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ARTICLE

Cerambycid pheromones affect catches of *Phymatodes aeneus* (Coleoptera: Cerambycidae) and *Thanasimus undatulus* (Coleoptera: Cleridae) in ethanol-baited multiple-funnel traps in the Pacific Northwest, United States of America

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Abstract

In 2012, we evaluated the effects of hardwood cerambycid pheromones (*syn*-2,3-hexanediol, 3-hydroxyhexan-2-one, and 3-hydroxyoctan-2-one) on catches of bark and woodboring beetles in ethanol-baited multiple-funnel traps in two field trials in Oregon and Washington, United States of America. Catches of *Phymatodes aeneus* LeConte (Coleoptera: Cerambycidae) in ethanol-baited traps increased with the addition of 3-hydroxyhexan-2-one lures or the 3,2-hydroxyketone lure blend (3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one). Catches of the predator *Thanasimus undatulus* (Say) (Coleoptera: Cleridae) in ethanol-baited traps increased with the addition of 3-hydroxyhexan-2-one lures but not *syn*-2,3-hexanediol lures or 3-hydroxyoctan-2-one lures. The 3,2-hydroxyketone lure blend decreased catches of the corthyline ambrosia beetle, *Gnathotrichus sulcatus* (LeConte) (Coleoptera: Curculionidae), but not the xyleborine ambrosia beetle, *Xyleborinus saxesenii* (Ratzeburg), (Coleoptera: Curculionidae). Catches of *Ptilinus basalis* LeConte (Coleoptera: Anobiidae) in ethanol-baited traps increased with the addition of the 3,2-hydroxyketone lure blend.

Introduction

In the past two decades, significant research efforts have been undertaken to discover attractants for woodboring beetles, due in no small part to invasions by the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) (Dodds and Orwig 2011), and the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) (Poland and McCullough 2006), into North America. With the exception of Millar *et al.* (2018), most trapping experiments with the cerambycid pheromones 2,3-hexanediols and 3,2-hydroxyketones have occurred in the eastern United States of America, likely because of the high diversity of beetle species associated with the high diversity of hardwoods found there. A few trapping efforts have focused on cerambycid species associated with conifer forests in the western United States of America, using compounds such as pheromones used by bark beetles (ipsenol and ipsdienol; Allison

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et al. 2001; Miller *et al.* 2013a) or *Monochamus* spp. of Cerambycidae (monochamol; Miller *et al.* 2016).

Our goal was to conduct a preliminary field assessment of three common hardwood cerambycid pheromones (*syn*-2,3-hexanediol, 3-hydroxyhexan-2-one, and 3-hydroxyoctan-2-one) on catches of bark and woodboring beetles in ethanol-baited traps in the Pacific Northwest of the United States of America. Given the low level of diversity and abundance of hardwood species in western forests, our expectations for responses by western species of Cerambycidae were low. To maximise our ability to detect such responses, we employed two different trapping designs based on successful designs employed in the southeastern United States of America (Miller *et al.* 2015, 2017). The two studies were conducted at two different locations (approximately 240 km apart) within the Cascade Mountain Range of Oregon and Washington states, United States of America to provide further opportunities for detection of responses.

Methods

We used black 10-unit multiple-funnel traps (Synergy Semiochemicals Inc.; Delta, British Columbia, Canada), modified to allow lures to be hung within the funnels (Miller *et al.* 2013b). Black ethanol ultra-high release pouches, white *syn*-2,3-hexanediol (racemic) lures, brown 3-hydroxyhexan-2-one (racemic), and brown 3-hydroxyoctan-2-one (racemic) lures were obtained from Contech Enterprises (Victoria, British Columbia, Canada). Release rates (determined by the manufacturer at 20–23 °C) from these lures were 0.5 g/d, 1.5 mg/d, 20–25 mg/d, and 20–25 mg/d, respectively.

