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Interactions between Ethanol, *syn*-2,3-Hexanediol, 3-Hydroxyhexan-2-one, and 3-Hydroxyoctan-2-one Lures on Trap Catches of Hardwood Longhorn Beetles in Southeastern United States

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Abstract

The effectiveness of a four-component "super lure" consisting of ethanol (E) and the cerambycid pheromones *syn*-2,3-hexanediol (D6), racemic 3-hydroxyhexan-2-one (K6), and racemic 3-hydroxyoctan-2-one (K8) on trap catches of Cerambycidae (Coleoptera) was determined in southeast United States with seven trapping experiments in 2011–2013. We captured 74 species of longhorn beetles in our three-year study. Ethanol significantly increased the mean catches of seven species and increased the number of cerambycid species detected. Traps with the "super lure" were effective for 8 of 13 species of Cerambycidae previously shown to be attracted to binary combinations of ethanol plus one of the three pheromones. However, the "super lure" was less effective for the remaining five species with catch reductions of 40–90% compared with combinations of ethanol and one or two of the pheromones. For example, K6 + K8 lures reduced catches of *Anelaphus villosus* (F.) in traps with E + D6 by 90%. Similarly, catches of *Anelaphus pumilus* (Newman) in traps with E + K6 + D6 were reduced by 50% with the addition of K8. Catches of *Knulliana cincta* (Drury) in traps with K6 + K8 lures were interrupted by D6, an effect negated by the addition of ethanol. Given the interruptive effects on trap catches of some species when lures are combined in a single trap, developing optimal lure blends to maximize detection efficacy will be a challenge for managers of detection programs for non-native invasive species of longhorn beetles.

Key words: Cerambycidae, hardwood, detection, non-native, invasive

Effective lures are required for trapping programs aimed at early detection of non-native, potentially invasive species of longhorn beetles (Coleoptera: Cerambycidae) (Liebhold et al. 2012, Hanks and Millar 2016). Traps baited with the cerambycid pheromones *syn*-2,3-hexanediol (D6, 50:50 mix of *R*,*R*- and *S*,*S*-2,3-hexanediol), racemic 3-hydroxyhexan-2-one (K6), and/or racemic 3-hydroxyoctan-2-one (K8) lures are attractive to ≥31 species of longhorn beetles in North America, primarily species of Cerambycinae (Hanks and Millar 2016). Of these 31 species, 20 are attracted to K6, 2 to K8, 7 to D6, and 4 to the combination of K6 + D6. The addition of ethanol (E) enhances trap catches of some species to these compounds (Hanks et al. 2012, Handley et al. 2015, Miller et al. 2015a).

Separate traps, each baited with a species-specific lure, could be expensive and possibly impractical in a detection program given the species richness of Cerambycidae at >35,000 worldwide (Nearns et al. 2016). One option for reducing costs is to combine active compounds into one "super lure" (Hanks et al. 2012, Sweeney et al. 2014, Wickham et al. 2014). "Super lure" combinations of multiple components placed in one trap have been used for broad detection of woodborers, particularly for determinations of species richness and phenologies (Hanks and Millar 2013, Hanks et al. 2014, Sweeney et al. 2014, Wickham et al. 2014, Handley et al. 2015).

"Super lures" should work best if responses by beetles to all compounds are additive and there is little, if any, negative interaction between compounds on attraction of beetles (Hanks and Millar 2016). For example, the lure combination of ethanol + α-pinene + ipsenol + ipsdienol is broadly effective for pine inhabiting Cerambycidae and Buprestidae in North America, with no evidence of interruption due to any one component

Table 1. Locations, coordinates, dominant tree species and trapping dates for each of seven experiments on flight responses of woodboring beetles to multiple-funnel traps baited with ethanol, *syn*-2,3-hexanediol, 3-hydroxyhexan-2-one, and 3-hydroxyoctan-2-one in southeast United States

Exp	Location	Coordinates	Tree species	Trapping dates
1	Oconee National Forest, Putnam Co., GA	33.344 N, 83.457 W	*Quercus alba* L., *Quercus falcata* Michaux, *Liquidambar styraciflua* L., *Pinus echinata* Miller, *Carya tomentosa* Sargent	3 June–12 July 2011
2	Oconee National Forest, Putnam Co., GA	33.237 N, 83.514 W	*Q. alba, Q. falcata, L. styraciflua, P. echinata, C. tomentosa*	12 July–23 Aug. 2012
3	Oconee National Forest, Greene Co., GA	33.744 N, 83.282 W	*Pinus taeda, Q. alba, L. styraciflua*	9 April–29 May 2013
4	Oconee National Forest, Greene Co., GA	33.742 N, 83.282 W	*P. taeda, Q. alba, P. echinata, L. styraciflua*	16 Mar.–7 May 2012
5	Harbison State Forest, Richland Co., SC	34.086 N, 81.116 W	*P. taeda*	16 May–10 July 2012
6	Oconee National Forest, Putnam Co., GA	33.237 N, 83.514 W	*Q. alba, Q. falcata, L. styraciflua, P. echinata, C. tomentosa*	26 April–14 June 2012
7	Oconee National Forest, Greene Co., GA	33.750 N, 83.261 W	*P. taeda, Q. alba, P. echinata*	2 Aug.–26 Sept. 2013

Table 2. Description of lures used in the study

Code	Compound	Release rate[a]
E	Ethanol UHR	0.5 g/d at 23 °C
D6	*syn*-2,3-hexanediol	1.5 mg/d at 20 °C
K6	racemic 3-hydroxyhexan-2-one	20–25 mg/d at 20 °C
K8	racemic 3-hydroxyoctan-2-one	20–25 mg/d at 20 °C

[a] Determined by manufacturer. All chemical purities >95%.

