Synthetic 3,5-Dimethyldodecanoic Acid Serves as a General Attractant for Multiple Species of *Prionus* (Coleoptera: Cerambycidae)

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ABSTRACT Males of the longhorned beetle *Prionus californicus* Motschulsky (Coleoptera: Cerambycidae) are significantly attracted to the female-produced sex pheromone (3*R*,5*S*)-3,5-dimethyldodecanoic acid. Males respond equally well to the synthetic blend of the four stereoisomers of 3,5-dimethyldodecanoic acid as to the single natural enantiomer, suggesting that the unnatural isomers are not inhibitory. Males of the congener *Prionus lecontei* Lameere also are attracted to the (3*R*,5*S*)-enantiomer but not to the (3*S*,5*R*)-enantiomer, suggesting that the (3*R*,5*S*)-enantiomer is also an important pheromone component of that species. Here, we report the results of field trials that test the hypothesis that synthetic 3,5-dimethyldodecanoic acid will serve as a general attractant for males of other *Prionus* species. We conducted field bioassays of synthetic 3,5-dimethyldodecanoic acid at study sites in six different regions of North America and one site in the United Kingdom. Traps baited with the synthetic pheromone blend captured males of *P. californicus* (southwestern Idaho, southern California, and northwestern Utah), *P. lecontei* (southern California and northwestern Utah), and six additional species of *Prionus*: *Prionus integer* LeConte (southwestern Idaho), *Prionus imbricornis* (L.) (Georgia), *Prionus laticollis* (Drury) (Georgia), *Prionus linsleyi* Hovore (north central Arizona), *Prionus aztecus* Casey (northern Mexico), and *Prionus coriarius* (L.) (East Anglia, United Kingdom). These findings demonstrate that synthetic 3,5-dimethyldodecanoic acid can be used to assess the geographic distribution and local abundance of many *Prionus* species and therefore may be of value for monitoring threatened and endangered species of this genus, and for managing those that are pests.

KEY WORDS Prioninae, semiochemical, cross attraction, pheromone-based pest management

Many species of cerambycid beetles in the subfamily Prioninae are economically important pests of crops and ornamental plants worldwide (Linsley 1962, Solomon 1995). The larvae usually feed on roots of plants, and host relationships and geographic ranges of many species are well established (for general information on biology, see Solomon 1995, volumes indexed in Linsley and Chemsak 1997). Adult prionines are relatively large beetles (often >5 cm long), are crepuscular or nocturnal, and usually do not feed. Earlier observational studies have indicated that females of some prionine species produce volatile sex pheromones (Rotrou 1936, Edwards 1961, Benham and Farrar 1976, Gwynne and Hostetler 1978), in contrast to the male-produced sex or aggregation pheromones of many cerambycid species in the subfamilies Cerambycinae and Spondylidinae (Silk et al. 2007; Lacey et al. 2009; Ray et al. 2009a,b; Millar et al. 2009, and references therein). We recently identified the female-produced sex pheromone of *Prionus californicus* Motschulsky as (3*R*,5*S*)-3,5-dimethyldodecanoic acid (Rodstein et al. 2009, 2011). In field bioassays, a blend of all four stereoisomers of 3,5-dimethyldodecanoic acid and the insect-produced (3*R*,5*S*)-enantiomer proved to be equally attractive to male *P. californicus*, indicating that the unnatural isomers did not inhibit attraction to the natural isomer (Rodstein et al. 2011). Males responded to as little as 10 μg of the synthetic blend when deployed in polyethylene sachet dispensers in dose–response field trials, and capture rate reached an upper threshold at 100 μg per trap lure.

During field bioassays that targeted *P. californicus* in southern California, males of the congener *Prionus lecontei* Lameere also were caught in large numbers by traps baited with synthetic 3,5-dimethyldodecanoic acid or (3*R*,5*S*)-3,5-dimethyldodecanoic acid, but not the (3*S*,5*R*)-enantiomer (Rodstein et al. 2011). These findings suggest that the (3*R*,5*S*)-enantiomer is an important pheromone component of *P. lecontei*, and that the absence of inhibition of the natural isomer by

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unnatural isomers again resulted in a general response to the synthetic blend of stereoisomers.

