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Acetophenone superior to verbenone for reducing attraction of western pine beetle *Dendroctonus brevicomis* to its aggregation pheromone

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Abstract

1 The western pine beetle *Dendroctonus brevicomis* LeConte (Coleoptera: Scolytidae) is one of the most damaging insect pests of ponderosa pines *Pinus ponderosa* Douglas ex P. & C. Lawson in Western U.S.A. We compared the effect of verbenone, a well known bark beetle anti-aggregation pheromone, with that of acetophenone on the attraction of *D. brevicomis* to its aggregation pheromone in a ponderosa pine forest in northern California. We tested the *D. brevicomis* aggregation pheromone alone and with three different release ratios of the aggregation pheromone (attractant) to verbenone or acetophenone (1 : 1, 1 : 2 and 1 : 5).

2 All treatments containing acetophenone or verbenone resulted in a significant reduction in the catch of *D. brevicomis* relative to the aggregation pheromone alone. When beetle responses to the three verbenone or three acetophenone treatments were pooled, the pooled verbenone treatment caught more *D. brevicomis* than the pooled acetophenone treatment.

3 There was no significant difference in the number of *D. brevicomis* caught among the three release rates of verbenone. By contrast, the 1 : 2 attractant : acetophenone ratio attracted significantly more *D. brevicomis* than the traps with the 1 : 5 attractant : acetophenone ratio.

4 Attraction of a major predator, *Temnochila chlorodia* (Mannerheim) (Coleoptera: Trogositidae), to the aggregation pheromone of *D. brevicomis* was reduced by verbenone, but not by acetophenone. Moreover, the *T. chlorodia* : *D. brevicomis* ratio for the pooled acetophenone treatment was 1.7-fold greater than that for the attractant alone and two-fold greater than the ratio for the pooled verbenone treatment, suggesting that acetophenone would not disrupt populations of this natural enemy. The importance of anti-attractants in the biology of *D. brevicomis* and other bark beetles is discussed.

Keywords Anti-attractant, bark beetles, Coleoptora, predators, release rates, Scolytidae.

Introduction

The western pine beetle *Dendroctonus brevicomis* LeConte (Coleoptera: Scolytidae) is an important mortality agent of ponderosa pines (*Pinus ponderosa* Douglas ex P. & C. Lawson) in Western North America (Furniss & Carolin, 1977). The beetle reproduces in the phloem of its host and, similar to other aggressive, tree-killing bark beetle species, it rapidly colonizes its host by producing an aggregation pheromone (Wood, 1982). Mass colonization of host trees is

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crucial for the survival of all the life stages of beetles in the phloem and bark. Responding beetles initiate additional attacks on the tree and release their own aggregation pheromones, thereby augmenting the total amount of pheromone released and helping ensure numbers of attacks adequate to overcome tree resistance (Wood, 1982).

When beetles reach a maximum attack density on the host, they produce other compounds that inhibit attraction of conspecifics (Bedard *et al.*, 1980a; Tilden *et al.*, 1981; Borden, 1982), causing newly-arriving beetles to shift their attacks to nearby host trees (Byers, 1989). Although the exact role of anti-attractants during host colonization by bark beetles is unclear, they are believed to have evolved to regulate attack densities of beetles for optimal beetle reproduction (Borden *et al.*, 1987; Byers, 1989). Aggregation of *D. brevicomis* is mediated by the pheromone components *exo*-brevicomin [(+)-*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 plant-produced compound, myrcene (Wood, 1982). *Dendroctonus brevicomis* also produces anti-attractant compounds that inhibit attraction of conspecifics (Renwick, 1967).

Many field investigations have lead to the use of some of the anti-aggregation pheromones and plant-produced compounds, such as green leaf volatiles, for preventing or minimizing economic damage caused by bark beetles (Dickens *et al.*, 1992; Zhang & Schlyter, 2004). Most research with anti-aggregation pheromones for bark beetles has focused on verbenone (Amman, 1994; Borden, 1996), but more recent papers report use of verbenone in combination with other potential semiochemicals, such as non-host volatiles (Fettig *et al.*, 2005, 2008). These studies have demonstrated a reduction in the attraction of beetles, but only by adding a large number of additional chemicals; such multi-component blends may be prohibitively expensive to register for operational use. Our approach, therefore, has been to focus on simpler blends and single-component semiochemicals. Verbenone is an anti-aggregation pheromone of many bark beetle species, including *D. brevicomis*, and it inhibits the response of *D. brevicomis* to its aggregation pheromone (Bedard *et al.*, 1980a; Byers & Wood, 1980, 1981; Paine & Hanlon, 1991; Bertram & Paine, 1994). Verbenone is produced as an oxidative product of yeasts in scolytid tissues (Leufvén *et al.*, 1984; Hunt & Borden, 1990), or as a two-step autoxidative product, via *trans*-verbenol from α-pinene found in pine resin (Hunt *et al.*, 1989; Grosman, 1995).

