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RAPID COMMUNICATION

Acetophenone as an Anti-attractant for the Western Pine Beetle, *Dendroctonus Brevicomis* LeConte (Coleoptera: Scolytidae)

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Abstract Host location and colonization by bark beetles is dependent upon the relative and absolute amounts of attractant and antiattractant compounds available. Many investigations have lead to use of antiattractants for the management of these pests and have been especially focused on verbenone. However, recent studies have identified new anti-attractants for several species of bark beetles. We report results of recent investigations of the response of western pine beetle, *Dendroctonus brevicomis* LeConte, to two recently identified antiattractants, acetophenone, and fenchyl alcohol, with verbenone as a standard of comparison, in northern California. Release of both acetophenone and verbenone resulted in significantly lower trap catches of *D. brevicomis* in aggregation pheromone-baited traps, while fenchyl alcohol was inactive. Acetophenone was the only antiattractant that did not reduce numbers of the most abundant predator of *D. brevicomis*, *Temnochila chlorodia* (Mannerheim), responding to the attractant pheromone of its prey. Aggregation pheromone-baited traps with acetophenone also had the highest predator/prey ratio. Our results suggest that acetophenone may be part of the intra- and interspecific interactions among sympatric species of bark beetles and may have application in their control.

Keywords Acetophenone · Verbenone · Fenchyl alcohol · Antiattractants · Bark beetles · Predators

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Introduction

The western pine beetle (WPB), *Dendroctonus brevicomis* LeConte (Coleoptera: Scolytidae), is indigenous to western conifer forests. Its hosts are ponderosa pine, *Pinus ponderosae* Lawson, and Coulter pine, *Pinus coulteri* D. Don. In order for WPB progeny to survive, the parent beetle population must kill the tree, so large numbers of beetles must aggregate in synchrony on a single host tree. Many species of bark beetles release aggregation pheromones that attract both mates and additional colonizers, thus enhancing their tree killing ability (Wood 1982). WPB releases *exo*-brevocomin ((+)-*exo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1] octane), (−)-frontalin ((−)-1,5-dimethyl−6,8-dioxabicyclo [3.2.1]octane), and a host terpene, myrcene, which attracts large numbers of conspecifics during the aggregation phase of host colonization. In addition, some bark beetles, including WPB, produce antiaggregation pheromones that inhibit attraction of conspecifics to aggregation pheromones to optimize resource partitioning (Paine and Hanlon 1991).

Many investigations have led to the use of antiaggregation pheromones, such as verbenone, to prevent or minimize damage caused by beetle infestation (Borden 1996). Verbenone, an antiaggregation pheromone of several bark beetle species, was the focus of earlier studies with WPB (Paine and Hanlon 1991). Earlier studies investigated the inhibitory effect of verbenone in combination with semiochemicals produced by heterospecific bark beetles (Paine and Hanlon 1991; Bertram and Paine 1994a,b). Tests of verbenone to interrupt host colonization by WPB, in some cases, reduced trap catch, but in others were inconsistent in protecting individual trees (Paine and Hanlon 1991; Bertram and Paine 1994a,b).

Recent investigations identified previously unknown semiochemicals for several species of bark beetles (Pureswaran and Borden 2004; Sullivan 2005). Some of these semiochemicals are potential inhibitors of bark beetle attraction (Pureswaran and Borden 2004; Sullivan 2005). In the current study, we tested two of these recently identified antiattractant compounds—acetophenone and fenchyl alcohol. Our objective was to compare the inhibition of WPB response by these compounds relative to verbenone to its aggregation pheromone.