Table 3. Treatments used in seven randomized-block experiments testing the interactive effects between ethanol, *syn*-2,3-hexanediol, 3-hydroxyhexan-2-one, and 3-hydroxyoctan-2-one (19) lures on catches of woodborers in north Georgia and central South Carolina

Exp	*n*	Treatments
1	8	1. **Blank** = No lures
		2. **D6** = *syn*-2,3-hexanediol lure
		3. **K6** = racemic 3-hydroxyhexan-2-one lure
		4. **K8** = racemic 3-hydroxyoctan-2-one lure
		5. **K6K8** = K6 lure + K8 lure
		6. **D6K6** = D6 lure + K6 lure
		7. **D6K8** = D6 lure + K8 lure
		8. **D6K6K8** – D6 lure + K6 lure + K8 lure
2–3	8, 8	1. **D6** = D6 lure
		2. **K** = K6 lure + K8 lure
		3. **D6K** = D6 lure + K lures
		4. **D6E** =D6 lure + ethanol UHR lure (**E**)
		5. **KE** = K lures + E lure
		6. **D6KE** = D6 lure + K lures + E lure
4–5	10, 10	1. **E** = E lure
		2. **ED6** = E lure + D6 lure
		3. **EK** = E lure + K6 lure + K8 lure
		4. **ALL** = E lure + D6 lure + K6 lure + K8 lure
6–7	9, 10	1. **ALL** - **K8** = E lure + K6 lure + D6 lure
		2. **ALL** - **D6** = E lure + K6 lure + K8 lure
		3. **ALL** - **K6** = E lure + D6 lure + K8 lure
		4. **ALL** = E lure + D6 lure + K6 lure + K8 lure

(Miller et al. 2013a, 2015b). In addition, the blend is attractive to six common species of ambrosia beetles and two common species of bark beetles (Coleoptera: Curculionidae: Scolytinae) in the southeast United States (Miller et al. 2011).

Interruption in attraction of some species of Cerambycidae to certain lure combinations is known for some hardwood-inhabiting species. Hanks et al (2012) found that attraction of *Neoclytus acuminatus* (F.) to a five-component blend that included D6, was interrupted by the presence of K6. When interactions between compounds reduce trap catches of one or more species, then it might be preferable to bait survey traps with two or more blends of mutually compatible compounds, rather than a single "super lure."

In Georgia, traps baited with ethanol plus D6, K6, or K8 are attractive to 13 species of Cerambycinae [*Anelaphus parallelus* (Newman), *Anelaphus pumilus* (Newman), *Anelaphus villosus* (F.), *Clytus marginicollis* Castelnau & Gory, *Eburia quadrigeminata* (Say), *Euderces pini* (Olivier), *Knulliana cincta* (Drury), *Megacyllene caryae* (Gahan), *N. acuminatus*, *Neoclytus jouteli jouteli* Davis, *Neoclytus mucronatus* (F.), *Neoclytus scutellaris* (Olivier), and *Xylotrechus colonus* (F.)] (Miller et al. 2015a). The current study assessed the interactions between these four compounds on catches of longhorn beetles in the Piedmont region of southeastern United States. These interactions have not been fully examined for these species, much less the 31 species in North America that respond to D6, K6, and K8 (Hanks and Millar 2016). Our goal is to develop optimal blend combinations that are the most effective in attracting the greatest number of cerambycid species in survey and detection programs.

Materials and Methods

Seven experiments were conducted in north Georgia and central South Carolina during 2011–2013. Modified 10-unit multiple-funnel traps (Miller et al. 2013b) were used in all experiments with protocols similar to those in Miller et al. (2015a) but with different experimental designs. Locations, host types, and trapping periods are noted in Table 1 for each experiment. Pouch lures used in our study (Table 2) were obtained from Contech Enterprises (Victoria BC). Ethanol lures were attached to the underside of the top canopy, whereas the remaining lures were attached to trap legs with black pipe cleaners, and allowed to hang within the funnels and below the ethanol lure in each trap. Four different experimental designs were used across the seven experiments (Table 3), with traps spaced 15- to 20-m apart in Experiment 1 and 8- to 12-m apart in the other experiments. No trap was located within 2 m of any standing tree. In 2012–2013, a piece (2.5 by 5.0 cm^2) of VaporTape II (a.i. 2,2-dichlorovinyl dimethyl phosphate, Hercon Environmental Corp.,

Table 4. Total numbers of longhorn beetles (Cerambycidae and Disteniidae) captured in seven experiments conducted in north Georgia and central South Carolina (2011–2013)

Family: subfamily, species	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Total
Cerambycidae: Parandrinae								
Neandra brunnea (F.)	–	24	–	–	–	–	4	27
Cerambycidae: Prioninae								
Mallodon dasystomus (Say)	–	–	–	–	–	–	1	1
Orthosoma brunneum (Forster)	2	1	–	–	–	–	1	4
Prionus imbricornis (L.)	11	3	–	–	1	–	–	15
Prionus pocularis Dalman	1	3	–	–	1	1	4	10
Sphenostethus taslei (Buquet)	47	–	–	–	–	–	–	47
Cerambycidae: Spondylidinae								
Asemum striatum (L.)	–	–	23	21	–	–	–	44
Cerambycidae: Lepturinae								
Acmaeops discoideus (Haldeman)	–	–	17	1	–	–	–	18
Bellamira scalaris (Say)	7	–	–	1	2	20	–	30
Centrodera sublineata LeConte	–	–	1	–	–	–	–	1
Gaurotes cyanipennis (Say)	–	–	19	14	–	–	–	33
Gaurotes thoracica (Haldeman)	–	–	1	–	–	1	–	2
Judolia cordifera (Olivier)	–	–	1	–	–	–	–	1
Rhagium inquisitor (L.)	–	–	8	15	–	–	–	23
Stenocorus cinnamopterus (Randall)	–	–	1	1	–	1	–	3
Strangalia bicolor (Swederus)	1	–	–	–	–	1	–	2
Strangalia famelica Newman	1	–	–	–	–	–	–	1
Strophiona nitens (Forster)	8	–	–	–	–	–	–	8
Typocerus lunulatus (Swederus)	1	–	–	–	1	1	–	3
Typocerus velutinus (Olivier)	2	–	–	–	–	–	–	2
Typocerus zebra (Olivier)	6	–	11	9	–	1	–	27
Cerambycidae: Cerambycinae								
Ancylocera bicolor (Olivier)	–	–	2	6	–	–	–	8
Anelaphus parallelus (Newman)	–	–	219	226	2	9	–	456
Anelaphus pumilus (Newman)	10	–	1,718	1,031	2	459	–	3,220
Anelaphus villosus (F.)	1	–	4	44	3	21	–	73
Batyle suturalis (Say)	–	–	–	1	–	–	–	1
Clytus marginicollis Castelnau & Gory	1	–	152	390	–	10	–	553
Clytus ruricola (Olivier)	1	–	–	–	–	5	–	6
Curius dentatus Newman	–	5	1	–	13	1	16	36
Cyrtophorus verrucosus (Olivier)	1	–	86	58	–	19	–	164
Eburia quadrigeminata (Say)	–	4	–	–	–	–	–	4
Elaphidion mucronatum (Say)	3	6	–	10	5	10	2	36
Elytroleptus floridanus (LeConte)	–	–	3	4	–	–	–	7
Enaphalodes atomarius (Drury)	5	–	–	–	–	–	1	6
Euderces picipes (F.)	1	–	–	–	5	1	–	7
Euderces pini (Olivier)	–	–	4,147	1,742	–	55	–	5,944
Heterachthes quadrimaculatus Haldeman	–	–	–	–	–	1	–	1
Knulliana cincta (Drury)	–	–	207	262	–	30	–	499
Megacyllene caryae (Gahan)	–	–	12	13	–	–	–	25
Molorchus bimaculatus Say	–	–	44	45	–	1	–	90
Neoclytus acuminatus (F.)	429	830	3,840	2,920	218	1,297	434	9,968
Neoclytus caprea (Say)	–	–	52	9	–	–	–	61
Neoclytus jouteli Davis	1	2	–	–	15	2	10	30
Neoclytus mucronatus (F.)	190	1,167	39	97	190	1,230	1,025	3,938
Neoclytus scutellaris (Olivier)	502	572	2	4	91	387	220	1,778
Obrium maculatum (Olivier)	–	–	1	7	48	12	–	68
Parelaphidion aspersum (Haldeman)	6	–	–	–	–	–	1	1
Phymatodes amoenus (Say)	–	–	1	–	–	–	–	1
Phymatodes varius (F.)	–	–	43	156	–	–	–	199
Tessaropa tenuipes (Haldeman)	–	–	–	1	1	–	–	2
Tilloclytus germinatus (Haldeman)	–	–	5	2	–	–	–	7
Tylonotus bimaculatus Haldeman	–	1	–	–	–	–	–	1
Xylotrechus colonus (F.)	36	39	56	189	26	50	30	426
Xylotrechus sagittatus (Germar)	–	–	1	–	–	2	–	3
Cerambycidae: Lamiinae								
Acanthocinus nodosus (F.)	–	–	–	1	–	–	–	1
Aegomorphus modestus (Gyllenhal)	1	–	–	1	–	1	–	3

