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Cerambycid Pheromones 3,2-Hydroxyketones Affect Catches of Some Bark and Ambrosia Beetles (Coleoptera: Curculionidae) in Ethanol-Baited Multiple-Funnel Traps in Southeastern United States

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Abstract

In 2012–2013, we assessed the interactive effects of the cerambycid pheromones *syn*-2,3-hexanediol, 3-hydroxyhexan-2-one, and 3-hydroxyoctan-2-one on catches of bark and ambrosia beetles (Coleoptera: Curculionidae) in ethanol-baited multiple-funnel traps in north Georgia and South Carolina. We found that catches for nine of eleven species of ambrosia beetles in ethanol-baited traps were either unaffected or enhanced by the addition of 3,2-hydroxyketones. Similarly catches of five species of bark beetles were either unaffected or enhanced by the addition of 3,2-hydroxyketones. In particular, catches of *Xylosandrus crassiusculus* (Motschulsky), *Cnestus mutilatus* (Blandford), and *Monarthrum fasciatum* (Say) in ethanol-baited traps increased with the addition of 3-hydroxyhexan-2-one and/or 3-hydroxyoctan-2-one. Catches of the bark beetles *Hylocurus rudis* (LeConte) and *Hypothenemus rotundicollis* (Eichhoff) were enhanced by the addition of 3-hydroxyhexan-2-one and 3-hydroxyoctan-2-one, respectively. *syn*-2,3-Hexanediol had no effect on catches of bark and ambrosia beetles in ethanol-baited traps. Our data provide support for the use of ethanol + cerambycid pheromones for targeting non-native species of bark and ambrosia beetles as well as cerambycids in detection programs.

Key words: Scolytinae, *Xylosandrus crassiusculus*, *Cnestus mutilatus*, *Hypothenemus rotundicollis*, *Monarthrum fasciatum*

Numerous species of non-native bark and woodboring species pose biosecurity threats to forests in countries worldwide, necessitating various levels of surveillance and detection tools such as baited traps (Poland and Rassati 2019, Rabaglia et al. 2019). Managers of detection programs desire cost-effective trap lure blends capable of attracting multiple species to a single trap thereby reducing program costs (Hanks and Millar 2013, 2016, Fan et al. 2019, Rice et al. 2020). Such lure combinations can also provide opportunities for determining biodiversity of the bark and woodboring guilds, critical groups in the sustainability of healthy forests (Dodds et al. 2015, DiGirolomo and Dodds 2017, Wickham et al. 2021).

Combining lures for bark and ambrosia beetles with those for woodborers in the same trap is one way to minimize costs while targeting two important groups (Sweeney et al. 2016). Ethanol is

a common attractant for bark, ambrosia, and woodboring beetles (Miller 2006, Miller and Rabaglia 2009, Sweeney et al. 2016). Attraction of cerambycids to traps baited with 2,3-hexanediols and 3,2-hydroxyketones can be increased with the addition of ethanol lures (Miller et al. 2015). The addition of cerambycid lures to traps baited with ethanol seemed to have minimal negative effects on catches of most ambrosia beetle species in a previous study conducted in Georgia (Miller et al. 2015). In western Canada, Noseworthy et al. (2012) found that the addition of 3-hydroxyoctan-2-one enhanced catches of the ambrosia beetle *Monarthrum scutellare* (LeConte) (Coleoptera: Curculionidae) in ethanol-baited traps. In the Russian Far East, catches of the ambrosia beetle *Scolytoplatus tycon* Blandford (Coleoptera: Curculionidae) in ethanol-baited traps were enhanced by the addition of 3-hydroxyhexan-2-one (Sweeney et al. 2016).

In 2011–2013, we conducted seven trapping experiments with *syn*-2,3-hexanediol (D6), racemic 3-hydroxy-2-hexan-2-one (K6), and racemic 3-hydroxyoctan-2-one (K8) in the Piedmont region of Georgia and South Carolina with an initial goal of assessing the interactions among these semiochemicals on trap catches of Cerambycidae (Miller et al. 2017). The secondary goal of these experiments was to assess the effects of these combinations on catches of bark and ambrosia beetles. Herein, we report results on this secondary goal for four of the seven experiments that resulted in sufficient numbers of bark and ambrosia beetles for analyses, likely due to the inclusion of the attractant ethanol in all lure treatments (Miller and Rabaglia 2009).

Materials and Methods

In 2012–2013, four trapping experiments were conducted in mature, closed-canopy, mixed pine-hardwood forests, previously described in Miller et al. (2017). Stands consisted primarily of a mix of *Quercus*, *Pinus*, and *Liquidambar* species. Lure treatments were applied using a randomized complete block design in each experiment (Table 1). Experiments 1–2 were additive designs to determine the interactive effects between *syn*-2,3-hexanediol and the blend of 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one. In contrast, experiments 3–4 were elimination designs to determine the contribution of *syn*-2,3-hexanediol, 3-hydroxyhexan-2-one, and 3-hydroxyoctan-2-one in a 4-component blend towards trap catches of bark and ambrosia beetles.

