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Synthesis and Field Tests of Possible Minor Components of the Sex Pheromone of *Prionus californicus*

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Abstract Earlier work has shown that adult male *Prionus californicus* Motschulsky (Coleoptera: Cerambycidae) are attracted to the female-produced compound (3*R*,5*S*)-3,5-dimethyldodecanoic acid, and to a synthetic mixture of the four stereoisomers of 3,5-dimethyldodecanoic acid. Here, we report the results of field trials that tested whether or not three structurally related compounds (methyl 3,5-dimethyldodecanoate, 3,5-dimethyltridecanoic acid, and 3,5-dimethylpentadecanoic acid), present in extracts of virgin females, are attractive, and whether or not they influence attraction to 3,5-dimethyldodecanoic acid. In a trial with single components, only traps baited with the acid or its methyl ester captured more beetles than did control traps; catches to the acid were five times higher than to the methyl ester. Another trial, excluding 3,5-dimethyldodecanoic acid, confirmed the activity of the methyl ester. Finally, addition of the three compounds to 3,5-dimethyldodecanoic acid, in the ratio found in extracts from female beetles, gave a catch similar to that of traps baited with 3,5-dimethyldodecanoic acid alone. Consequently, the function of these minor compounds remains undetermined.

Key Words Prioninae · Pest management · Pheromone · (3*R*,5*S*)-3,5-dimethyldodecanoic acid · Coleoptera · Cerambycidae

Introduction

A number of insects including the herald moth, *Scoliopteryx libatrix* L, and the peach twig borer, *Anarsia lineatella* Zeller, produce novel, branched alkanes, alkenes, and alcohols (Francke et al., 2000; Schlamp et al., 2005). Adult females of the cerambycid beetle *Prionus californicus* Motschulsky produce a volatile sex pheromone, (3*R*,5*S*)-3,5-dimethyldodecanoic acid, to which males are strongly attracted, and which has potential as a management tool (Rodstein et al., 2009, 2011; Maki et al., 2011). Males responded as strongly to a synthetic mixture of the four stereoisomers of 3,5-dimethyldodecanoic acid as they did to the natural enantiomer, indicating that the unnatural stereoisomers were not inhibitory (Rodstein et al., 2011). Antennae of male *P. californicus* responded most strongly to 3,5-dimethydodecanoic acid in coupled gas chromatography-electroantennography (GC-EAD) studies, and also responded to three other compounds, the methyl ester of 3,5-dimethyldodecanoic acid and the homologs 3,5-dimethyltridecanoic acid and 3,5-dimethylpentadecanoic acid, that were present in solid phase microextraction (SPME) wipe samples of ovipositors

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(Rodstein et al., 2009). Here, we report field trials testing these compounds.

Methods and Materials

Field sites were commercial hop yards in Canyon Co., ID, USA, which were infested with *P. californicus*. We used pitfall traps made of polypropylene buckets (19 l, 38 cm tall×30 cm diam.) fitted with aluminum funnels (model 2815B, BioQuip, Rancho Dominguez, CA, USA). Traps were buried between rows of hop plants, with tops of funnels flush with the soil surface, at least 27 m from the margins of hop yards. We conducted three independent bioassays at different times during 3–15 July 2009 (max. air temp: 28–36°C; average wind speed: 2.4–15.5 $km.h^{-1}$; 0.18 cm of precipitation on 15 July).

In Bioassay 1, we compared the attractiveness of the four individual compounds. Compounds were synthesized non-stereoselectively as mixtures of all four possible stereoisomers in approximately equal proportions (≥90% chemical purity), using the methods of Rodstein et al. (2009), and described in the online Supplementary Information. Lures were clear, low-density, polyethylene press-seal bags (Bagette model 14770, 5.1×7.6 cm, 0.05 mm wall thickness, Cousin Corp., Largo, FL, USA), with 10 mg of synthetic compounds, diluted to 100 µl in hexane (HPLC grade, Chromasolv®, Sigma-Aldrich, St. Louis, MO, USA), or 100 µl hexane alone (controls). Lures were attached to the wire bucket handles, which had been modified to remain upright. Treatments were randomly assigned to traps, positioned 18 m apart in each of three commercial hop yards, which were at least 0.4 km apart. The bioassay was repeated for another 3 d in two of the hop yards, re-randomizing treatments daily, to yield nine replicates. Each morning we recorded the number of male *P. californicus* trapped during a night. Differences between treatments in the number of beetles caught were tested with the nonparametric Friedman's test (blocked by day and hop yard; Proc Freq with CMH option; SAS Institute, 2001). We tested differences between pairs of means with the REGWQ means separation test to control maximum experiment-wise error rates (SAS Institute, 2001).

Because 3,5-dimethyldodecanoic acid could affect attraction of males by the other compounds, either positively (by attracting beetles into the vicinity of traps) or negatively (by competing against the other compounds), we compared the activity of 3,5-dimethyldodecanoate, 3,5-dimethyltridecanoic acid, and 3,5-dimethylpentadecanoic acid, in the absence of 3,5-dimethyldodecanoic acid, in Bioassay 2. We used the same methods and hop yards as in Bioassay 1. The bioassay was replicated in two hop yards over 4 d, re-randomizing treatments daily, to yield eight replicates. Data were analyzed as above, excluding one replicate that caught no beetles.