Here, we report the results of research at field sites in six different regions of North America, and one area in the United Kingdom, that tested the hypothesis that synthetic 3,5-dimethyldodecanoic acid serves as a general attractant for males of other species in the genus *Prionus.*

Materials and Methods

We produced the synthetic blend of four stereoisomers by nonstereoselective synthesis of 3,5-dimethyldecanoic acid (96% chemical purity; the four stereoisomers in approximately equal proportions) as described by Rodstein et al. (2009). Lures consisted of clear polyethylene sachets (press-seal bags, Bagette model 14770, 5.1 by 7.6 cm, 0.05 mm wall thickness, Cousin Corp., Largo, FL) that were loaded with 10 mg of the synthetic blend in 100 μl of HPLC grade hexane or absolute ethanol. Adult *P. californicus* do not respond to either of these solvents (Rodstein et al. 2009, 2011), but we cannot yet rule out the possibility that one or both of these solvents synergizes attraction to 3,5-dimethyldecanoic acid. The pheromone lures remain active for at least 14 d under field conditions (J.D.B., unpublished data; and see below). Control lures contained 100 μl of solvent. Lures were suspended with wires above pitfall traps or in the center of panel traps, with dry collecting basins, the design and placement of which differed somewhat between study sites (see below). Care was taken in working with the pheromone because traps contaminated with even minute quantities subsequently attract male beetles for several years (A.M.R., unpublished data).

Study sites in North America were selected to target a variety of *Prionus* species (based on regional records of Monné and Hovore 2005), but selection of sites also was contingent on availability of local collaborators. In some cases logistical problems limited the duration of field bioassays to only a few days.

Study sites in Idaho (Canyon Co.) consisted of fields of commercial hop (*Humulus lupulus* L.) that were infested with *P. californicus* (see Cervantes et al. 2006; Barbour et al. 2006; approximate center of study site: 43° 42′57.88″ N, 116° 55′29.26″ W; elevation, ≈735 m). Other species of *Prionus* known from this region include *P. lecontei* and *Prionus integer* LeConte (Monné and Hovore 2005). Bioassays were conducted 8–26 July 2008 (maximum air temperature, 28–36°C; no precipitation). Pitfall traps that were used in this bioassay, and at some of the other sites, were constructed from 19-liter polypropylene buckets (38 cm in height by 30 cm in diameter) fitted with aluminum funnels (model 28 15B, BioQuip, Rancho Dominguez, CA) that were buried with funnel tops flush with the soil surface. Lures were attached to the wire bucket handles that had been modified to remain upright. One trap baited with pheromone and one control trap were set up 15 m apart between rows of hop plants in each of five sites that were at least 0.4 km apart. We checked traps for beetles every 2 d, replacing lures and switching the position of treatments within each field. Captured beetles were identified and sexed, then released at least 30 m from the nearest trap. Thus, some beetles probably were captured more than once during the study (see Maki et al. 2011).

There were four study sites in Riverside Co., CA, that represented a range of elevations and habitats (for detailed information about local plant communities, see Baldwin et al. 2002). The only *Prionus* species that are known to be common in the area are *P. californicus* and *P. lecontei* (Monné and Hovore 2005). Bioassays were conducted 7–9 July 2008 by using panel traps constructed from black corrugated plastic (1.2 m in height by 0.3 m in width; model PT Intercept, APTIV, Portland, OR), with the supplied collection basin replaced with a plastic jar and funnel, and traps were suspended from L-shaped frames of PVC pipe (for details, see Graham et al. 2010). At each site, we set up one pheromone trap and one control trap, ≈10 m apart. Traps were set up at three elevations in the mountains of San Bernardino National Forest: a ≈2.5-ha area that was wooded primarily with oaks that recently had been burned (referred to as Burned Oaks; 33° 52′31.51″ N, 116° 50′32.16″ W; 1,207-m elevation), in a pine forest near the Vista Grande ranger station (Vista Grande; 33° 50′21.49″ N, 116° 48′28.41″ W; 1,482 m elevation), and in a mixed conifer and hardwood forest at James San Jacinto Mountains Reserve (James Reserve; University of California Reserve System; 33° 48′30.00″ N, 116°46′40.02″ W; 1,638-m elevation). We also set up a pair of traps in a Colorado subsection of the Sonoran Desert: a transitional habitat of oaks, juniper, and cacti (Pinyon Flats; 33° 34′56.58″ N, 116° 27′13.10″ W; 1,230-m elevation). Traps were set up in late mornings and run for 3 d, with the exception of the Pinyon Flats site, where we ended the bioassay after a single day of trapping that yielded sufficient beetles for a statistical test (see Results).