We used black 10-unit multiple-funnel traps (Synergy Semiochemicals, Delta, BC), modified to allow lures to hang within the funnels (Miller et al. 2013); trap spacing was 8–12 m within blocks and blocks spaced 8–12 m apart. Contech Enterprises (Victoria, BC) supplied sealed plastic pouch lures for releasing ethanol, racemic *syn*-2,3-hexanediol, racemic 3-hydroxyhexan-2-one, and racemic 3-hydroxyoctan-2-one. Release rates (determined by the manufacturer) were 0.3 g/d, 1.5 mg/d, 20–25 mg/d, and 20–25 mg/d at 20–23 °C, respectively. Collection cups contained an aqueous solution of propylene glycol (Splash RV & Marine Antifreeze, Splash Products Inc., St. Paul, MN) (Miller and Duerr 2008). Pinned voucher specimens were deposited in the University of Georgia Collection of Arthropods (Athens, GA).

Experiments 1 and 4 were conducted at two separate sites on the Oconee National Forest, Greene Co., GA (33.742° N, 83.282° W and 33.750° N, 83.261° W, respectively) on 16 March–7 May 2012 and 2 August–26 September 2013, respectively. Experiment 2 was conducted on the Harrison State Forest, Richland Co., SC (34.086° N, 81.116° W) on 16 May–10 July 2012 whereas experiment 3 was conducted on the Oconee National Forest, Putnam Co., GA (33.237° N, 83.514° W) on 26 April–14 June 2012.

We used the SYSTAT (ver. 13) and SigmaStat (ver. 3.01) statistical packages (SYSTAT Software Inc., Point Richmond, CA) to analyze data for species with total catches ≥ 30 . Data were transformed by $\ln(Y + 1)$ as needed to meet assumptions of normality and homoskedasticity verified with the Shapiro-Wilk and Equal Variances test, respectively. All data were analyzed by mixed-model ANOVA with lure treatment as the fixed factor, followed by the Holm-Sidak multiple comparison test ($\alpha = 0.05$). For data with a significant treatment effect in experiments 1–2, a mixed model ANOVA was applied with the following fixed factors: (1) *syn*-2,3-hexanediol (D6), (2) 3-hydroxyhexan-2-one lure + 3-hydroxyoctan-2-one lure blend (K); and (3) D6 $\times$ K.

Results

We caught > 22,000 bark and ambrosia beetles across the four experiments, representing 8 species of non-native ambrosia beetles, three species of native ambrosia beetles, and five species of native bark beetles (Table 2). The most common species of ambrosia beetles was the non-native *Cnestus mutilatus* (Blandford) (Coleoptera: Curculionidae) accounting for 35% of the total catch of ambrosia beetles ($N = 14,321$); non-native species accounted for 95% of the total catch of ambrosia beetles. The most abundant species of bark beetles was *Hypothenemus rotundicollis* (Eichhoff) (Coleoptera: Curculionidae), accounting for 85% of total catch of bark beetles ($N = 7,839$).

Ambrosia Beetles (Coleoptera: Curculionidae)

Catches of *Xylosandrus crassiusculus* (Motschulsky) were affected by the K blend in experiments 1–2 (Table 3). In both experiments, catches in traps baited with ethanol + K blend, with or without D6, were greater than those in traps baited with the other two lure treatments (Fig. 1A–B). There was no effect of D6 on catches of *Xylo. crassiusculus* in the two experiments (Table 3). In experiment 1, catches of *Cn. mutilatus* were affected by the K blend but not D6 (Table 3). Catches of *Cn. mutilatus* in traps with ethanol + K blend (with or without D6) were greater than those in traps baited solely with ethanol (Fig. 1C); too few were caught in experiment 2 for analyses (Table 2). Similarly, catches of *Monarthrum fasciatum* (Say) in experiment 1 were affected by the K blend but not D6 (Table 3). Adding the K blend to ethanol-baited traps significantly increased mean catches of *M. fasciatum* whether D6 was present or not (Fig. 1D); *M. fasciatum* was not caught in experiment 2 (Table 2).

Catches of *Dryoxylon onoharaense* (Murayama) were affected by D6 and the interaction between D6 and the K blend in experiment 1 (Table 3); catches in traps baited with ethanol + K blend

Table 1. Treatments used in four randomized-block experiments testing the effects of *syn*-2,3-hexanediol, 3-hydroxyhexan-2-one and 3-hydroxyoctan-2-one lures on catches of bark and ambrosia beetles in ethanol-baited traps

Exp	<i>n</i>	Treatments
1, 2	10, 10	1.Et = Ethanol UHR lure (Et) 2.D6 Et = Et lure + <i>syn</i> -2,3-hexanediol lure (D6) 3.K Et = Et lure + 3,2-hydroxyketone blend [3-hydroxyhexan-2-one lure + 3-hydroxyoctan-2-one lure] (K lure blend) 4.K D6 Et = Et lure + K lure blend + D6 lure
3, 4	9, 10	1.K6 K8 D6 E = E lure + K6 lure + K8 lure + D6 lure 2.K8 D6 E = E lure + K8 lure + D6 lure 3.K6 D6 E = E lure + K6 lure + D6 lure 4.K6 K8 E = E lure + K6 lure + K8 lure

n = Number of replicate blocks.