(continued)

Table 4. continued

Family: subfamily, species	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Total
Astylopsis arcuatus (LeConte)	–	–	–	–	–	–	1	1
Astylopsis sexguttata (Say)	–	–	–	–	–	–	1	1
Ataxia crypta (Say)	–	–	22	46	–	–	–	68
Ecyrus dasycerus (Say)	–	–	–	2	1	8	1	12
Eupogonius pauper LeConte	–	–	–	–	1	–	–	1
Eupogonius tomentosus (Haldeman)	–	–	–	–	1	–	1	2
Goes tigrinus (DeGeer)	–	–	–	–	–	1	–	1
Graphisurus fasciatus (DeGeer)	3	2	–	–	–	2	–	7
Leptostylopsis planidorsus (LeConte)	–	–	–	–	1	–	–	1
Leptostylus asperatus (Haldeman)	–	1	8	13	3	4	8	37
Lepturges confluens (Haldeman)	–	–	–	–	12	–	–	12
Monochamus carolinensis (Olivier)	–	–	2	–	–	–	–	2
Monochamus titillator (F.)	–	–	–	4	1	1	2	8
Psenocerus supernotatus (Say)	–	–	5	6	–	1	–	12
Saperda lateralis F.	1	–	–	2	–	–	–	3
Sternidius alpha (Say)	–	–	–	1	2	5	–	8
Styloleptus biustus (LeConte)	–	–	–	–	–	–	11	11
DISTENIIDAE								
Elytrimitatrix undata (F.)	17	–	–	–	6	1	–	24
Total number of beetles	1,296	2,660	10,754	7,355	652	3,650	1,774	28,141
Total number of species	29	15	34	38	26	35	20	74

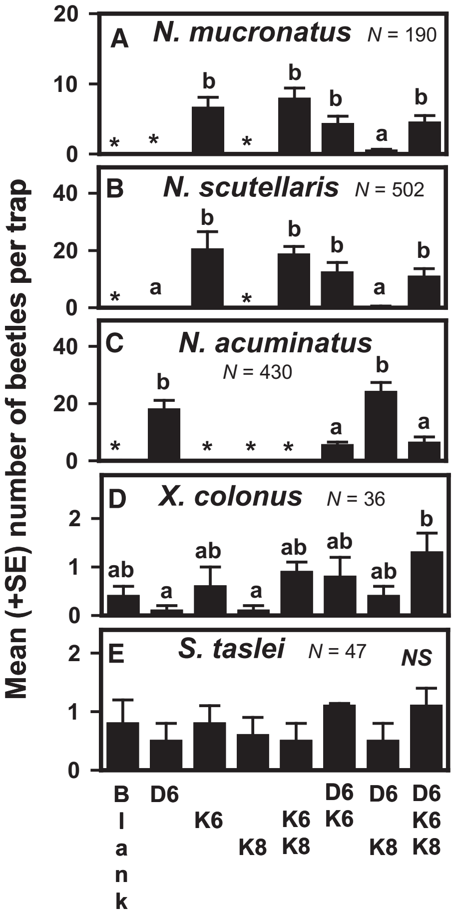

Fig. 1. Interaction of *syn*-2,3-hexanediol (**D6**), 3-hydroxyhexan-2-one (**K6**), and 3-hydroxyoctan-2-one (**K8**) on trap catches of Cerambycidae in north Georgia (Experiment 1). For each species, means followed by the same letter are not significantly different at *P* = 0.05 (Holm–Sidak multiple-comparison test). Treatments with an asterisk had zero catches. ***N*** = Total trap catch of beetles per location, ***NS*** = *P* > 0.05.

Emigsville, PA) was attached to the underside of the canopy of each trap to prevent nesting by paper wasps, *Polistes* spp. (Hymenoptera: Vespidae). Each collection cup contained 150–200 ml of Splash RV & Marine Antifreeze (SPLASH Products Inc., St. Paul, MN). Treatments were replicated in randomized complete blocks in all experiments. Cerambycid species were identified using Chemsak (1996) and Lingafelter (2007), with current names as noted in Bezark (2016). Vouchers were deposited in the Museum of Natural History, University of Georgia (Athens, GA).