The study site in Utah was a commercial orchard of sweet cherry, *Prunus avium* L., that was infested with *P. californicus*, in Box Elder Co. (41° 25′36.07″ N, 112° 01′58.47″ W; 1,326-m elevation). *Prionus* species known to occur in the area include *P. californicus*, *P. lecontei*, *P. integer*, and *Prionus rhodocerus* Linsley (Monné and Hovore 2005). The bioassay was run from 2 July to 2 September 2009 (maximum air temperature, 26–38°C; total precipitation, 5 mm). Pitfall traps and cross-vane panel traps (as described above) were alternated along a transect with ≈12-m spacing, and treatment and control lures were assigned randomly to traps within trap type. Panel traps were suspended from tree branches (top of traps ≈2.5 m above the ground). Lures were replaced on 17 July 2009, and the replacement lures lasted throughout the remainder of the trapping period. Traps were checked for beetles, and lures were moved between traps (within trap type) twice weekly from 2 July to 10 August, and then once weekly until 2 September 2009.

The bioassay in Arizona targeted the rare species *Prionus linsleyi* Hovore and was conducted in its type locality, an area of sand dunes in Coconino Co. (Hovore 1981; 36° 01′39″ N, 111°11′7″ W; 1,662-m eleva-

tion). Other *Prionus* species known from the area include *P. californicus*, *P. lecontei*, *P. integer*, *Prionus heroicus* Semenov, *P. rhodocerus*, and *Prionus palparis* Say (Monné and Hovore 2005). The bioassay was conducted 18–21 July 2009 (maximum daily temperature, 31–39°C; no precipitation). We initially set up sentinel traps to determine whether adult *P. linsleyi* would be captured. Pitfall traps were baited with 100 μl of either a 12.5 or 25 mg/ml solution of 3,5-dimethyldecanoic acid in ethanol, but with no control traps. Four traps were positioned ≈5 m apart in a linear transect with treatments alternating. After capturing one male *P. linsleyi* (see Results), control traps were added to the trap line, but no more beetles were captured.

Study sites in Georgia were two orchards of pecan, *Carya illinoisensis* (Wangenheim) K. Koch, one orchard in Tift County (31° 30′34.24″ N, 83° 38′18.47″ W; 110-m elevation) and the other orchard in Crisp County (31° 59′9.38″ N, 83°55′24.32″ W; 76-m elevation). Bioassays were conducted 26 May–17 July 2009 (maximum daily temperature, 26–38°C; total precipitation, ≈135 mm). The orchards harbored infestations of *Prionus imbricornis* (L.) and *Prionus laticollis* (Drury) (J.D.D., personal observation). The only other *Prionus* species known to be present in the region is *Prionus pocularis* Dalman in Schoenherr (Monné and Hovore 2005). Six pitfall traps were buried 20–100 m apart in each orchard, with pheromone-baited traps alternating with control traps. Traps were checked for beetles three to five times weekly, and the number, sex, and species of captured beetles were recorded.

The study site in Mexico was in Sonoran oak–pine woodland on Mesa de Campañero near Yécora, Sonora. *Prionus* species known from the area include *P. californicus*, *Prionus aztecus* Casey, *Prionus curticollis* Casey, and *Prionus flohri* Bates (Monné and Hovore 2005). The bioassay was run 7–9 July 2008 (maximum air temperature, ≈15–20°C; periodic rainfall). Pitfall traps were baited with lures that were loaded with 100 μl of a 100 mg/ml solution of synthetic pheromone in absolute ethanol, or ethanol alone (controls). One pheromone trap and one control trap were set up, 5 m apart, at each of two sites that were ≈1 km apart (28° 21′60″ N, 109° 01′38″ W; 2,183-m elevation; 28° 22′ 20″ N, 109° 02′ 4″ W; 2,152-m elevation). Traps were checked for beetles daily.

The study site in the United Kingdom was an old pasture near the University of East Anglia, Norwich, England (52° 37′25.52″ N, 1° 14′09.03″ E; 27-m elevation). The study site harbored a population of *Prionus coriarius* (L.), and the adults were known to be active at the site during early August (M.R., personal observation). *P. coriarius* is the only species of the genus that is recorded from that region (Bense 1995). Bioassays were run 11–17 August 2009 (maximum air temperature, 18–25°C; no precipitation). Pitfall traps were similar in design to those used in the Idaho bioassay but with a large plastic flower pot in place of the 19-liter bucket. Three pheromone traps were positioned at equal distances (≈410 m) in a triangle pattern but varying in their proximity to dead trees from which adult beetles might emerge (M.R., personal observation): Trap A near a dead *Quercus robur* L.; trap B near a dead *Aesculus hippocastanum* L.; and trap C near living trees of both species (apparently not infested with *P. coriarius*). There were three control traps, one trap positioned approximately between pheromone traps A and B, one trap ≈410 m from pheromone traps A and C, and one trap that was >500 m from the nearest pheromone trap. The last control trap was located only 40 m from the point where captured beetles subsequently were released, and next to a dead *Q. robur*, and therefore provided a conservative assessment of the likelihood that beetles would be captured by control traps. Traps were checked for beetles daily. Dispersal distance of the adult males was assessed by marking the elytra of captured individuals with a spot of fingernail polish, releasing them, and recording where they were subsequently recaptured.