In 2012, we conducted two trapping experiments in mature stands of Douglas-fir, *Pseudotsuga menziesii* (Mirbel) Franco (Pinaceae), in the Cascade Mountain Range in the Pacific Northwest, United States of America: one in Oregon and the other in Washington. In both experiments, 40 traps were deployed in a randomised complete block design, with 10 blocks of four traps per block. Traps were hung by twine between trees, such that the collection cup of each trap was approximately 0.5 m above ground level. Traps were spaced 10–25 m apart within blocks; blocks were spaced 20–30 m apart. Each collection cup contained approximately 300 mL of an aqueous solution of propylene glycol to kill and preserve insects (Miller and Duerr 2008). The solution was changed after each two-week collection period. Voucher specimens were deposited in the University of Georgia Collection of Arthropods, Athens, Georgia, United States of America.

Experiment 1 was conducted from 25 June to 11 September 2012 in the Mount Baker–Snoqualmie National Forest, King County, Washington (47.398° N, 121.493° W). One of the following four lure combinations was randomly applied to a trap within each block: (1) ethanol alone; (2) ethanol + *syn*-2,3-hexanediol (D6); (3) ethanol + 3-hydroxyhexan-2-one (K6); and (4) ethanol + 3-hydroxyoctan-2-one (K8). Experiment 2 was conducted from 21 June to 15 August 2012 in the Mount Hood National Forest, Clackamas County, Oregon (45.444 °N, 122.157 °W). One of the following four lure combinations was randomly applied to a trap within each block: (1) ethanol alone; (2) ethanol + *syn*-2,3-hexanediol (D6); (3) ethanol + 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one (K); and (4) ethanol + *syn*-2,3-hexanediol (D6) + 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one (K). Ethanol was used as the control for comparing additional lures because ethanol alone is attractive to bark and woodboring beetles (Miller 2006; Miller and Rabaglia 2009; Sweeney *et al.* 2016) and increases attraction of cerambycids to pheromone-baited traps (Miller *et al.* 2015). In each experiment, *syn*-2,3-hexanediol, 3-hydroxyhexan-2-one, and 3-hydroxyoctan-2-one lures were replaced after six weeks.

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Statistical analyses for species caught in sufficient numbers ($N \geq 30$) were conducted with the SYSTAT, version 13, and SigmaStat, version 3.01, statistical packages (SYSTAT Software Inc., Point Richmond, California, United States of America). As needed, data were transformed by ln (Y + 1) to attain normality and homoscedasticity, verified by the Shapiro–Wilk and equal variance tests, respectively. In both experiments, data were analysed by mixed-model analysis of variance, with treatment as the fixed factor, followed by the Holm–Sidak multiple-comparison test for species showing effects of treatments ($\alpha = 0.05$). For species affected by treatments in experiment 2, data were further analysed by a mixed-model analysis of variance using the following model factors: (1) *syn*-2,3-hexanediol (D6); (2) 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one (K); and (3) *syn*-2,3-hexanediol (D6) × 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one (K; *i.e.*, D6 + K).

Results and discussion

We captured a total of 478 longhorned beetles across the two experiments, detecting 20 cerambycid species (Table 1). Only *Phymatodes aeneus* LeConte (Coleoptera: Cerambycidae) was caught in sufficient numbers for statistical analyses. Similar to Millar *et al.* (2018), lure treatments had a significant effect on catches of *Ph. aeneus* ($N = 95$) in experiment 1 ($F_{3,27} = 33.89$, $P < 0.001$). Traps baited with ethanol + 3-hydroxyhexan-2-one caught more beetles than traps baited with the other three treatments did (Fig. 1A). In experiment 2, the 3, 2-hydroxyketone blend had a significant effect on catches of *Ph. aeneus* (Table 2). Catches were greatest in traps baited with the 3,2-hydroxyketone blend (with or without *syn*-2,3-hexanediol; Fig. 1E).

A common feature among Cerambycidae is the use of the same pheromones among related species (Hanks and Millar 2013), allowing for the operational use of lure blends to detect multiple cerambycid species simultaneously (Hanks *et al.* 2012). 3-Hydroxyhexan-2-one is a pheromone or attractant for several congeners of *Ph. aeneus* in North America: *Phymatodes aereus* Newman, *Ph. amoenus* Say, *Ph. lecontei* Linsley, *Ph. lengi* Joutel, *Ph. obliquus* Casey, *Ph. testaceus* Linnaeus, and *Ph. varius* (Hanks *et al.* 2007, 2019; Hanks and Millar 2013; Mitchell *et al.* 2015; Millar *et al.* 2018; Collignon *et al.* 2019).