Statistical procedures in the SYSTAT (ver. 13) and SigmaStat (ver. 3.01) statistical packages (SYSTAT Software Inc., Point Richmond, CA) were used for species with total numbers ≥30 in an experiment. Unless otherwise noted, trap catch data were transformed by ln(Y + 1) to ensure homoskedasticity and normality (Pepper et al. 1997). Normality and homoskedasticity were verified with the Shapiro–Wilk and Equal Variance tests, respectively, before analyses. Because of issues with heterogeneity in variances, any treatment with zero total catch for a given species was omitted from the analysis (Reeve and Strom 2004). The following analysis of variance (ANOVA) models were used to analyze the data:

A. Model factors for Experiment 1 = (1) replicate, (2) *syn*-2,3-hexanediol treatment (D6), (3) 3-hydroxyhexan-2-one treatment (K6), (4) 3-hydroxyoctan-2-one treatment (K8), (5) D6 × K6; (6) D6 × K8; (7) K6 × K8, and (8) D6 × K6 × K8.

B. Model factors for Experiments 4–5 = (1) replicate, (2) D6, (3) K6 + K8 (K blend) and (4) D6 × K blend.

C. Model factors for all experiments = (1) replicate and (2) treatment. Analyses were followed by the Holm–Sidak multiple comparison procedure (α = 0.05). The Holm–Sidak procedure controls the experiment-wise error rate at 0.05 (Glantz 2005).

Results

We captured >28,000 longhorn beetles representing 74 species over three years of field trials, with Cerambycinae accounting for 92% of the total catch (Table 4). In Experiment 1, we caught sufficient numbers of five species for statistical analyses, although ANOVA Model

Table 5. Total numbers of longhorn beetles (Cerambycidae and Disteniidae) captured in Experiments 2–3 comparing the effects of ethanol on traps baited with *syn*-2,3-hexanediol, 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one blend (2012–2013)

Family: subfamily, species	Ethanol absent	Ethanol present
Acmaeops discoideus	7	10
Ancylocera bicolor	–	2
Anelaphus parallelus	84	135
Anelaphus pumilus	882	836
Anelaphus villosus	–	4
Asemum striatum	11	12
Ataxia crypta	3	19
Centrodera sublineata	1	–
Clytus marginicollis	73	81
Curius dentatus	–	6
Cyrtophorus verrucosus	38	48
Eburia quadrigeminata	–	4
Elaphidion mucronatum	–	6
Elytroleptus floridanus	–	3
Euderces pini	2,233	1,914
Gaurotes cyanipennis	8	11
Gaurotes thoracica	1	–
Graphisurus fasciatus	–	2
Judolia cordifera	–	1
Knulliana cincta	39	170
Leptostylus asperatus	3	6
Megacyllene caryae	6	6
Molorchus bimaculatus	5	40
Monochamus carolinensis	1	1
Neandra brunnea	14	10
Neoclytus acuminatus	2,198	2,472
Neoclytus caprea	25	28
Neoclytus jouteli	–	2
Neoclytus mucronatus	302	906
Neoclytus scutellaris	201	374
Obrium maculatum	–	1
Orthosoma brunneum	–	1
Phymatodes amoenus	–	1
Phymatodes varius	21	23
Prionus imbricornis	2	1
Prionus pocularis	1	2
Psenocerus supernotatus	1	4
Rhagium inquisitor	5	8
Stenocorus cinnamopterus	1	–
Tilloclytus germinatus	–	5
Tylonotus bimaculatus	–	1
Typocerus zebra	5	6
Xylotrechus colonus	12	83
Xylotrechus sagittatus	–	1
Total number of beetles	6,183	7,246
Total number of species	29	41

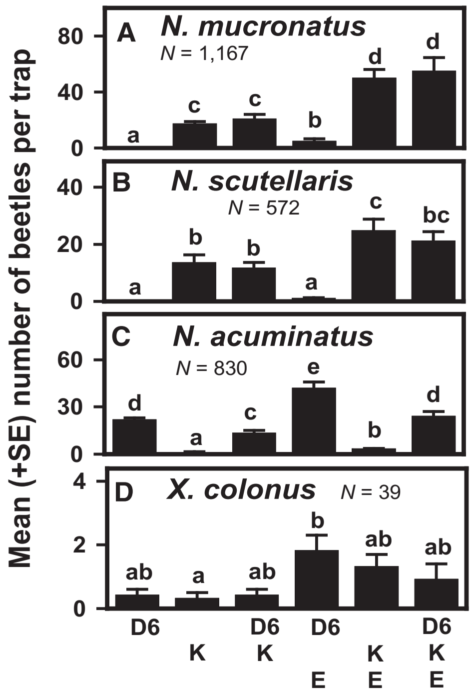

Fig. 2. Interaction of ethanol (**E**), *syn*-2,3-hexanediol (**D6**), and 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one blend (**K**) on trap catches of Cerambycidae in north Georgia (Experiment 2). For each species, means followed by the same letter are not significantly different at $P = 0.05$ (Holm–Sidak multiple-comparison test). ***N*** = Total trap catch of beetles per location.

A could not be used on data for *N. mucronatus*, *N. scutellaris*, and *N. acuminatus* because two to four treatments per species had zero catches (Fig. 1A–C). Using Model C, we found that catches of *N. mucronatus* and *N. scutellaris* were the highest in traps with K6 with no interruptive effects from D6 or K8 (Fig. 1A–B). In contrast, catches of *N. acuminatus* were the highest in traps baited solely with D6 or the combination of D6 + K8 (Fig. 1C). The addition of K6 reduced catches of *N. acuminatus* in traps with D6.

Catches of *X. colonus* were higher in traps with the full three-component blend than in traps baited solely with either D6 or K8 (Fig. 1D). Analysis with ANOVA model A found a significant treatment effect from K6 ($F_{1,49} = 13.555$, $P = 0.001$) but not from D6 ($F_{1,49} = 0.365$, $P = 0.549$) or K8 ($F_{1,49} = 1.809$, $P = 0.185$). There were no significant interactions between D6 and K6 ($F_{1,49} = 0.365$, $P = 0.549$), D6 and K8 ($F_{1,49} = 1.148$, $P = 0.289$), and K6 and K8 ($F_{1,49} = 1.809$, $P = 0.185$), or among D6, K6, and K8, ($F_{1,49} = 0.637$, $P = 0.429$). Using Model C, we found no significant effect of treatments on catches of *Sphenostethus taslei* (Buquet) (Prioninae) ($F_{7,49} = 0.934$, $P = 0.489$).