were less than those in traps baited with ethanol + D6 (Fig. 1E). In experiment 2, catches of *D. onoharaense* were affected only by the K blend (Table 3) but with a similar negative effect as in Experiment 1; adding the K blend to traps baited with ethanol + D6 significantly reduced mean catch of *D. onoharaense* (Fig. 1F). The K blend also negatively affected catches of *Xyleborus*

spp in experiment 1 (Table 3); adding K blend to ethanol-baited traps (with or without D6) significantly reduced mean catch of *Xyleborus* spp (Fig. 1G). In contrast, catches of *Xyleborus* spp were unaffected by lure treatments in experiment 2 (Table 4). Lure treatments had no effects on catches of *Cyclorhipidion bodoanum* (Reitter) and *Xyleborinus saxesenii* (Ratzeburg) in experiments 1–2 (Table 4). Similarly, catches of *Monarthrum mali* (Fitch) were unaffected by lure treatments in experiment 2 (Table 4) and none were caught in experiment 1 (Table 2).

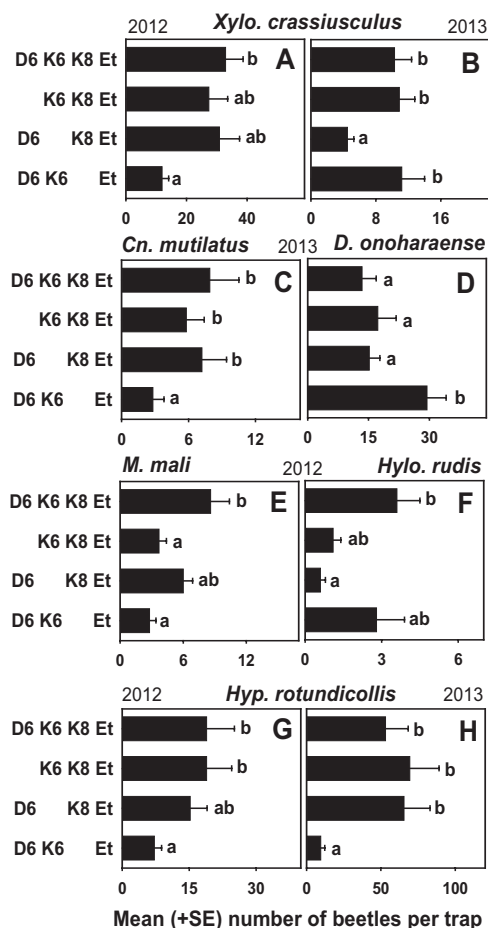


Fig. 2. Effects of syn-2,3-hexanediol lure (D6), 3-hydroxyhexan-2-one lure (K6) + 3-hydroxyoctan-2-one lure (K8) on catches of *Xylosandrus crassiusculus* (A–B), *Cnestus mutilatus* (C), *Dryoxylon onoharaense* (D), *Monarthrum mali* (E), *Hylocurus rudis* (F), and *Hypothenemus rotundicollis* (G–H) in traps baited with ethanol (E) in 2012 (Exp 3) and 2013 (Exp 4) in Georgia.

Table 2. Numbers of bark and ambrosia beetles (Coleoptera: Curculionidae) captured in experiments 1–4 (2012–2013)

Species	Exp 1	Exp 2	Exp 3	Exp 4
<i>Ambrosiodmus</i> spp ^a	-	24	-	-
<i>Cnestus mutilatus</i> (Blandford) ^a	4,599	10	196	237
<i>Cyclorhipidion bodoanum</i> (Reitter) ^a	433	70	228	15
<i>Dryoxylon onoharaense</i> (Murayama) ^a	329	-	473	753
<i>Euwallacea interjectus</i> (Blandford) ^a	-	13	-	-
<i>Hylastes tenuis</i> Eichhoff ^c	183	32	5	-
<i>Hylocurus rudis</i> (LeConte) ^c	-	75	72	-
<i>Hypothenemus rotundicollis</i> (Eichhoff) ^c	3,984	200	542	1,976
<i>Monarthrum fasciatum</i> (Say) ^b	156	-	-	-
<i>Monarthrum mali</i> (Fitch) ^b	-	156	192	20
<i>Pityophthorus</i> spp ^c	-	-	-	253
<i>Stenoscelis brevis</i> (Boheman) ^c	-	243	274	-
<i>Xyleborinus saxesenii</i> (Ratzeburg) ^a	850	1,315	435	122
<i>Xyleborus</i> spp ^b	134	-	21	41
<i>Xylosandrus crassiusculus</i> (Motschulsky) ^a	583	1,615	928	369
<i>Xylosandrus germanus</i> (Blandford) ^a	-	-	-	4

^a Ambrosia beetles, non-native.