We found that *syn*-2,3-hexanediol had an effect on catches of *Ph. aeneus* in experiment 2 (Table 2), with catches in traps baited with ethanol + *syn*-2,3-hexanediol greater than those in traps baited solely with ethanol (Fig. 1E). In contrast, Millar *et al.* (2018) found that attraction of *Ph. aeneus* to traps baited with 3-hydroxyhexan-2-one in Oregon was interrupted by the addition of the blend of *syn*- + *anti*-2,3-hexanediols; the effect of *syn*-2,3-hexanediol alone on catches of beetles in traps baited with 3-hydroxyhexan-2-one was not determined. Hanks and Millar (2013) and Hanks *et al.* (2019) previously noted the congeners *Ph. aereus* and *Ph. varius* were attracted to traps baited with *syn*-2,3-hexanediol in the United States of America.

A thorough understanding of the chemical ecology of this genus will require determinations of pheromone production by all these species; determinations have only been conducted for a few species. Additional studies testing the effects of host kairomones and other pheromones such as 2-methylbutanol on behavioural responses need to be conducted (Collignon *et al.* 2016). Dose and chemical composition can affect trap catches (Collignon *et al.* 2019). Enantiomeric composition may be an important issue for these species because the following compounds are chiral, existing in (+) and (–) forms: *syn*-2,3-hexanediol, *anti*-2,3-hexanediol, 3-hydroxyhex-2-one, and 3-hydroxyoctan-2-one (Ray *et al.* 2012; Mitchell *et al.* 2013, 2015; Hanks *et al.* 2019). Such studies should help to clarify the effects of interspecific competition, geographic variation, host-specific kairomones, and predation on these species.

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Table 1. Catches of Cerambycidae (Coleoptera) in two experiments with multiple-funnel traps baited with ethanol (Et), syn-2,3-hexanediol (D6), 3-hydroxyhexan-2-one (K6), 3-hydroxyoctan-2-one (K8), and a blend of K6 + K8 (K) in the Pacific Northwest, United States of America.

	Experiment 1, Washington					Experiment 2, Oregon				
Species	Et	Et + D6	Et + K6	Et + K8	Total	Et	Et + D6	Et + K	Et + D6 + K	Total
Asemum striatum (Linnaeus)	–	–	1	–	1					
Brachyleptura dehiscens (LeConte)	1	–	–	–	1					
Clytus pacificus (Van Dyke)	–	–	–	2	2					
Clytus planifrons (LeConte)	–	–	1	6	7	–	–	5	5	10
Etorofus obliteratus (Haldeman)	–	2	2	–	4	2	1	5	2	10
Evodinus vancouveri Casey	–	3	4	2	9	1	–	2	–	3
Hybodera tuberculata LeConte	–	–	1	–	1					
Leptalia macilenta (Mannerheim)	–	–	–	1	1					
Lepturopsis dolorosa (LeConte)	1	2	3	–	6					
Megasemum aspersum (LeConte)	2	1	–	1	4					
Necydalis laevicollis LeConte	3	7	1	3	13					
Phymatodes aeneus LeConte	2	6	79	8	95	3	14	71	116	204
Phymatodes vilitatis Linsley	–	–	4	–	4					
Pidonia quadrata Hopping	7	5	1	16	29	2	–	–	–	2
Pidonia scripta (LeConte)	14	1	11	3	29	–	3	2	–	5
Xestoleptura behrensii (LeConte)	4	–	2	–	6	–	1	2	–	3
Xestoleptura crassicornis (LeConte)	2	2	1	–	5	1	2	–	–	3
Xestoleptura crassipes (LeConte)	1	–	1	–	2	1	–	–	–	1
Xestoleptura tibialis (LeConte)	3	–	–	–	3					
Xylotrechus undulatus (Say)	6	3	1	2	12	–	1	–	2	3