In Experiments 2–3, traps with ethanol detected 41% more species of Cerambycidae than those without ethanol (Table 5). In Experiment 2, ethanol had a significant effect on *N. acuminatus*, *N. mucronatus*, and *N. scutellaris*, increasing catches in traps baited with D6 and K blend (Fig. 2). The largest catches of *N. mucronatus* and *N. scutellaris* were in traps with ethanol + K blend, with no interruptive effect from D6 (Fig. 2A–B). The largest catches of *N. acuminatus* were in traps with E + D6; the K blend interrupted response of *N. acuminatus* to D6 (Fig 2C). Catches of *X. colonus* were higher in traps with E + D6 than in traps baited solely with the K blend (Fig 2D).

In Experiment 3, ethanol had a significant effect on the five species of Cerambycidae, whereas nine species were affected by D6 and/or K blend (Fig. 3). Catches of *Molorchus bimaculatus* Say were higher in traps with E + D6 than in those with D6 alone or D6 + K blend (Fig. 3A), whereas catches of *X. colonus* were the highest in any trap with ethanol (Fig. 3B). Catches of *A. parallelus* in traps with D6 were higher than those in traps with the K blend, regardless of the addition of ethanol (Fig. 3C). The K blend interrupted response of *A. parallelus* to traps baited with E + D6, whereas ethanol increased attraction of *A. parallelus* to traps baited with either D6 or the K blend but not both. Traps with the K blend were more attractive to *K. cincta* than those baited with D6 (Fig. 3D). D6 interrupted the response of *K. cincta* to traps with the K blend but not when ethanol was present. Ethanol enhanced catches of both

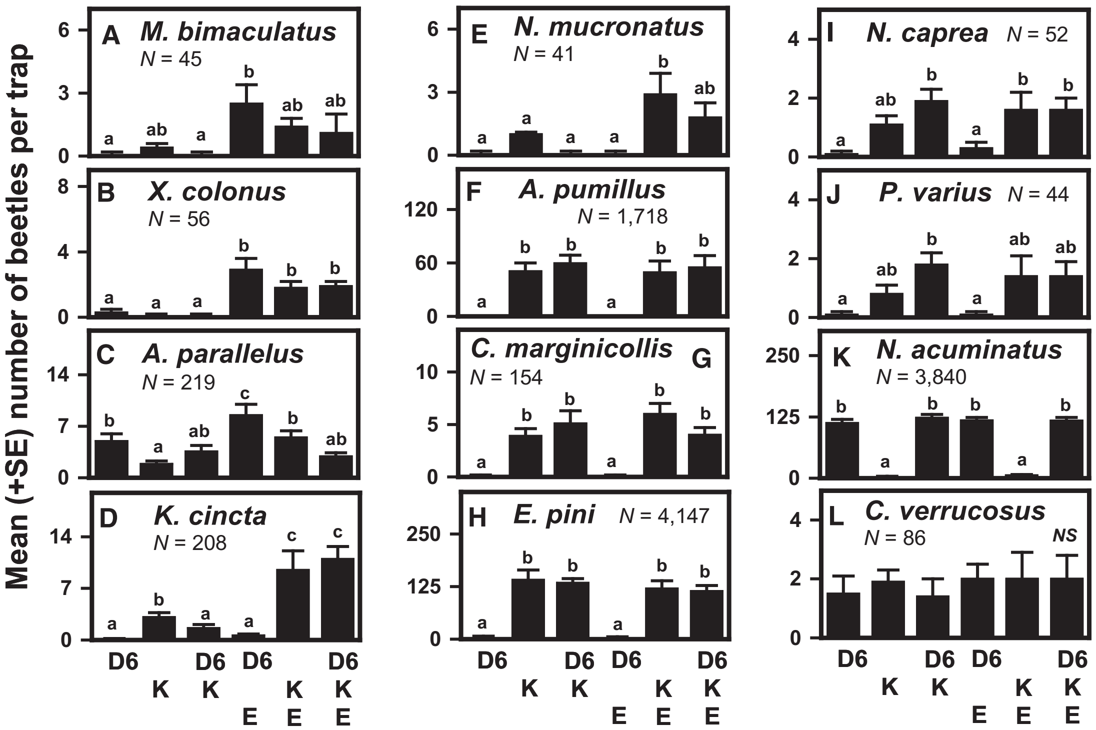

Fig. 3. Interaction of ethanol (**E**), *syn*-2,3-hexanediol (**D6**), and 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one blend (**K**) on trap catches of Cerambycidae in north Georgia (Experiment 3). For each species, means followed by the same letter are not significantly different at $P = 0.05$ (Holm–Sidak multiple-comparison test). ***N*** = Total trap catch of beetles per location, ***NS*** = $P > 0.05$.

Table 6. ANOVA (Model B) significance levels (*P*) for *syn*-2,3-hexanediol treatment (D6), 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one treatment (K blend), interaction between the two treatments (D6 x K blend), and replicate on catches of Cerambycidae in north Georgia (Experiments 4–5).

	P			
	Rep	D6	K	D6 × K
Expt 4				
Anelaphus parallelus	0.006	0.229	0.002	0.040
Anelaphus pumilus	0.271	0.203	<0.001	0.857
Anelaphus villosus	0.169	0.004	0.001	0.012
Euderces pini	0.018	0.870	<0.001	0.784
Knulliana cincta	0.009	0.562	<0.001	0.042
Neoclytus acuminatus	0.029	<0.001	<0.001	<0.001
Neoclytus mucronatus	0.403	0.659	<0.001	0.835
Phymatodes varius	0.024	0.488	<0.001	0.872
Expt 5				
Neoclytus acuminatus	0.487	<0.001	0.380	0.167
Neoclytus mucronatum	0.436	0.980	<0.001	0.341
Neoclytus scutellaris	0.293	0.790	<0.001	0.409

K. cincta and *N. mucronatus* in traps baited with the K blend (Fig. 3 D–E).

The following five species exhibited a similar response profile in Experiment 3, with catches generally highest in traps with the K blend, regardless of the addition of ethanol or D6: *A. pumilus*, *C. marginicollis*, *E. pini*, *Neoclytus caprea* Say, and *Phymatodes varius* (F.) (Fig 3F–J). One exception to the generalization was that mean catches of *N. caprea* in traps with the K blend alone were not different from that of any other treatment (Fig. 3I). In the same fashion, the only significant differences in mean catches of *P. varius* was between traps baited with D6 + K blend and traps baited with D6 or E + D6 (Fig. 3J). Catches of *N. acuminatus* were highest in traps with D6 and unaffected by the addition of ethanol or the K blend (Fig. 3 K), analyzed with non-transformed data as transformed data failed to meet normality assumption (Shapiro–Wilk test, $P < 0.05$). Catches of *Cyrtophorus verrucosus* (Olivier) were unaffected by all treatments ($F_{5,35} = 0.437$, $P = 0.820$, ANOVA Model C) (Fig. 3L).