^b Ambrosia beetles, native.

^c Bark beetles.

Table 3. ANOVA Table for effects of syn-2,3-hexanediol treatment (D6), 3,2-hydroxyketone blend treatment (K), and the interaction between the two treatments (D6 × K) for bark and ambrosia beetles affected by lure treatments in experiments 1–2

Species	Exp	D6		K		D6 × K	
		$F_{1,27}$	P	$F_{1,27}$	P	$F_{1,27}$	P
<i>Cnestus mutilatus</i>	1	1.390	0.249	14.76	0.001	1.279	0.268
<i>Dryoxylon onoharaense</i>	1	5.755	0.024	3.528	0.071	0.006	0.938
	2	0.114	0.738	8.545	0.007	1.432	0.242
<i>Hylastes tenuis</i>	1	0.594	0.448	8.685	0.007	0.569	0.457
<i>Hylocurus rudis</i>	2	0.192	0.664	26.37	<0.001	0.017	0.896
<i>Hypothenemus rotundicollis</i>	1	59.38	<0.001	287.9	<0.001	76.87	<0.001
	2	1.157	0.292	44.19	<0.001	2.749	0.109
<i>Monarthrum fasciatum</i>	1	0.230	0.636	12.19	0.002	0.360	0.553
<i>Xyleborus</i> spp	1	0.221	0.642	13.15	0.001	0.025	0.876
<i>Xylosandrus crassiusculus</i>	1	0.198	0.660	44.51	<0.001	2.064	0.162
	2	0.105	0.749	23.07	<0.001	0.308	0.583

Catches of *Xylo. crassiusculus* were affected by lure treatments in experiments 3 ($F_{3,24} = 3.773$, $P = 0.024$) and 4 ($F_{3,27} = 4.559$, $P = 0.010$). In experiment 3, catches were reduced with the elimination of K8 from the 4-component blend whereas in experiment 4 reduction in catches occurred with the elimination of K6, not K8 (Fig. 2B). In experiment 4, lure treatments affected catches of *Cn. mutilatus* ($F_{3,27} = 6.857$, $P = 0.001$) and *D. onoharaense* ($F_{3,27} = 10.152$, $P < 0.001$). Elimination of K8 from the blend resulted in lower catches of *Cn. mutilatus* (Fig. 2C) but increased catches of *D. onoharaense* (Fig. 2D). Lure treatments affected catches of *M. mali* in experiment 3 ($F_{3,24} = 8.719$, $P < 0.001$) with reductions in catches resulting from the elimination of D6 or K8 from the blend (Fig. 2E); too few *M. mali* were caught in experiment 4 for analyses (Table 2). Lure treatments had no effect on catches of *Cy. bodoanum* and *Xyle. saxesenii* in experiment 3 nor on catches of *Xyle. saxesenii* and *Xyleborus* spp in experiment 4 (Table 5). Too few *Xyleborus* spp and *Cy. bodoanum* were caught in experiments 3 and 4, respectively, for analyses (Table 2).

Bark Beetles (Coleoptera: Curculionidae)

The K blend affected catches of *Hylocurus rudis* (LeConte) in experiment 1, and *Hypothenemus rotundicollis* (Eichhoff) in experiments 1 and 2 (Table 3). Adding the K blend to ethanol-baited traps significantly increased catches of *Hylo. rudis* regardless of the presence of D6 (Fig. 1H); *Hylo. rudis* was not captured in experiment 1 (Table 2). Similarly, the K blend boosted catches of *Hyp. rotundicollis* when added to traps baited with ethanol alone or ethanol + D6 (Fig. 1I–J). Catches of *Hyp. rotundicollis* were also affected by D6 and the interaction between D6 and K blend but only in experiment 1 (Table 2); adding D6 to ethanol-baited traps increased catches of *Hyp. rotundicollis* (Fig. 1I). Catches of *Hylastes tenuis* Eichhoff were negatively affected by the K blend in experiment 1 (Table 3) although the Holm-Sidak test was unable to separate treatment means (Fig. 1K). There was no treatment effect on catches of *Hyla. tenuis* in experiment 2 (Table 4). Similarly, treatments had no effect of catches of *Stenoscellis brevis* (Boheman) in experiment 2 (Table 4); none were caught in experiment 1 (Table 2).

Catches of *Hylo. rudis* in experiment 3 were affected by lure treatments ($F_{3,24} = 4.637$, $P = 0.011$). The elimination of K6 from the 4-component blend resulted in a decrease in trap catches (Fig. 2F); *Hylo. rudis* was not captured in experiment 4 (Table 2). Lure treatments affected catches of *Hyp. rotundicollis* in experiment 3 ($F_{3,24} = 4.326$, $P = 0.014$) and experiment 4 ($F_{3,27} = 41.12$, $P < 0.001$). In both experiments, the elimination of K8 from the blend resulted in decreases in trap catches (Fig. 2G–H). Treatments had no effect on catches of *S. brevis* in experiment 3 or *Pityophthorus* spp in experiment 4 (Table 5). There were no catches of *S. brevis* in experiment 4 nor *Pityophthorus* spp in experiment 3 (Table 2).