For each study site, we report the total number of beetles of *Prionus* species that were captured by pheromone-baited and control traps. Differences between treatments in the total number of beetles that were captured were tested, separately for each study site, with the log likelihood (G) goodness-of-fit test (Sokal and Rohlf 1995).

We have submitted voucher specimens of all of the *Prionus* species that were captured in North American bioassays to the W. F. Barr Entomology Museum at the University of Idaho; the Entomology Research Museum at the University of California, Riverside; or the Collection of Arthropods at the University of Georgia Museum of Natural History, Athens. All adult *P. coriarius* that were captured during the bioassay were released at the study site.

Results

We captured eight species of *Prionus* over the 11 study sites (Table 1), including *P. californicus*, *P. lecontei*, *P. integer*, *P. linsleyi*, *P. imbricornis*, *P. laticollis*, *P. aztecus*, and *P. coriarius*. In almost every case pheromone-baited traps captured significantly greater numbers of beetles than did unbaited control traps.

In the Idaho bioassay, we captured 832 male *P. californicus*, all but six in pheromone-baited traps (Table 1). Two male *P. integer* also were captured by pheromone-baited traps.

Pheromone-baited traps at the different sites in Riverside Co., CA, captured male *P. californicus* and *P. lecontei*, but no beetles were captured in control traps (Table 1). We captured both species at the lower elevation Burned Oaks site but only *P. californicus* at the higher elevation Vista Grande and James Reserve sites. The 56 male *P. californicus* at Pinyon Flats (Table 1) were captured by the single trap in only one night of trapping.

In the Utah bioassay, we captured 264 male *P. californicus*, 90% of which were in pheromone-baited traps (Table 1). Pitfall traps captured 78% of the *P. californicus* (191 pheromone:16 control), the remain-

Table 1. Total numbers of beetles of *Prionus* species that were captured in traps baited with synthetic 3,5-dimethyldecanoic acid diluted in solvent (pheromone-baited traps), or with solvent alone (control traps) at various study sites in North America and the United Kingdom

Location	Study sites	*Prionus* spp.	No. in pheromone/control traps
North America			
Idaho		*californicus*	826/6***[a]
		integer	2/0
California	Burned Oaks	*californicus*	32/0***
		lecontei	12/0***
	Vista Grande	*californicus*	33/0***
	James Reserve	*californicus*	7/0**
	Pinyon Flats	*californicus*	56/0***
Utah		*californicus*	238/26***
		lecontei	1/0
Arizona		*linsleyi*	1/0
Georgia	Crisp Co.	*imbricornis*	141/0***
		laticollis	17/0***
	Tift Co.	*imbricornis*	98/0***
Mexico		*aztecus*	26/3***
United Kingdom			
East Anglia		*coriarius*	12/0***

All captured beetles were males except for one female *P. coriarius* at the United Kingdom site.

[a] Asterisks indicate statistically significant differences between treatments and controls (*G*-test for goodness of fit): *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

der being captured in the panel traps (47 pheromone:10 control). A single male *P. lecontei* was captured in a pheromone-baited pitfall trap.

The bioassay conducted in Arizona captured one male *P. linsleyi* but no beetles of other *Prionus* species. In Crisp Co., GA, we captured significant numbers of male *P. imbricornis* and *P. laticollis* with pheromone-baited pitfall traps, with none in control traps (Table 1). Only male *P. imbricornis* were captured in Tift Co., GA (Table 1). In northern Mexico, we captured 29 male *P. aztecus*, 90% of which were in pheromone-baited traps (Table 1).

In the bioassay in East Anglia, United Kingdom, we captured 11 male *P. coriarius* in pheromone-baited traps and none in control traps (Table 1). A single female *P. coriarius* was captured in one of the pheromone-baited traps. Approximately equal numbers of males were captured by the three pheromone-baited traps (not significantly different, $P > 0.05$; *G*-test), suggesting that the likelihood of catching male beetles was not strongly influenced by the proximity of traps to trees from which they might have emerged. Only one of the marked and released males was recaptured, and it had traveled at least 400 m.