In Experiment 4, the K blend significantly affected catches of nine longhorn beetle species (Table 6; Fig. 4), with the following six species exhibiting a similar response profile: *A. pumilus*, *C. marginicollis*, *E. pini*, *K. cincta*, *N. mucronatus*, and *P. varius* (Fig. 4A–E, G). For each of these six species, traps with ethanol + K blend had the highest catches with no effect from D6. Similarly, catches of *A. parallelus* were greater in traps with E + K blend or E + D6 + K blend than in those with only E (Fig. 4F). Catches of *A. villosus* and *N. acuminatus* were affected by D6 and the K blend with significant interaction between D6 and the K blend (Table 6). The highest catches of *A. villosus* were into traps baited with E + D6 with significant interruption by the K blend (Fig. 4H). There were no differences among the other three treatments. Catches of *N. acuminatus* were significantly higher in traps with E + D6 regardless of the presence or absence of the K blend (Fig. 4I). The K blend increased the attraction of *N. acuminatus* when D6 was absent but not when it was present. Neither D6 nor the K blend affected mean catches of *Ataxia crypta* (Say) ($F_{3,27} = 1.091$, $P = 0.370$), *C. verrucosus* ($F_{3,27} = 0.865$, $P = 0.471$), *M. bimaculatus* ($F_{3,27} = 0.477$, $P = 0.701$), and *X. colonus* ($F_{3,27} = 0.515$, $P = 0.675$) in ethanol-baited traps (Fig. 4J–M) (ANOVA Model C).

In Experiment 5, catches of *N. acuminatus* were affected by D6, whereas those of *N. mucronatus* and *N. scutellaris* were affected by the K blend with no significant interactions for any species

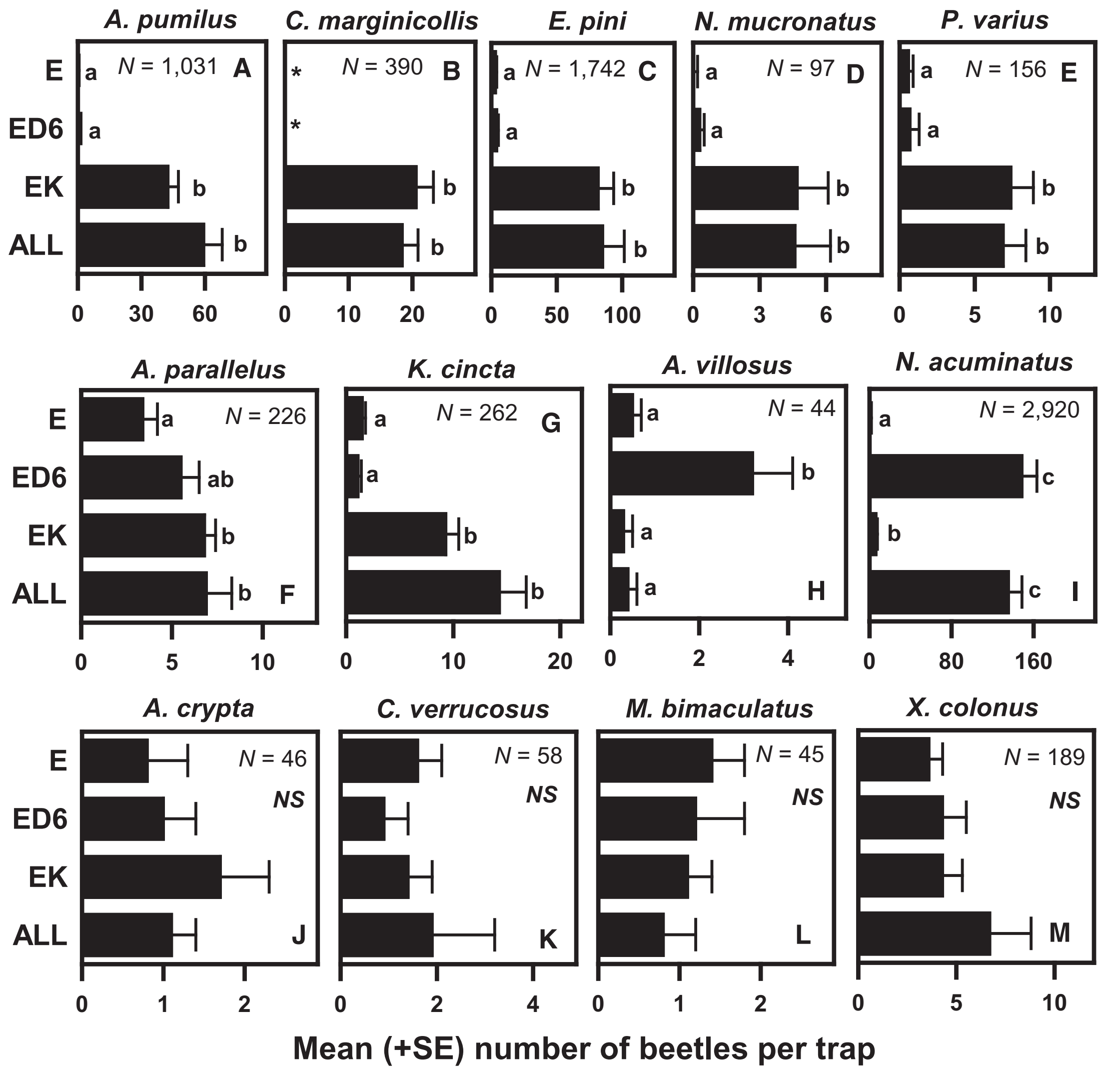

Fig. 4. Interaction of *syn*-2,3-hexanediol (**D6**) and 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one blend (**K**) on catches of Cerambycidae ethanol (**E**)-baited traps in north Georgia (Experiment 4). For each species, means followed by the same letter are not significantly different at $P = 0.05$ (Holm–Sidak multiple-comparison test). Treatments with an asterisk had zero catches. ***N*** = Total trap catch of beetles per location.