The use of verbenone to protect forest stands, however, has been limited partly by a lack of effective release systems to disperse the pheromone in forest stands and partly by the high cost of chemically pure verbenone. In addition, some experiments using verbenone to protect trees from infestation have produced inconsistent results for *D. brevicomis* (Renwick & Vité, 1970; Bedard *et al.*, 1980a; Paine & Hanlon, 1991; Bertram & Paine, 1994) and *Dendroctonus ponderosae* Hopkins (Bentz *et al.*, 1989; Gibson *et al.*, 1991; Shea *et al.*, 1991; Shore *et al.*, 1991; Amman & Lindgren, 1995; Borden *et al.*, 2003). Recent investigations have identified other potential anti-attractants for economically important bark beetles (Pureswaran *et al.*, 2000; Pureswaran & Borden, 2004; Sullivan, 2005; Erbilgin *et al.*, 2007), some of which could potentially lower the cost of large-scale applications (Erbilgin *et al.*, 2007).

We have been investigating alternative anti-attractants to verbenone for the western pine beetle in California for the last 2 years. The results obtained in an earlier study (Erbilgin *et al.*, 2007) were promising enough to warrant further research on acetophenone as an anti-attractant for *D. brevicomis*. In the present study, we report the results of investigations on the comparative effects of verbenone and acetophenone on the attraction of *D. brevicomis* in northern California.

Materials and methods

Site location, experimental design and treatments

Three release rates of (1S)-(−)-verbenone (enantiomeric purity: 97% (−); chemical purity: ≥93%) and acetophenone (chemical purity: ≥98%), each combined with the aggregation pheromone of *D. brevicomis*, as well as the aggregation pheromone alone, were presented to *D. brevicomis* in a replicated field test in a ponderosa pine *P. ponderosa* Douglas ex P. & C. Lawson forest (owned by Hancock Forest Management), at latitude 41°23.75′N and longitude 122°03.83′W in Siskiyou County, northern California. The site is a mixed-conifer forest dominated by ponderosa pine and incense cedar *Calocedrus* (= *Libocedrus*) *decurrens* Torr.

All chemicals except for the aggregation pheromone were obtained from Sigma-Aldrich Chemical Co. (St Louis, Missouri). The aggregation pheromone components [(+)-*exo*-brevicomin, (–)-frontalin, and the achiral host compound, myrcene, chemical purity: > 97%] was obtained from Phero Tech International, Inc. (Delta, BC, Canada). Acetophenone and verbenone were released from 15-mL polyethylene bottle dispensers (Synergy Semiochemicals, BC, Canada). Each dispenser was filled with 10 mL of verbenone or acetophenone. To ensure that the entire contents of each vial were exposed to the release surface, the bottles were squeezed to remove headspace air until the bottles totally collapsed, and then were tightly capped. Each bottle also contained 100 mg of the antioxidant butylhydroxytoluene (Sigma-Aldrich Chemical Co.). The approximate release rates of verbenone and acetophenone from 15-mL polyethylene bottles are shown in Table 1. The aggregation pheromone was released from bubble cap reservoirs with membranes. The release rates of *D. brevicomis* pheromone components, *exo*-brevicomin, frontalin and myrcene, were 3.0, 3.0 and 18.0 mg/day at 24 °C, respectively (data provided by Phero Tech International, Inc.). The release rates of anti-attractants were measured against the release rate of *exo*-brevicomin and frontalin.