Discussion

Our results support the hypothesis that the synthetic blend of all four stereoisomers of 3,5-dimethyldodecanoic acid can serve as a general attractant for males of *Prionus* species, with our pheromone-baited traps capturing seven of the 22 species of *Prionus* that occur in North America (Monné and Hovore 2005), and the one species native to the United Kingdom (Bense 1995). The use of similar or the same pheromone components by closely-related species also has been reported in other groups of cerambycids, including genera and tribes in the subfamilies Aseminae (Silk et al. 2007) and Cerambycinae (Hanks et al. 2007, Lacey et al. 2009).

Trap captures were sex-specific, with only males of the various *Prionus* species being caught, with the exception of a single female *P. coriarus.* It seems likely that females of these various other *Prionus* species also produce one or more stereoisomers of 3,5-dimethyldodecanoic acid as their sex pheromones, given that male *P. californicus* and *P. lecontei* were attracted only to (3*R*,5*S*)-3,5-dimethyldodecanoic acid, and not to the other, unnatural stereoisomers of 3,5-dimethyldodecanoic acid, or to analogs that differed by one or more carbons in chain length or in the position(s) of methyl branches (Rodstein et al. 2011).

It also seems likely that the capture of a few males in control traps in Utah were probably the result of contamination, because at this site, lures were rotated between traps to control for positional effects. Despite the fact that lures were suspended from wires, with no actual contact between the lure and the trap, sufficient pheromone may have been adsorbed on trap surfaces to result in the attraction of a few male beetles. Similarly, the few male beetles caught in control traps in Mexico may have been the result of treatment and control traps being relatively close together, so that a few males responding to the pheromone-baited traps accidentally blundered into the controls.

The North American species that we captured represent the subgenera *Prionus* (*P. californicus, P. coriarius, P. laticollis,* and *P. lecontei*), *Neopolyarthron* (*P. aztecus* and *P. imbricornis*), and *Homaesthesis* (*P. integer* and *P. linsleyi;* Monné and Hovore 2005). We did not capture beetles of species in other prionine genera (e.g., *Archodontes* and *Ergates*), including those in the same tribe as *Prionus*, the Prionini (e.g., species of *Derobrachus*), even though species in those genera occur in the areas of our studies (Monné and Hovore 2005). These findings, however, should not be taken as evidence that these other species do not respond to the compound, because the adults may not have been active during our bioassays. Such phenological effects also could account for the absence of *P. lecontei* at some of our study sites in southern California. Slight differences between *P. lecontei* and *P. californicus* in flight period (see Hovore and Giesbert 1976) could account for *P. lecontei* not being active at higher elevations during the few days that our bioassays were deployed. Furthermore, the more restricted larval host range of *P. lecontei* (a few *Quercus* species) might result in its having a more limited distribution than the highly polyphagous *P. californicus* (see Hovore and Giesbert 1976), which also could account for our findings.

Similarly, our capture of only a few individuals *P. integer* in Idaho, *P. lecontei* in Utah, and *P. linsleyi* in Arizona does not necessarily indicate a weak response to the compound but rather could be due to seasonal

phenology and/or low local abundance. For example, the small number of specimens of *P. integer* in the Orma J. Smith Museum of Natural History (College of Idaho, Caldwell, ID) suggests that it is rare in that state. *P. linsleyi* also is very rarely encountered, as evidenced by its being known from only two specimens that were collected in the 1970s (S. W. Lingafelter, personal communication). Our contribution of a third specimen, collected in a field bioassay of very limited scope, demonstrates the potential utility of pheromone-baited traps for detecting rare and cryptic species of Cerambycidae, as was noted recently with other rare Coleoptera (e.g., Larsson and Svensson 2009).

We conclude from our results that the synthetic blend of all four stereoisomers of 3,5-dimethyldodecanoic acid may be useful for characterizing the geographic distribution of a number of *Prionus* spp., including rare species such as *P. linsleyi*, and species that are threatened, such as *P. coriarius* in the United Kingdom (UICN 1994). In addition, the synthetic blend of all four stereoisomers of 3,5-dimethyldodecanoic acid is readily available and will be useful for monitoring the phenology and abundance of pest species such as *P. californicus*, *P. imbricornis* and *P. laticollis*, Finally, it is noteworthy that the European species *P. coriarius* was attracted to this compound, despite the fact that it has been separated from its North American congeners for millions of years.

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