Discussion

Numerous species of Cerambycidae are attracted to traps baited with 2,3-hexanediols and 3,2-hydroxyketones, in North America and overseas (Hanks and Millar 2013, 2016, Sweeney et al. 2014, Bobadoye et al. 2019, Fan et al. 2019, Molander et al. 2019, Wickham et al. 2021). The addition of ethanol lures to traps baited with cerambycid pheromones can significantly increase trap catches of cerambycids thereby improving their detectability in trapping surveillance programs for non-native species (Miller et al. 2015, 2017). Ethanol is also a general attractant for ambrosia beetles (Miller and Rabaglia 2009, Sweeney et al. 2016). Ambrosia beetles are an

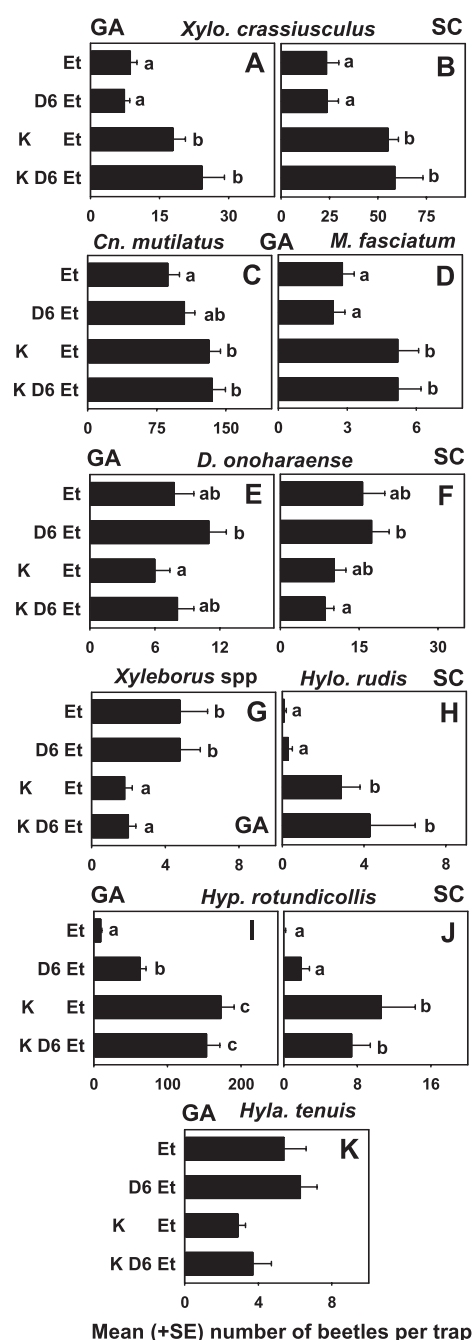


Fig. 1. Effects of syn-2,3-hexanediol lure (D6) and 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one lure blend (K) on catches of *Xylosandrus crassiusculus* (A–B), *Cnestus mutilatus* (C), *Monarthrus fasciatum* (D), *Dryoxylon onoharaense* (E–F), *Xyleborus* spp. (G), *Hylocurus rudis* (H), *Hypothenemus rotundicollis* (I–J), and *Hylastes tenuis* (K) in traps baited with ethanol (Et) in Georgia (Exp 1) and South Carolina (Exp 2).

important group targeted in detection programs for non-native species (Brockerhoff et al. 2006); > 60 species of non-native ambrosia beetles have become established in the United States (Rabaglia et al. 2019). The opportunity to detect non-native species of both cerambycids and ambrosia beetles is an important one for reducing costs of detection programs (Sweeney et al. 2016). Yet, only a few studies testing cerambycid pheromones have reported the effects of these compounds on catches of bark and ambrosia beetles (e.g., Miller et al. 2015).

Our data and those of others (Noseworthy et al. 2012, Miller et al. 2015, 2022, Sweeney et al. 2016) clearly provide support for the use of ethanol with cerambycid pheromones for targeting non-native species of bark and ambrosia beetles as well as cerambycids in detection programs. As in Miller et al. (2022), *syn*-2,3-hexanediol had no effect on catches of ambrosia beetles in the present study. In the present study, we found that catches for nine of eleven species of ambrosia beetles in ethanol-baited traps were either unaffected or enhanced by the addition of 3,2-hydroxyketones. Similarly catches of five species of bark beetles were either unaffected or enhanced by the addition of 3,2-hydroxyketones. The same broad pattern was noted in Miller et al. (2015, 2022) for the same species in southeastern United States. In the Russian Far East, Sweeney et al. (2016) found that attraction of seven of ten ambrosia beetle species were unaffected or enhanced by the addition of 3,2-hydroxyketone lures. Unaffected species in the Russian Far East included three common non-native species of ambrosia beetles in North America: *Anisandrus dispar* (F.), *Xyle. saxesenii* and *Xylosandrus germanus* (Blandford).