Methods and Materials

(*1S*)-(−)-Verbenone (chemical purity: ≥93%, enantiomeric purity ~97%) and two antiattractant compounds, acetophenone (chemical purity: ≥98%) and (*1R*)-(+)-fenchyl alcohol (chemical purity: ≥97%, enantiomeric purity ~97%), each combined with the aggregation pheromone of WPB, as well as WPB aggregation pheromone alone, were presented to WPB in a replicated test in a ponderosa pine forest managed by Hancock Forest Management, Siskyou County in northern California. All chemicals except for the aggregation pheromone were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The aggregation pheromone (racemic, chemical purity: >97%) was obtained from Phero Tech International, Inc. (Delta, British Columbia, Canada). All antiattractant compounds were released from 15-ml polyethylene bottles (Synergy Semiochemicals, Burnaby, British Columbia, Canada), each filled with 8 ml of one compound. The aggregation pheromone was released from membranes with bubble cap reservoirs. To ensure that the entire contents of each vial were exposed to the release surface, the bottles were squeezed until the liquid reached the shoulder, and then were tightly capped leaving the bottles slightly collapsed. Each bottle also contained 100 mg of the antioxidant BHT (butylhydroxytoluene) (Sigma-Aldrich Chemical Co.). Approximate release rates of verbenone, fenchyl alcohol, and acetophenone were 2.5, 4.9, and 7.25 mg/d, respectively, at 15.1±4.3°C with a wind velocity of 16.2 km/hr±5.3 for at least 30 d in the field. The release rates of chemicals were determined by daily weight loss under the same conditions. The release rates of different compounds of WPB pheromone, frontalin, *exo*-brevicomin, and myrcene are 3.0, 3.0, and 18.0 mg/d at 24°C, respectively (data provided by Phero Tech International).

The experimental design consisted of six replications/treatment (six traps), completely randomized with repeated measurements of one data collection per week during 5 wk (30 counts/treatment samples for pheromone or pheromone with acetophenone; 29 counts for pheromone with verbenone or pheromone with fenchyl alcohol due to missing data for one trap on the third and second collection period, respectively); in some sampling occasions, the traps were rotated between sites. Release devices were suspended in the middle of flight intercept traps (Advanced Pheromone Technologies, Marylhurst, OR, USA). Traps were suspended from metal standards more than 2 m above ground and spaced at least 15 m from any other trap. An insecticidal strip (Hercon Environmental Co., Emigsville, PA, USA) was placed in each collection cup to prevent predation. Trapped beetles were collected every 7 d from June 21 to July 27, 2006. Beetles captured in each trap were identified, counted, and recorded; voucher specimens were stored at the USDA Forest Service Institute of Forest Genetics, Placerville, CA, USA, and the University of California, Berkeley.

Statistical Analyses Mean numbers of beetles per trap per sampling intervals (week) were analyzed with Poisson Regression Models for overdispersed Poisson distributed responses (counts) to address potential overdispersion arising from the repeated measurements (McCulloch and Searle 2001). This Poisson Regression model belongs to the family of the Mixed Generalized Linear Model (G LM). Model:

$$\text{Expected}\left[\text{count}_{i,j,\text{site}}|\varepsilon_{\text{site}}\right] = e^{\text{Treat}*\text{week}_{ij}+\varepsilon_{\text{site}}}$$

Where i=1,2,3,4 (four treatments) and j=1,2,3,4,5 (five weekly periods), ε is the overdispersion error due to the same site being used for measurements in different weeks (Julian days), "|" means "conditioned to", and "*" means "interaction".

The predator/prey ratio was estimated by using a similar model for the predator counts with the logarithm of number of prey as an offset:

$$\text{Expected}\left[\text{Predator count}_{i,j,\text{site}}|\varepsilon_{\text{site}}\right] = e^{\text{Treat}_i+\text{week}_j+\log\left(\text{Prey count}_{i,j,\text{site}}\right)+\varepsilon_{\text{site}}}$$

The generalized estimating equations (Liang and Zeger 1986) method was used to estimate the model's parameters with SAS (v. 9.1.3) GENMOD procedure, and the Wald chi-square test with the Bonferroni adjustment was used for multiple comparisons for an experiment-wise error rate of 0.05. The data per trap are not independent because the same site is measured during the five occasions (five sampling periods). The statistical analysis accounted for these repeated measurements. The analysis was done per week as is shown in the statistical model above. The counts for the whole period per trap were obtained by summing the estimated weekly counts.