We tested three ratios of each of anti-attractant compounds in this study. To account for the differences in release rates between acetophenone and verbenone (release rate of acetophenone is approximately three-fold greater than that of verbenone), we used three polyethylene bottles to release

Table 1 Pheromone : anti-attractant ratios assayed for responses by *Dendroctonus brevicomis* in a ponderosa pine forest in northern California

Approximate pheromone/anti-attractant ratios targeted	Number of dispensers used to achieve target ratios[a]	Compound released/ dispenser (mg/day)[b]	Approximate total compound released (mg/day)
Pheromone alone	NA	NA	3
1 : 1 Pheromone : verbenone	3	1	3
1 : 2 Pheromone : verbenone	6	1	6
1 : 5 Pheromone : verbenone	15	1	15
1 : 1 pheromone : acetophenone	1	3	3
1 : 2 Pheromone : acetophenone	2	3	6
1 : 5 Pheromone : acetophenone	3	3	15

[a] Each dispenser consisted of a 15-mL polyethylene bottle. The release rate of acetophenone is approximately three-fold faster than that of verbenone; thus, we used different numbers of bottles to account for the differences in release rates.

[b] Release rates were estimated based on the weight loss per dispenser in the laboratory. Na, not applicable.

verbenone and one polyethylene bottle to release acetophenone. The treatments were: (i) aggregation pheromone alone (called 'attractant' hereafter); (ii) 1 : 1 attractant : verbenone ratio; (iii) 1 : 2 attractant:verbenone ratio; (iv) 1 : 5 attractant: verbenone; (v) 1 : 1 attractant:acetophenone ratio; (vi) 1 : 2 attractant:acetophenone ratio; and (vii) 1 : 5 attractant : acetophenone ratio.

The experimental design consisted of seven traps/treatment, completely randomized with repeated measurements of one collection per week for 7 weeks (total 49 counts/ treatment). Treatments were rotated (position 1 to position 2, position 49 to position 1, etc.) at each collection to minimize possible effects due to trap positions within a site. Flight intercept traps consisted of two thin panels of black colored plastic (height 100 cm; width 25 cm) mounted vertically and crosswise over a plastic collection cub (diameter 22 cm) fitted at the bottom of the trap (Advanced Pheromone Technologies, Marylhurst, Oregon), were suspended from metal standards approximately 2 m above ground and spaced at least 15 m from the nearest trap. Dispensers were suspended in the middle of each trap. Two insecticidal strips (Hercon Environmental Co., Emigsville, Pennsylvania) were placed in each collection cup to prevent predation. Trapped beetles were collected from 17 May to 6 July 2007. Beetles captured in each trap were identified, counted and recorded; voucher specimens were stored at the USDA Forest Service Institute of Forest Genetics, Placerville, California, and the University of California, Berkeley. Baits were replaced with new ones every 3 weeks to maintain constant release rate over time.

Statistical analysis

When analyzing insect count data, it is important to select a model that handles the particular properties of this type of data, which is often characterized by excess zero values and heterogeneous variances (Sileshi, 2006). In the Poisson distribution, the variance is equal to the mean; however, insect trap catch data usually have the variance greater than the mean, implying an over-dispersion error. Although log transformations of insect count data have often been used for this type of analysis, the assumption that log-transformed counts are normally distributed is not appropriate because that transformation models the variance poorly, violating the assumptions and yielding a less rigorous test of treatment differences (Williamson & Gaston, 2005). Furthermore, because the log of zero does not exist, such transformations require inflating zeroes (adding a constant) (Sileshi, 2006). The Poisson model with correction for over-dispersion provided the best fit for our data, so we analyzed mean numbers of beetles per trap (counts) per sampling interval (week) with Poisson regression models for over-dispersed Poisson-distributed responses to address: (i) the discrete nature of the counts; (ii) the variance heterogeneity of the counts (increasing variance with increasing means); and (iii) the potential overdispersion arising from repeated sampling from the same trap positions (McCulloch & Searle, 2001). Based on exploratory analyses, a second degree polynomial for time (week) was used as an explanatory variable in the analysis. The Poisson regression model belongs to the family of the mixed generalized linear models (GLM):

$$\log\left\{\text{Expected}\left[\text{count}_{i,t,p}|\varepsilon_p\right]\right\} = a + b_i + c_i * \text{week}_{i,t} + d_i * \text{week}^2_{i,t} + \varepsilon_p \qquad (1)$$

where $i = 1, 2, \ldots, 7$ (7 treatment levels), $p =$ trap position (49 trap positions), $t = 1, 2, \ldots, 7$ (7-weekly sampling periods), a is the overall intercept, b_i is the coefficient for the treatment, c_i is the coefficient for the treatment · time interaction, d_i is the coefficient for the treatment · time^2 interaction, and ϵ is the overdispersion error due to repeated measures being taken at the same trap position in different weeks. This over-dispersion error is assumed to be normally distributed (McCulloch & Searle, 2001) ['|' means 'conditioned to']. The numbers of trapped beetles at each position were assumed to have the Poisson distribution with an expected count as defined in Eq. (1).