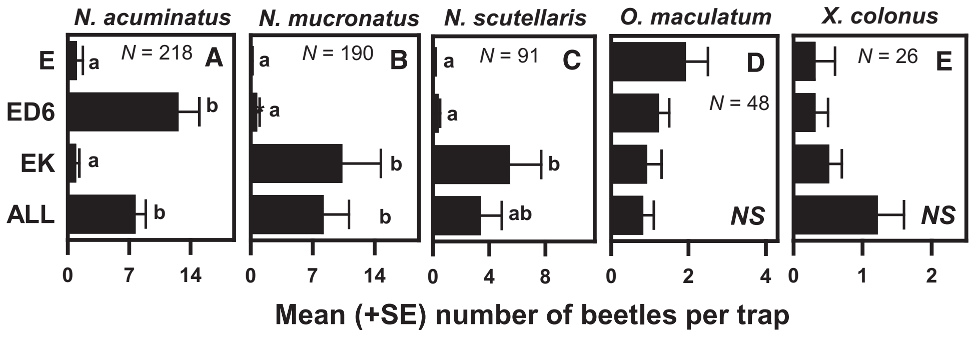

Fig. 5. Interaction of *syn*-2,3-hexanediol (**D6**) and 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one blend (**K**) on catches of Cerambycidae in ethanol (**E**)-baited traps in central South Carolina (Experiment 5). For each species, means followed by the same letter are not significantly different at $P = 0.05$ (Holm–Sidak multiple-comparison test). ***N*** = Total trap catch of beetles per location, ***NS*** = $P > 0.05$.

(Table 6). Traps with E + D6 caught the greatest number of *N. acuminatus*, regardless of the presence or absence of the K blend (Fig. 5A). Similarly, the greatest numbers of *N. mucronatus* and *N. scutellaris* were in traps with ethanol + K blend (Fig. 5B–C). Neither D6 nor the K blend affected catches of *Obrium maculatum* (Olivier) ($F_{3,27} = 1{,}158$, $P = 0.344$) and *X. colonus* ($F_{3,27} = 1.713$, $P = 0.188$) in ethanol-baited traps (Fig. 5D–E) (ANOVA Model C).

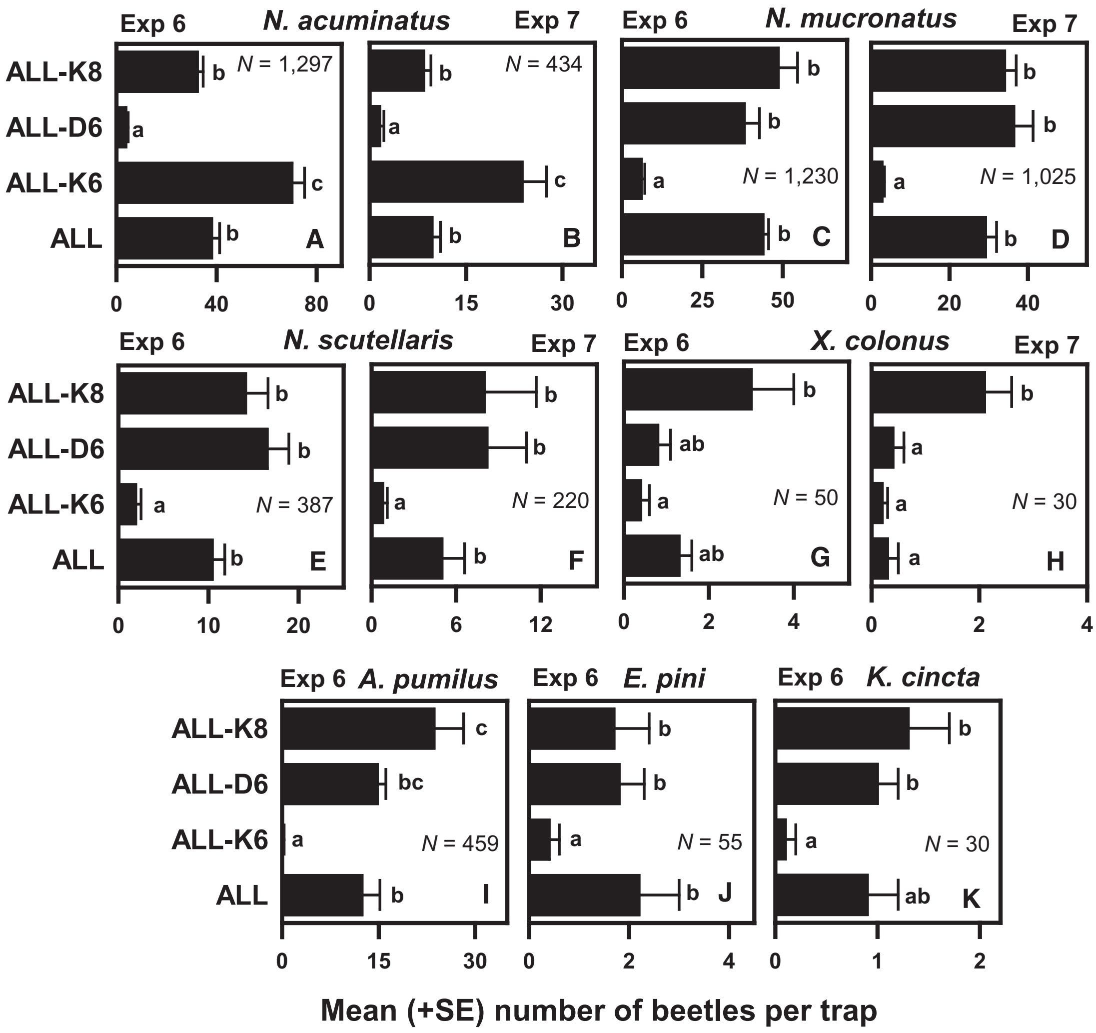

Fig. 6. Effects of eliminating 3-hydroxyoctan-2-one (**ALL - K8**), *syn*-2,3-hexanediol (**ALL - D6**), or 3-hydroxyhexan-2-one (**ALL - K6**) from traps baited with the four-component blend of ethanol + *syn*-2,3-hexanediol + 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one (**ALL**) (Experiments 6–7). For each species, means followed by the same letter are not significantly different at $P = 0.05$ (Holm–Sidak multiple-comparison test). ***N*** = Total trap catch of beetles per location, ***NS*** = $P > 0.05$.