Several species of ambrosia beetles appear to be attracted to traps baited with 3-hydroxyhexan-2-one or 3-hydroxyoctan-2-one. For example, the blend of 3,2-hydroxyketones increased catches of the xyleborine ambrosia beetle *Xylo. crassiusculus* to ethanol-baited traps (Fig. 1A–B). However, it is unclear whether the increase in attraction is based on the presence of 3-hydroxyhexan-2-one or 3-hydroxyoctan-2-one (Figs 2A–B). Miller et al. (2022) found that attraction of *Xylo. crassiusculus* to traps baited with ethanol + *syn*-2,3-hexanediol was enhanced by the addition of 3-hydroxyoctan-2-one. Both 3,2-hydroxyketones were inactive in a previous study (Miller et al. 2015) when added separately to ethanol-baited traps, possibly due to low power associated with low numbers of beetles caught in that study compared to the present study.

Variability in the effects of 3,2-hydroxyketones was noted for several species of xyleborine ambrosia beetles. Catches of *Xyleborus*

spp were reduced by addition of 3,2-hydroxyketones in Georgia (experiment 1) (Fig. 1G) but not in South Carolina (experiment 2) (Table 4). In Miller et al. (2022) catches of *Xyleborus* spp in traps baited with ethanol + *syn*-2,3-hexanediol were reduced by the addition of 3-hydroxyoctan-2-one lures. Attraction of *Cn. mutilatus* to ethanol-baited traps was enhanced by the addition of 3,2-hydroxyketones in experiment 1 (Fig. 1C) with maximum catches in traps baited with 3-hydroxyoctan-2-one (Fig. 2C). Miller et al. (2022) also found that catches of *Cn. mutilatus* were increased by adding 3-hydroxyoctan-2-one to traps baited with ethanol + *syn*-2,3-hexanediol. However, Miller et al. (2015) observed no effect on catches of *Cn. mutilatus* when 3-hydroxyoctan-2-one was added to ethanol-baited traps. Similar types of variation between our present study and those of Miller et al. (2015, 2022) exist for other species such as *D. onoharaense* and *Xyle. saxesenii*.

For xyleborine species of ambrosia beetles, their responses to cerambycid pheromones such as 3-hydroxyhexan-2-one and 3-hydroxyoctan-2-one are likely kairomonal in nature; basically eavesdropping on the communication channels of other species in search of feeding and breeding sites (Hanks and Millar 2013, Silva et al. 2017, Miller et al. 2022). Xyleborine species of ambrosia beetles are typically haplo-diploid with haploid males that are diminutive, uncommon, and flightless. There is little need for sex pheromones to draw the two sexes together other than contact pheromones within brood galleries. Pheromones have yet to be discovered for any xyleborine species (El-Sayed 2021). Kairomonal preferences for species with broad host ranges such as *Xylo. crassiusculus* may change over time and space in response to local availability of brood hosts, possibly accounting for variation in trapping results.

In contrast, corthyline species of ambrosia beetles are diploid with males capable of flight and using pheromones as found for *Gnathotrichus sulcatus* (LeConte) (Byrne et al 1974, Borden et al. 1976). It is possible that 3,2-hydroxyketones may be used by both

Table 4. Mean ($\pm$ SE) number per trap, and *F* and *P* values (ANOVA) for bark and ambrosia beetles not affected by lure treatments in experiments 1–2

Species	Exp	Mean $\pm$ SE	<i>F</i> _{3,27}	<i>P</i>
<i>Cyclorhipidion bodoanum</i>	1	10.8 $\pm$ 0.9	1.199	0.329
	2	1.8 $\pm$ 0.3	1.496	0.238
<i>Hylastes tenuis</i>	2	0.8 $\pm$ 0.2	0.679	0.572
<i>Monarthrum mali</i>	2	3.9 $\pm$ 1.6	0.399	0.755
<i>Xyleborinus saxesenii</i>	1	21.2 $\pm$ 1.3	0.970	0.421
	2	32.9 $\pm$ 2.2	0.575	0.636
<i>Xyleborus</i> spp	2	12.1 $\pm$ 1.3	1.340	0.282
<i>Stenoscelis brevis</i>	2	6.1 $\pm$ 1.4	1.567	0.220

Table 5. Mean ($\pm$ SE) number per trap, and *F* and *P* values (ANOVA) for bark and ambrosia beetles not affected by lure treatments in experiments 3–4

Species	Exp	Mean $\pm$ SE	<i>F</i> ^a	<i>P</i>
<i>Cnestus mutilatus</i>	3	5.4 $\pm$ 1.1	1.789	0.176
<i>Cyclorhipidion bodoanum</i>	3	6.3 $\pm$ 0.8	1.700	0.194
<i>Dryoxylon onoharaense</i>	3	13.1 $\pm$ 1.1	1.043	0.392
<i>Pityophthorus</i> spp	4	6.3 $\pm$ 0.8	0.408	0.748
<i>Xyleborinus saxesenii</i>	3	12.1 $\pm$ 0.8	0.763	0.526
	4	3.1 $\pm$ 0.3	0.345	0.793
<i>Xyleborus</i> spp	4	1.0 $\pm$ 0.2	0.257	0.856
<i>Stenoscelis brevis</i>	3	7.6 $\pm$ 1.0	0.676	0.575

^adf = 3, 24 for Exp 3; 3, 27 for Exp 4.

ambrosia beetles and woodborers as pheromones rather than kairomones. For example, in North America sulcatol is a pheromone for several species of *Gnathotrichus* ambrosia beetles (Byrne et al 1974, Borden et al. 1976) as well as several lamiine species of Cerambycidae (Meier et al. 2019, 2020).