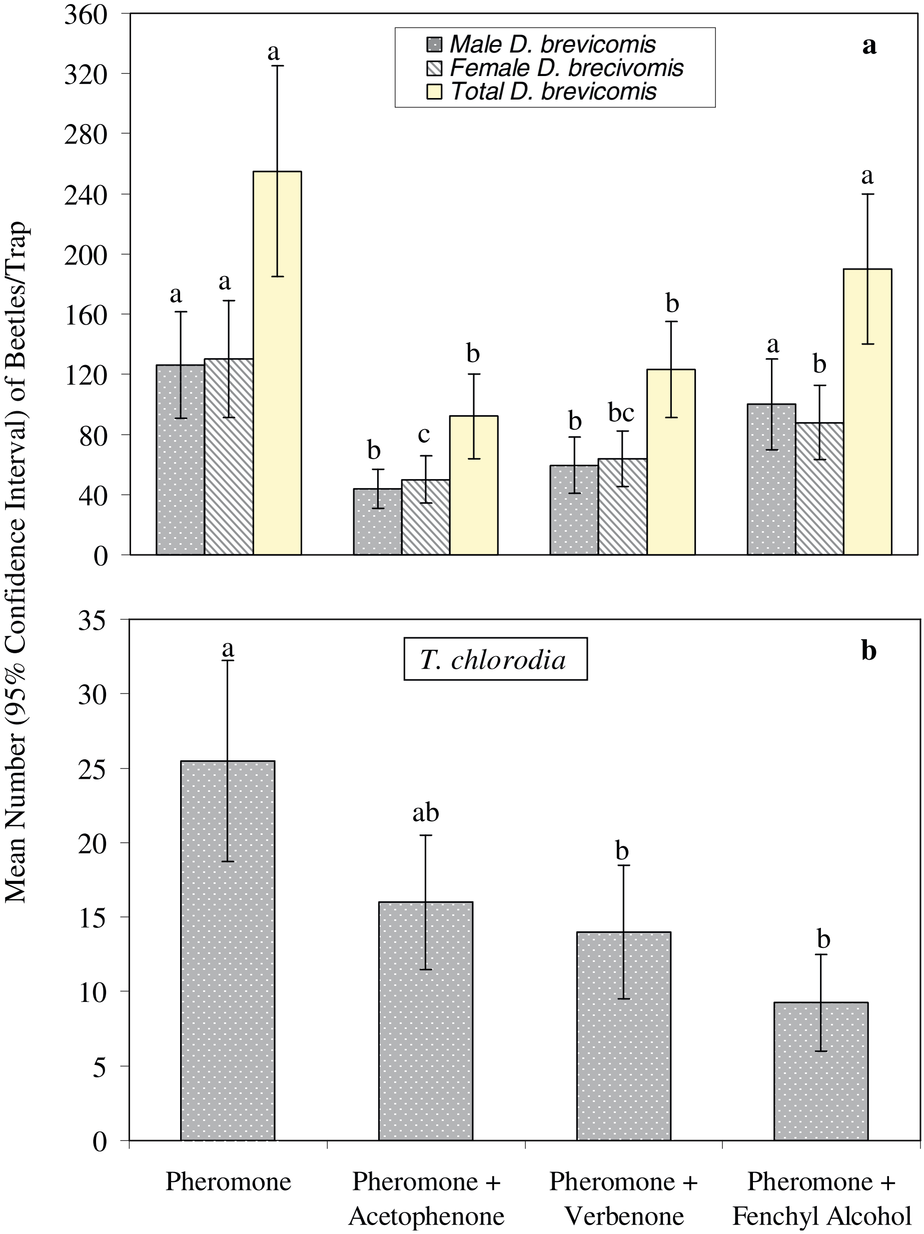

Fig. 1 Field responses of *Dendroctonus brevicomis* and *Temnochila chlorodia* to flight intercept traps baited with aggregation pheromone (P) of *D. brevicomis* alone or with acetophenone (A), verbenone (V), or fenchyl alcohol (F) in a ponderosa pine forest in Northern California. Data show mean numbers (95% lower and upper confidence interval) of beetles per trap summed over the sampling period (five sampling periods) for each treatment level from June 28 to July 27. There were a total of six traps/treatment (30 counts for P or P+A, 29 counts for P+V or P+F due to missing data for one trap on the third and second collection period, respectively). Means followed by the same letter are not significantly different from each other ($P-$ values $< 0.05/6 = 0.008$, Bonferroni adjusted). The weekly mean counts per trap were estimated assuming the Generalized Linear Models (GLM) for overdispersed Poisson counts. **(a)** Mean numbers of males and females and total numbers of *D. brevicomis*. Note that intervals can overlap and still the treatment level can be significant as for the case F versus V. **(b)** Mean numbers of *T. chlorodia*

Results and Discussion

A total of 4,461 beetles representing two species were caught over the experimental period. The WPB was 91.3% of total catch, and the remaining 8.7% was the predator *Temnochila chlorodia* (Mannerheim) (Coleoptera: Trogositidae). The overall male/female ratio of *D. brevicomis* was 1.008.