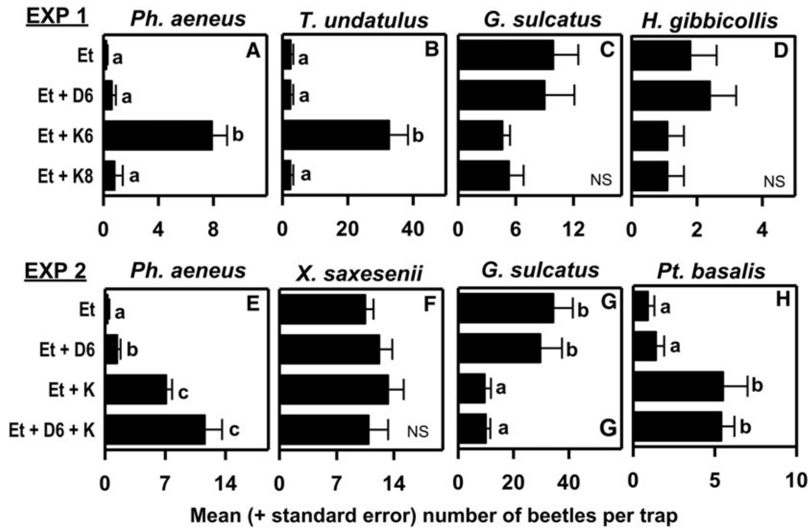

Fig. 1. Effects on mean (± standard error) catches of **A**, *Phymatodes aeneus*, **B**, *Gnathotrichus sulcatus*, **C**, *Thanasimus undatulus*, and **D**, *Hemicoelus gibbicollis* by *syn*-2,3-hexanediol (D6), 3-hydroxyhexan-2-one (K6), and 3-hydroxyoctan-2-one (K8) in experiment 1, and of **E**, *Ph. aeneus*, **F**, *G sulcatus*, **G**, *Xyleborinus saxesenii*, and **H**, *Ptilinus basalis* in traps baited with *syn*-2,3-hexanediol (D6) and 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one (K) in experiment 2. Traps were control baited with ethanol (Et). Means for a species followed by the same letter are not significantly different at $P = 0.05$ (Holm–Sidak test).

Catches of *Thanasimus undatulus* (Say) (Coleoptera: Cleridae) in experiment 1 ($N = 399$) were affected by lure treatments ($F_{3,27} = 23.79$, $P < 0.001$), with the greatest number captured in traps baited with ethanol + 3-hydroxyhexan-2-one (Fig. 1B), mirroring the response profile of *P. aeneus* (Fig. 1A). *Thanasimus undatulus* is a generalist predator of bark and woodboring beetles, with adults feeding on adult prey and larval *T. undatulus* feeding on larval prey (Furniss and Carolin 1980).

Prey and predators attracted by the same compounds to the same sites likely increase the likelihood of predation events. Understanding the chemical ecology of predators can provide insights into their ecological roles with native species of bark and woodboring beetles, as well as their potential role in mitigating the impacts of future nonnative species. Responses of predators mirroring the pheromone responses of cerambycids are not uncommon with traps baited with *syn*-2,3-hexanediol and 3,2-hydroxyketones. In the southeastern United States of America, 3-hydroxyhexan-2-one was attractive to six common species of Cerambycidae and to the predators *Temnoscheila virescens* (Fabricius) (Coleoptera: Trogossitidae) and *Apiomerus crassipes* (Fabricius) (Hemiptera: Reduviidae); *A. crassipes* and the woodborer *Clytus marginicollis* Laporte and Gory (Coleoptera: Cerambycidae) were attracted to traps baited with 3-hydroxyoctan-2-one (Miller *et al.* 2015, 2022).

Similarly, catches of the predators *Chariessa pilosa* (Forster), *Madoniella dislocata* (Say), and *Enoclerus ichneumoneus* (Fabricius) (all Coleoptera: Cleridae) were greatest in traps baited with *syn*-2,3-hexanediol; catches of all three species were reduced by the addition of the blend of 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one (Miller *et al.* 2022). The same response profile was found for three species of woodborers at the same sites (Miller *et al.* 2015, 2017, 2022). The cerambycid pheromone sulcatol (Meier *et al.* 2019) attracted the woodborer *Leptostylus asperatus* (Haldemann) (Coleoptera: Cerambycidae) and four species of predators in Georgia (Miller and Crowe 2020). In Europe, traps baited with blends of 3,2-hydroxyketones were

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Table 2. Analysis of variance results for effects of *syn*-2,3-hexanediol (D6) and 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one (K), and the interaction of syn-2,3-hexanediol (D6) × 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one (K; *i.e.*, D6 × K) on catches of beetles in 10-unit modified multiple-funnel traps baited with ethanol. *N*, total number of beetles.