In the present study, catches of the corthyline species *M. fasciatum* in ethanol-baited were increased by the addition of the 3,2-hydroxyketone blend (Fig. 1D). In British Columbia, catches of the congener *M. scutellare* in ethanol-baited traps were enhanced by the addition of 3-hydroxyoctan-2-one (Noseworthy et al. 2012); 3-hydroxyoctan-2-one was needed to maximize catches of the congener *M. mali* in the present study (Fig. 2E). The same pheromones are often found among species within the same genus: ipsenol and ipsdienol among *Ips* species of bark beetles (Ipina); frontalin among *Dendroctonus* bark beetles (Hylurgina); and lineatin among *Trypodendron* ambrosia beetles (Xyloterina) (El-Sayed 2021).

The need to clarify the roles of kairomone and pheromone for 2,3-hexanediols and 3,2-hydroxyketones is also relevant to responses observed with two species of bark beetles in our study. As in Miller et al. (2015), *Hyp. rotundicollis* was attracted to traps baited with 3-hydroxyoctan-2-one (Figs. 1I,J, 2G,H). *Hylo. rudis* was attracted to traps baited with 3-hydroxyhexan-2-one (Figs. 1H, 2F). Both species have been collected from numerous species of hardwood shrubs and trees (Atkinson 2022). Clearly, verification of pheromone status of 3,2-hydroxyketones for bark and ambrosia beetles captured in our study will require determinations of pheromone production by these species.

