants for several of the species captured in our study, including *Sceleocantha glabricollis* Newman, *B. tillides*, *Rhytiphora* spp., and *Coptocerus* species 1 (Stone et al. 2010). In the northern hemisphere, host plant volatiles are often effective in attracting conifer-feeding cerambycids, but may be less likely to attract species associated with deciduous trees (e.g., Hanks et al. 2012, Wong et al. 2012). In Australia, where forests are dominated by eucalypts, in which the monoterpenes α- and β-pinene are common components of the odor of leaves and bark (Dunlop et al. 1999; Asante et al. 2001; Brophy and Southwell 2002; Nahrung et al. 2009, 2012; Gilles et al. 2010; Hayes et al. 2013, 2014; Bett et al. 2016), these volatiles may well be attractive to cerambycid beetles.

Few species were trapped in sufficiently large numbers to allow analyses of responses of individual species. However, a few apparent trends were identified which warrant further study. Specifically, during Experiment 1, 97% of *B. tillides* and all *X. australis* were caught in traps baited with C6-ketol. In subsequent experiments, these species were caught in all traps baited with this pheromone, either as a single component or in blends with other components. C6-ketol is a male-produced aggregation pheromone component of several congeners (e.g., *X. colonus* [F.]; Lacey et al. 2009). *Bethelium tillides* was also caught in traps baited with host plant volatiles. The presence of other pheromone compounds did not significantly affect catches of this species, implying no apparent inhibitory effect.

During Experiment 1, all but one of the 12 *Adrium* species 1 were caught in traps baited with C6-ketol. In subsequent experiments, this species also was caught in traps baited with this pheromone in combination with *syn*-C8-diol, host volatiles, or both, but not in traps baited with the pheromone blend (Experiment 3). During Experiment 1, 21% of all individuals trapped were from the predominantly *Acacia*-feeding genus *Ancita* (Duffy 1963), with beetles being caught in traps baited with eight of the nine lures tested. In subsequent experiments, with a more restricted range of lures, only 1.6% of specimens were from this genus. The lack of specificity to a specific lure might suggest that catches were merely random, and the high catch rate may have resulted from adults of these species emerging during Experiment 1, but not the following year, although the fact that these beetles were trapped at all three sites used in Experiment 1 makes this seem unlikely.

Our study supports the view that there is considerable parsimony in pheromone biosynthesis and use within the Cerambycidae worldwide (Hanks et al. 2014). While cross-attraction to shared pheromone

Table 5. Total number of species and individuals trapped in Experiment 3, sorted with respect to lure treatment

	Control	Host	Blend	Blend + host	Total
Total no. of individuals	7	14	21	42	84
Total no. of species	5	8	10	15	20
Total no. of tribes	3	7	6	8	12
Total no. of cerambycine species (individuals)	3 (5)	5 (10)	7 (12)	9 (34)	12 (61)
Total no. of lamiine species (individuals)	2 (2)	3 (4)	3 (9)	5 (6)	7 (21)
Total no. of unique species	0	0	3	6	

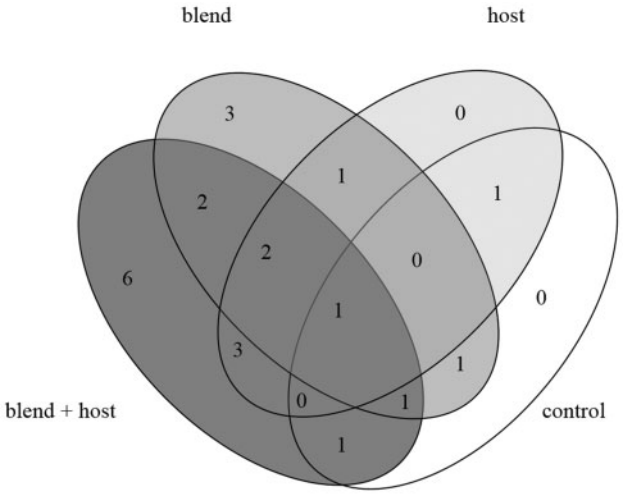

Fig. 4. Venn diagram representing the number of species trapped by each lure treatment, represented as an overlapping ellipse in Experiment 3.

components used by sympatric species may facilitate host location (Lacey et al. 2009) or mating (Handley et al. 2015), the flight activity of different species is often partitioned temporally (Mitchell et al. 2015) or spatially (Graham et al. 2012, Handley et al. 2015). Our experimental design did not allow this to be tested, but up to 15 sympatric species were captured in traps containing C6-ketol, with a maximum of five overlapping species in traps baited with lures containing this component on any one trap count.

The attractiveness of C6-ketol to a broad range of tribes and species of Australian cerambycid beetles, and to a range of species in the northern hemisphere (e.g., Wickham et al. 2014, Mitchell et al. 2015) demonstrates its utility as a multispecies attractant. This lure could potentially be used for early detection of invasive species, and for helping to elucidate communities of wood-boring insects in native habitats. However, some caution must be used when drawing conclusions from the findings of the current study because we have no knowledge of the entire cerambycid community that could have been sampled, only a knowledge of what was caught in our traps. Our assertion that C6-ketol is an effective attractant is only relative to the other treatments, and future lures may be more effective still. The continued development of attractive cerambycid lures, including specific lures for target biosecurity pests, is the next objective of this research.

Acknowledgments

We sincerely thank Simon Lawson, F. Ross Wylie, and Philipp Kirsch for critical discussions on the design of these experiments. We thank Janet McDonald, Jennifer Nahrung, Lance Maddock, Alex Glauerdt, and Bryn Hughes for assistance with field work. We thank Justin Bartlett and F. Ross Wylie for assistance with beetle identification. We thank Justin Bartlett and Susan House for assistance with preparation of figures. This research was supported by a grant from The Alphawood Foundation to L.M.H., and USDA-APHIS grant 14-8130-1422-CA to J.G.M. and L.M.H.

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