Results in Experiments 6–7 were similar to each other. In both experiments, removal of D6 from the four-component blend of ethanol + D6 + K6 + K8 reduced trap catches of *N. acuminatus*, whereas removal of K6 increased trap catches; removal of K8 had no effect on catches of *N. acuminatus* (Fig. 6A–B). Removal of K6 from the four-component blend reduced catches of both *N. mucronatus* and *N. scutellaris* in both experiments, whereas removal of D6 or K8 had no effect on either species (Fig. 6C–F). Data for *N. mucronatus* were analyzed with non-transformed data, as transformed data failed to meet normality assumption (Shapiro–Wilk test, $P < 0.05$).

Mean catches of *X. colonus* were greatest in traps without K8 in both experiments but the difference between the four-component blend and the blend without K8 was significant only in Experiment 7 (Fig. 6G, H). Similarly, for *A. pumilus*, removal of K8 from the "super lure" blend resulted in an increase in trap catches compared with those in traps baited with the "super lure" blend, whereas removal of K6 resulted in a decrease in trap catches (Fig. 6I). Compared with catches in traps with the "super lure", catches of *E. pini* were reduced by the removal of K6, whereas removal of K8 or D6 had no effect (Fig. 6J). Catches of *K. cincta* were higher in traps with either K8 or D6 removed than in traps with K6 removed (Fig. 6K).

Discussion

Our trapping results on attraction of Cerambycidae to *syn*-2,3-hexanediol, 3-hydroxyhexan-2-one and/or 3-hydroxyoctan-2-one in the Piedmont region of southeastern United States are consistent with those found for the same species in the northeast, mid-west and southeast regions of the United States (Miller et al. 2015a, Hanks and Millar 2016). Previously unreported results found in our study include: 1) attraction of *M. bimaculatus* to traps with D6 + ethanol (Fig. 3A); 2) K8 interrupted attraction of *X. colonus* and *A. pumilus* to traps with E + D6 + K6 (Fig. 6G–H); 3) *K. cincta* was attracted to K blend with interruption from D6 (Fig. 3D); and 4) K8 negated the interruptive effect of K6 on catches of *N. acuminatus* in traps with D6 (Fig. 6A–B).

In our study, combining all four compounds into one trap as a "super lure" was effective for 8 of 13 species captured in sufficient numbers for statistical analyses. Catches of four species (*C. marginicollis*, *E. pini*, *N. caprea*, and *P. varius*) were as high in traps with the "super lure" as in traps with K6 and/or K8 with no effect from E or D6 (Figs. 3G–J, 4B–C, 4E, 6J). Traps with E + K6 caught the highest numbers of *N. mucronatus* and *N. scutellaris* with no interruptive effect from either K8 or D6 (Figs. 2A–B, 3E, 4D, 5B–C, 6C–F).

Attraction of *M. bimaculatus* to traps with ethanol + D6 was unaffected by the addition of K6 + K8 (Figs. 3A, 4L). The interruptive effect of D6 on catches of *K. cincta* in traps baited with K6 + K8 was negated by ethanol (Figs. 3D, 4G, 6K). The captures of five species (*A. crypta*, *C. verrucosus*, *M. bimaculatus*, *O. maculatum*, and *S. taslei*) in traps baited with ethanol were unaffected by the addition of D6, K6, or K8 (Figs. 1E, 3L, 4J–L, 5D), an important consideration if a manager wants to add these compounds to an existing program using ethanol-baited traps.

For the remaining five species, the use of a "super lure" did not result in maximum trap catches. Removing K8 from traps baited with the "super lure" increased trap catches of *A. pumilus* by 50% (Fig. 6I). Catches of *A. villosus* in traps baited with ethanol + D6 were decreased by 90% with the addition of the K blend (Fig. 4H). The same was true for *A. parallelus* in one experiment with a reduction of 66% (Fig. 3C) but not in a second experiment (Fig. 4F). The preferred lure blend for *N. acuminatus* was E + D6 + K8; the addition of K6 to the blend reduced trap catches by 46–59% (Figs. 1C, 2C, 6A–B). Catches of *X. colonus* in traps baited with the blend of E + K6 + D6 were reduced by 57–86% with the addition of K8 in some experiments (Fig. 6G–H) but not in another experiment (Fig. 1D).

Managers of early detection programs need to recognize the tradeoffs in deploying various trapping schemes. The use of a "super lure" like the one tested in this study could reduce the need for large numbers of traps or allow traps to be deployed at more locations if the inventory of traps is fixed. However, the effectiveness of the "super lure" would be lower for a significant number of species (5 of 13 in our study). Typically, managers want to detect invasive non-native species when they first invade a country, typically in low numbers. For target species, managers might want to maximize the effectiveness of their program for those species. Interactions between D6, K6, and K8 could be eliminated by using three separate traps, each baited with only one compound plus ethanol. Separate traps would also ensure capture of other common species in Georgia such as *E. quadrigeminata* to E + K6, and *M. caryae* and *N. j. jouteli* to E + D6 (Miller et al. 2015a).

At present, we cannot predict which species will invade any given country or which species will cause significant damage. Therefore, there might be a significant advantage in using "super lure" combinations that maximize species richness, particularly for rare species (Wong et al. 2012, Dodds et al. 2015, Hanks and Millar 2016). Ethanol should always be a component of such a "superlure," as it synergized responses of some species to D6, K6, and/or K8 with no evidence of interruption. In our study, we found that traps co-baited with ethanol detected 41 species, whereas those without ethanol detected only 29 species (Table 5).

In the absence of a clear direction, managers could use traps with "super lures" at some locations and traps baited with separate lures in other locations. As they obtain data, managers could shift the relative use of the two tactics or include additional components. Additional pheromone attractants such as fuscumol, fuscumol acetate, methylbutanol, monochamol, and ipsenol (Mitchell et al. 2011, Miller et al. 2015b, 2016, Ryall et al. 2015, Hanks and Millar 2016) warrant consideration in a detection program. Host volatiles such as floral, leaf, and stem volatiles may also play a role in optimal trap lures for Cerambycidae (Allison et al. 2004, Ryall et al. 2015, Wong et al. 2017). However, interactions among these lures have not been assessed for most species of Cerambycidae. The challenge for managers will be to use an adaptive approach in determining blend–trap combinations that best satisfy their requirements for detection efficacy over time.

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