In Bioassay 3, we compared the activity of 3,5-dimethyldodecanoic acid alone vs. a blend of all four compounds that approximated their proportions in extracts of ovipositors (Rodstein et al., 2009). For the latter treatment, lures were loaded with 100 µl of a stock solution of 100 mg of 3,5-dimethyldodecanoic acid, 2.4 mg of methyl 3,5-dimethyldodecanoate, 36 mg of 3,5-dimethyltridecanoic acid, and 133 mg of 3,5-dimethylpentadecanoic acid, diluted to 1 ml in hexane. We used the methods described above, replicating the experiment in four commercial hop yards (including two used in the other bioassays). Treatments were re-randomized daily for 3 days and on a 4th day in one yard, yielding 13 replicates. Differences between treatments in catches of male *P. californicus* were tested with a paired *t*-test, after the normality of data was confirmed using the Shapiro-Wilk test for normality (SAS Institute, 2001). We present means ± 1 SE throughout.

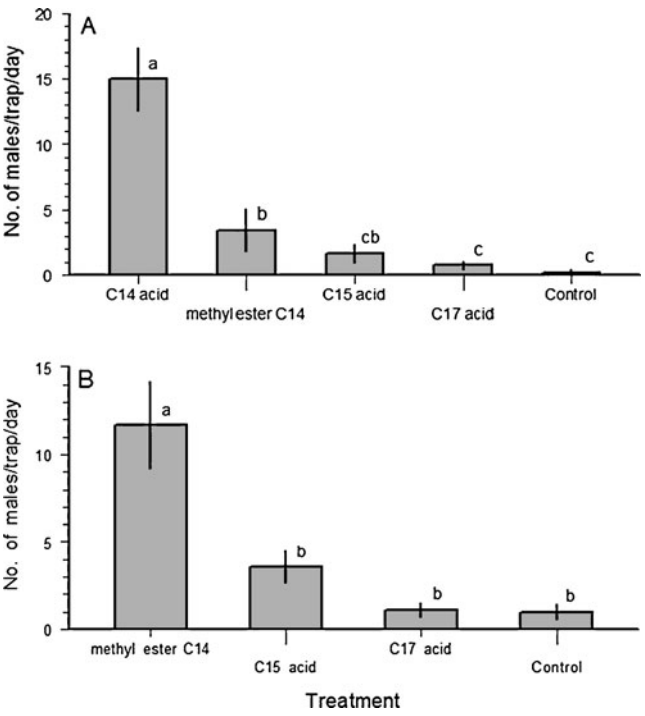

Fig. 1 Mean (± 1 SEM) numbers of male *Prionus californicus* captured by traps baited with various compounds (as mixtures of stereoisomers) that are released by females: **A**) Bioassay 1, **B**) Bioassay 2. Compounds tested were: 3,5-dimethyldodecanoic acid (C14 acid), methyl 3,5-dimethyldodecanoate (methyl ester C14), 3,5-dimethyltridecanoic acid (C15 acid), and 3,5-dimethylpentadecanoic acid (C17 acid). Control traps had lures of hexane only. Bars with the same letters are not different (REGWQ means-separation test) at $P<0.05$

Results and Discussion

No female *Prionus californicus* were captured in any bioassay. In Bioassay 1, only traps baited with 3,5-dimethyldodecanoic acid or its methyl ester captured more male *P. californicus* than did controls. Traps baited with the acid captured more than five times as many beetles as those baited with the methyl ester (Fig. 1A; Friedman's $Q_{4,45}$= 27.9, P<0.001). The activity of the methyl ester, in the absence of 3,5-dimethyldodecanoic acid, was confirmed in Bioassay 2 (Fig. 1B; Friedman's $Q_{3,28}$=115.1, P<0.002). These results suggest that the methyl ester is moderately attractive to male beetles, consistent with a stronger antennal response in GC-EAD analyses to that compound, relative to homologs of the acid (Rodstein et al., 2009).

Despite the activity of the methyl ester when tested alone, the reconstructed blend of components that mimicked the ovipositor extracts was no more attractive to males than 3,5-dimethyldodecanoic acid alone (means: 8.3±1.9 and 9.0±2.1 males/trap/day, respectively; not different, paired t-test P= 0.45). Although it is possible that one or more of the pure stereoisomers of these analogs might affect attraction to 3,5-dimethyldodecanoic acid, this seems unlikely, as any antagonistic effect from a particular stereoisomer would likely be manifested despite the presence of its stereoisomers. Similarly, it is unlikely that any synergistic effect from a particular stereoisomer would exactly cancel out effects by one or more of the other stereoisomers.

That possible minor components have no apparent effect on attraction of beetles to 3,5-dimethyldodecanoic acid, coupled with the fact that the unnatural stereoisomers of (3*R*,5*S*)-3,5-dimethyldodecanoic acid have no effect on its activity (Rodstein et al., 2011), will simplify development of the pheromone for management of *P. californicus*.

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