		D6		K		D6 × K	
Species	*N*	$F_{1,27}$	*P*	$F_{1,27}$	*P*	$F_{1,27}$	*P*
Anobiidae							
Ptilinus basalis	132	1.312	0.262	31.57	< **0.001**	0.031	0.861
Cerambycidae							
Phymatodes aeneus	204	8.038	**0.009**	127.2	< **0.001**	0.683	0.416
Curculionidae							
Gnathotrichus sulcatus	837	0.234	0.663	25.01	< **0.001**	0.328	0.571
Xyleborinus saxesenii	469	0.032	0.860	0.145	0.706	1.084	0.307

attractive to two cerambycid species and to the predator *Clerus mutillarius* Fabricius (Coleoptera: Cleridae) (Imrei *et al.* 2021).

Lure treatments had no effect on catches of the corthyline ambrosia beetle, *Gnathotrichus sulcatus* (Coleoptera: Curculionidae), ($N = 288$) in experiment 1 ($F_{3,27} = 1.620$, $P = 0.208$; Fig. 1C). In contrast, the 3,2-hydroxyketone blend affected catches of *G. sulcatus* in experiment 2 (Table 2). Traps baited with the blend (with or without *syn*-2,3-hexanediol) caught fewer beetles than traps baited with ethanol (with or without *syn*-2,3-hexanediol) did (Fig. 1G). In contrast, the xyleborine ambrosia beetle, *Xyleborinus saxesenii* (Coleoptera: Curculionidae), was unaffected by lure treatments in experiment 2 (Table 2; Fig. 1F). In Georgia, catches of *X. saxesenii* in ethanol-baited traps were unaffected by the addition of *syn*-2,3-hexanediol, 3-hydroxyhexan-2-one, or 3-hydroxyoctan-2-one individually (Miller *et al.* 2015) but increased by the addition of the blend of 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one (Miller *et al.* 2022).

Catches of the xylophagous species *Hemicoelus gibbicollis* (Coleoptera: Anobiidae) ($N = 64$) were unaffected by lure treatments in experiment 1 ($F_{3,27} = 1.248$, $P = 0.312$; Fig. 1D). In contrast, lure treatments affected catches of *Ptilinus basalis* (Coleoptera: Anobiidae) in experiment 2 (Table 2). Traps baited with the 3,2-hydroxyketone blend (with or without *syn*-2,3-hexanediol) caught the most beetles (Fig. 1H). In Georgia, catches of another xylophagous beetle, *Xylobiops basilaris* (Say) (Coleoptera: Bostrichidae), were higher in traps baited with ethanol + *syn*-2,3-hexanediol than in those baited with ethanol or ethanol + 3,2-hydroxyketones (Miller *et al.* 2015, 2022); 3-hydroxyoctan-2-one interrupted attraction of beetles to traps baited with ethanol + *syn*-2,3-hexanediol (Miller *et al.* 2022).

Bark and woodboring beetles are broadly attracted to traps baited with ethanol (Miller 2006; Miller and Rabaglia 2009; Sweeney *et al.* 2016). Determining the effects of cerambycid pheromones on responses of phloeo- and xylophagous species to ethanol-baited traps is important in optimising the use of traps baited with ethanol and cerambycid pheromones to detect nonnative species of bark and woodboring beetles simultaneously. The use of multispecies trap lure combinations is a cost-effective method for assessing the biodiversity of cerambycids (Dodds *et al.* 2015; Handley *et al.* 2015; Wickham *et al.* 2021). This approach may also be a cost-effective method for assessing the biodiversity of bark and ambrosia beetles (and associated predators) simultaneously. Determining the biodiversity of these groups could be important as an environmental monitoring tool in assessing subtle forest health impacts arising from climate change, invasions by nonnative species, harvesting practices, and wind disturbance events.

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