We found significant treatment effects in the response of both WPB and *T. chlorodia* ($P-$ values $< 0.05/6 = 0.008$, Bonferroni adjusted). Both acetophenone and verbenone reduced the catch of WPB attracted to its aggregation pheromone, whereas fenchyl alcohol did not (Fig. 1a). Although the mean number of WPB caught in traps baited with the aggregation pheromone and acetophenone was about 30% lower than that in traps baited with the aggregation pheromone and verbenone, this difference was not significant. The sex ratio of WPB trapped by the aggregation pheromone was not altered by combination with any antiattractant compound except fenchyl alcohol (Fig. 1a), which significantly reduced the relative proportion of females trapped by the aggregation pheromone alone (M:F= 1.15:1).

Inhibition of the response of WPB to its aggregation pheromone by acetophenone is the first report for this species. Acetophenone has been shown to inhibit attraction of the southern pine beetle, *Dendroctonus frontalis* Zimmermann, and the female Douglas-fir beetle, *Dendroctonuspseudotsugae* Hopkins, to their aggregation pheromones in the field (Pureswaran and Borden 2004; Sullivan 2005).

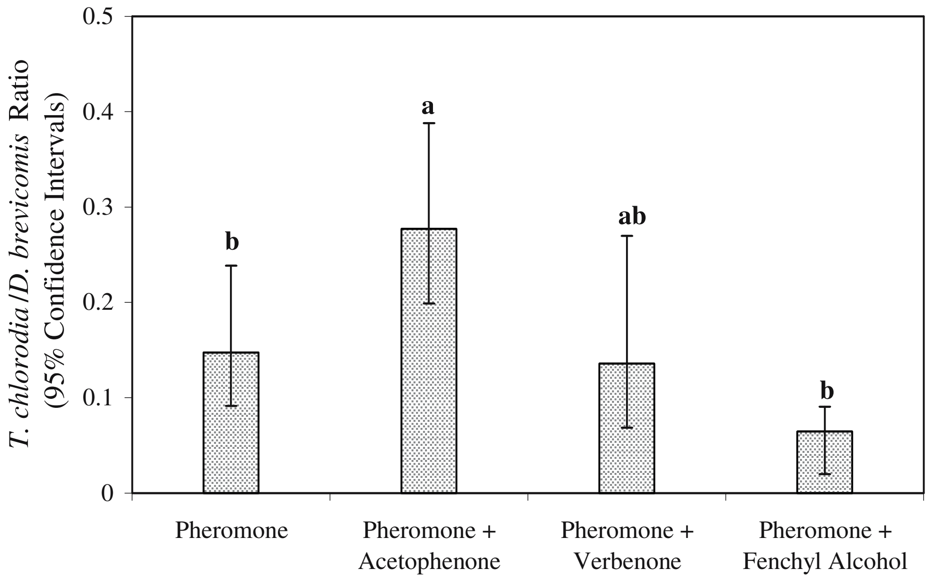

Fig. 2 Estimated mean and 95% confidence intervals of *T. chlorodia*/*D. brevicomis* ratio to flight intercept traps baited with aggregation pheromone (P) of *D. brevicomis* alone or with acetophenone (A), verbenone (V), or fenchyl alcohol (F) in a ponderosa pine forest in Northern California. Data show mean numbers (95% lower and upper confidence interval) of beetles per trap summed over the sampling period (five sampling periods) for each treatment level from June 28 to July 27. There were a total of six traps/treatment (30 counts for P or P+A, 29 counts for P+V or P+F due to missing data for one trap on the third and second collection period, respectively). Means followed by the same letter are not significantly different from each other ($P-$ values $< 0.05/6 = 0.008$, Bonferroni adjusted)

We do not know if WPB produces acetophenone, but it has been identified in volatiles from female *D. pseudotsugae* Hopkins, *Dendroctonus rufipennis* (Kirby), and *Dryocoetes confuses* Swaine; male *Taphrorychus bicolor* (Herbst); and both male and female *D. ponderosae* and *Ips pini* (Say) (references in Pureswaran et al. 2000). The inhibitory effect of acetophenone on WPB attraction suggests that either it is a part of the antiaggregation pheromone of WPB, as suggested for *D. ponderosae* (Pureswaran et al. 2000), or it is used to avoid competition with other bark beetle species. *Ips paraconfusus* Lanier, *I. Pini*, and *D. ponderosae* compete with WPB in the same host trees. Both *I. pini* and *D. ponderosae* produce and respond to acetophenone (Pureswaran et al. 2000). It has also been demonstrated that pheromones of competitors of WPB reduced trap catches of WPB responding to its pheromone (Wood 1982; Paine and Hanlon 1991; Bertram and Paine 1994a,b). Hence, production of acetophenone combined with aggregation pheromones of coinhabiting species may indicate unsuitable hosts for further colonization (Bertram and Paine 1994a,b; Pureswaran et al. 2000).