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References Cited

- Atkinson, T. H. 2022. *Bark and ambrosia beetles of the Americas*. Available from <http://www.barkbeetles.info>. Accessed 25 February 2022.
- Bobadoye, B., B. Torto, A. Fumbong, Y. Zou, K. Adlbauer, L. M. Hanks, and J. G. Millar. 2019. Evidence of aggregation-sex pheromone use by longhorned beetles (Coleoptera: Cerambycidae) species native to Africa. *Environ. Entomol.* 48: 189–192.
- Borden, J. H., L. Chong, J. A. MacLean, K. N. Slessor, and K. Mori. 1976. *Gnathotrichus sulcatus*: synergistic response to enantiomers of the aggregation pheromone sulcatol. *Science*. 192: 894–896.
- Brockerhoff, E. G., J. Bain, M. Kimberley, and M. Knížek. 2006. Interception frequency of exotic bark and ambrosia beetles (Coleoptera: Scolytinae) and relationship with establishment in New Zealand and worldwide. *Can. J. For. Res.* 36: 289–298.
- Byrne, K. J., A. A. Swigar, R. M. Silverstein, J. H. Borden, and E. Stokkink. 1974. Sulcatol: population aggregation pheromone in the scolytid beetle, *Gnathotrichus sulcatus*. *J. Insect Physiol.* 20: 1895–1900.
- DiGirolomo, M. F., and K. J. Dodds. 2017. Comparison of the species richness and abundance of Cerambycidae (Coleoptera) and Scolytinae (Coleoptera: Curculionidae) captured in aerial malaise traps with and without a bottom collector. *Can. Entomol.* 149: 408–412.
- Dodds, K. J., J. D. Allison, D. R. Miller, R. P. Hanavan, and J. Sweeney. 2015. Considering species richness and rarity when selecting optimal survey traps: Comparisons of semiochemical baited flight intercept traps for Cerambycidae in eastern North America. *Agric. For. Entomol.* 17: 36–47.
- El-Sayed, A. M. 2021. The pherobase: database of pheromones and semiochemicals. Available from <http://www.pherobase.com>. Accessed 29 December 2021.
- Fan, J. T., O. Denux, C. Courtin, A. Bernard, M. Javal, J. G. Millar, L. M. Hanks, and A. Roques. 2019. Multi-component blends for trapping native and exotic longhorn beetles at potential points-of-entry and in forests. *J. Pest Sci.* 92: 281–297.
- Hanks, L. M., and J. G. Millar. 2013. Field bioassays of cerambycid pheromones reveal widespread parsimony of pheromone structures, enhancement by host plant volatiles, and antagonism from heterospecifics. *Chemoecology*. 23: 21–44.
- Hanks, L. M., and J. G. Millar. 2016. Sex and aggregation-sex pheromones of cerambycid beetles: Basic science and practical implications. *J. Chem. Ecol.* 42: 631–654.
- Meier, L. R., J. G. Millar, J. A. Mongold-Diers, and L. M. Hanks. 2019. (*S*)-Sulcatol is a pheromone component for two species of cerambycid beetles in the subfamily Lamiinae. *J. Chem. Ecol.* 45: 447–454.
- Meier, L. R., Y. Zou, J. A. Mongold-Diers, J. G. Millar, and L. M. Hanks. 2020. Pheromone composition and chemical ecology of six species of cerambycid beetles in the subfamily Lamiinae. *J. Chem. Ecol.* 46: 30–39.
- Miller, D. R. 2006. Ethanol and (–)- α -pinene: attractant kairomones for some large wood-boring beetles in southeastern USA. *J. Chem. Ecol.* 32: 779–794.
- Miller, D. R., and D. Duerr. 2008. Comparison of arboreal beetle catches in wet and dry collection cups with Lindgren multiple funnel traps. *J. Econ. Entomol.* 101: 107–113.
- Miller, D. R., and R. J. Rabaglia. 2009. Ethanol and (–)- α -pinene: attractant kairomones for bark and ambrosia beetles in the southeastern US. *J. Chem. Ecol.* 35: 435–448.
- Miller, D. R., C. M. Crowe, B. F. Barnes, K. J. K. Gandhi, and D. A. Duerr. 2013. Attaching lures to multiple-funnel traps targeting saproxylic beetles (Coleoptera) in pine stands: inside or outside funnels? *J. Econ. Entomol.* 106: 206–214.
- Miller, D. R., C. M. Crowe, P. D. Mayo, P. J. Silk, and J. D. Sweeney. 2015. Responses of Cerambycidae and other insects to traps baited with ethanol, 2,3-hexanediol, and 3,2-hydroxyketone lures in north-central Georgia. *J. Econ. Entomol.* 108: 2354–2365.
- Miller, D. R., C. M. Crowe, J. D. Sweeney, P. D. Mayo, and P. J. Silk. 2017. Interactions between 2,3-hexanediol and 3,2-hydroxyketone lures on trap catches of hardwood beetles in Georgia. *J. Econ. Entomol.* 110: 2119–2128.
- Miller, D. R., C. M. Crowe, P. D. Mayo, P. J. Silk, and J. D. Sweeney. 2022. Interactions between *syn*- and *anti*-2,3-hexanediol lures on trap catches of woodboring beetles and associates in southeastern United States. *Environ. Entomol.* 51: 83–93.
- Molander, M. A., I. B. Winde, J. Burman, F. N. Nyabuga, T. U. T. Lindblom, L. M. Hanks, J. G. Millar, and M. C. Larsson. 2019. Common cerambycid pheromone components as attractants for longhorn beetles (Cerambycidae) breeding in ephemeral oak substrates in northern Europe. *J. Chem. Ecol.* 45: 537–548.
- Noseworthy, M. K., L. M. Humble, J. Sweeney, P. Silk, and P. Mayo. 2012. Attraction of *Monarthrum scutellare* (Coleoptera: Curculionidae: Scolytinae) to hydroxy ketones and host volatiles. *Can. J. For. Res.* 42: 1851–1857.
- Poland, T. M., and D. Rassati. 2019. Improved biosecurity surveillance on non-native forest insects: a review of current methods. *J. Pest Sci.* 92: 37–49.
- Rabaglia, R. J., A. I. Cognato, E. R. Hoebeke, C. W. Johnson, J. R. Labonte, M. E. Carter, and J. L. Vlach. 2019. Early detection and rapid response—a 10-year summary of the USDA Forest Service program of surveillance for non-native bark and ambrosia beetles. *Am. Entomol.* 65: 29–42.
- Rice, M. E., Y. Zou, J. G. Millar, and L. M. Hanks. 2020. Complex blends of synthetic pheromones are effective multi-species attractants for

- longhorned beetles (Coleoptera: Cerambycidae). *J. Econ. Entomol.* 113: 2269–2275.
- Silva, W. D., Y. Y. Zou, J. M. S. Bento, L. M. Hanks, and J. G. Millar. 2017. Aggregation-sex pheromones and likely pheromones of 11 South American cerambycid beetles, and partitioning of pheromone channels. *Front. Ecol. Evol.* 5: 101.
- Sweeney, J. D., P. J. Silk, and V. Grebennikov. 2014. Efficacy of semiochemical-baited traps for detection of longhorn beetles (Coleoptera: Cerambycidae) in the Russian Far East. *Eur. J. Entomol.* 111: 397–406.
- Sweeney, J. D., P. J. Silk, V. Grebennikov, and M. Mandelshtam. 2016. Efficacy of semiochemical-baited traps for detection of Scolytinae species (Coleoptera: Curculionidae) in the Russian Far East. *Eur. J. Entomol.* 113: 84–97.
- Wickham, J. D., R. D. Harrison, W. Lu, L. M. Hanks, and J. G. Millar. 2021. Rapid assessment of cerambycid beetle biodiversity in a tropical rainforest in Yunnan Province, China, using a multicomponent pheromone lure. *Insects.* 12: 277.