Catches of *T. chlorodia* were also altered by addition of antiattractants to the aggregation pheromone of WPB (Fig. 1b). Acetophenone was the only antiattractant that did not reduce numbers of *T. chlorodia* responding to the attractant pheromone of its prey. Moreover, the *T. chlorodia*/WPB ratio was highest for traps with acetophenone (0.28), which was 1.9 times higher than with the aggregation pheromone alone (0.15), 2.2 times higher than with verbenone (0.13), and 4.7 times higher than with fenchyl alcohol (0.06) (Fig. 2). Likewise, Sullivan (2005) found no difference in attraction of predators of *D. frontalis* to its pheromone when released with various antiattractants produced by *D. frontalis*, including acetophenone, in southern US. This result has important implication for the pest management of WPB. Although both acetophenone and verbenone reduced the catches of WPB, they did not completely stop WPB from reaching traps baited with its aggregation pheromone. Hence, arrival of higher number of predators may help to reduce WPB population in WPB attacked trees. However, the relationship between trapping the WPB and subsequent tree mortality needs to be evaluated with respect to the impact on predator populations.

For three reasons, our results suggest that acetophenone has promise for pest management strategies against the WPB. First, among the compounds we tested, acetophenone appears to be at least as effective as verbenone in inhibiting the response of WPB. Second, acetophenone was the only compound tested that did not interfere with the attraction of predators, and it produced the highest predator/prey ratio of the four treatments tested. Third, acetophenone is currently much more economical than verbenone. The cost for 1 kg of acetophenone and verbenone are \$34 and \$400, respectively, as of September, 2006 (Sigma-Aldrich Chemical Co.). This price difference may allow acetophenone to be used for large-scale experiments. Our study is the first step in a series of field bioassays for demonstrating the antiattractant activity of acetophenone for WPB. Before the effectiveness and utility of a potential treatment can be determined, further experiments are needed to determine the threshold ratio of aggregation pheromone to acetophenone and verbenone.

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References

Bertram, S. L. and Paine, T. D. 1994a. Influence of aggregation inhibitors (verbenone and ipsdienol) on landing and attack behavior of *Dendroctonus brevicomis* (Coleoptera: Scolytidae). *J. Chem. Ecol.* 20:1617–1629.

Bertram, S. L. and Paine, T. D. 1994b. Response of *Dendroctonus brevicomis* Le Conte (Coleoptera: Scolytidae) to different release rates and ratios of aggregation semiochemicals and the inhibitors verbenone and ipsdienol. *J. Chem. Ecol.* 20:2931–2941.

Borden, J. H. 1996. Disruption of semiochemicals-mediated aggregation in bark beetles, pp. 421–438, *in* R. T. Cardé and A. K. Minks (eds.). Pheromone Research: New Directions. Chapman & Hall, New York.

Liang, K. Y. and Zeger, S. L. 1986. Longitudinal data analysis using generalized linear, models. *Biometrika* 73:13–22.

McCulloch, C. E. and Searle, S. R. 2001. Generalized, Linear, and Mixed Models. John Wiley & Sons, Inc., New York, 325 pp.

Paine, T. D. and Hanlon, C. C. 1991. Response of *Dendroctonus brevicomis* and *Ips paraconfusus* (Coleoptera: Scolytidae) to combinations of synthetic pheromone attractants and inhibitors verbenone and ipsdienol. *J. Chem. Ecol.* 17:2163–2176.

Pureswaran, D. S. and Borden, J. H. 2004. New repellent semiochemicals for three species of *Dendroctonus* (Coleoptera: Scolytidae). *Chemoecology* 14:67–75.

Pureswaran, D. S., Gries, R., Borden, J. H., and Pierce, H. D. Jr. 2000. Dynamics of pheromone production and communication in the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, and the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). *Chemoecology* 10:153–168.

Sullivan, B. T. 2005. Electrophysiological and behavioral responses of *Dendroctonus frontalis* (Coleoptera: Curculionidae) to volatiles isolated from conspecifics. *J. Econ. Entomol.* 98:2067–2078.

Wood, D. L. 1982. The role of pheromones, kairomones and allomones in the host selection and colonization behavior of bark beetles. *Annu. Rev. Entomol.* 27:411–446.