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

$$\log\left\{\text{Expected}\left[\text{Predator count}_{i,p}|\varepsilon_p\right]\right\} = \text{Treat}_i + \log\left(\text{Prey count}_{i,p}\right) + \varepsilon_p \qquad (2)$$

To better understand the information in Eq. (2), this equation can be re-written as:

$$\frac{\text{Expected}\left[\text{Predator count}_{i,p}|\varepsilon_p\right]}{\text{Prey count}_{i,p}} = e^{\text{Treat}_i + \varepsilon_p} \qquad (3)$$

This equation models predator/prey ratios.

The generalized estimating equations method (Liang & Zeger, 1986) was used to estimate the parameters of the model with the SAS Institute Inc. (2003) (version 9.1.3) GENMOD procedure, and the Wald chi-square test with the Bonferroni adjustment was used for pairwise comparisons for an experiment-wise error rate of $\alpha = 0.05$. The results per trap are not independent because the same site is measured repeatedly seven times, but the over-dispersion error as described above accounted for these repeated measurements. Because the mean beetle counts per trap were averaged over the 7 weeks, the resulting estimates are mean counts per trap and per week. The predator/prey ratio is the ratio of counts per trap and per week.

We did not test all the pairwise comparisons among seven treatments ($7!/(5! \cdot 2!) = 21$ comparisons) because our objective was to test three null hypotheses for mean numbers of prey and predators and predator/prey ratio: (i) there is no difference between attractant, verbenone and acetophenone; (ii) there is no difference between the verbenone doses; and (iii) there is no difference between the acetophenone doses. Therefore, for each null hypothesis, only three means were compared using a Bonferroni adjusted individual $\alpha = 0.05/3 = 0.0167$.

Results

A total of 16 157 beetles from three species were caught over the experimental period. *Dendroctonus brevicomis* represented 87.1% of total catch followed by the predator *T. chlorodia* (Mannerheim) (Coleoptera: Trogositidae) (11.5%) and one woodboring species, *Chalcophora angulicollis* (LeConte) (Coleoptera: Bubrestidae) (1.4%). The overall female : male ratio of *D. brevicomis* was 1.008. The response by *D. brevicomis* to treatments varied by time (treatment · interaction effect: $\chi^2 = 24.66$, d.f. = 6, $P < 0.001$), but there was no sex by time interaction ($\chi^2 = 7.34$, d.f. = 6, $P = 0.29$).

To test whether each compound (verbenone or acetophenone) provided significant reduction in beetle numbers relative to those in the control, *D. brevicomis* responses to the three verbenone or three acetophenone treatments were pooled and compared with those in the control treatment (hypothesis 1). Pairwise comparisons between the mean of the attractant alone, the mean of pooled verbenone, and the mean of pooled acetophenone indicated that both verbenone and acetophenone significantly reduced attraction of *D. brevicomis* to its aggregation pheromone (Table 2; Fig. 1A). The mean number of *D. brevicomis* caught in traps baited with attractant alone was approximately 1.8-fold greater than that of the pooled verbenone, and approximately 2.5-fold greater than that of the pooled acetophenone. Furthermore, the pooled verbenone had 1.4-fold as many *D. brevicomis* as did the pooled acetophenone treatments. There was no significant difference in the numbers of *D. brevicomis* caught among the three release rates of verbenone (hypothesis 2). By contrast, pairwise comparisons of means indicated that the 1 : 5 attractant : acetophenone ratio had significantly fewer *D. brevicomis* than the 1 : 2 attractant : acetophenone ratio (hypothesis 3) (Table 2; Fig. 1A). The sex ratio was not altered by any treatments ($\chi^2 = 0.875$, d.f. = 1, $P = 0.35$).

There were also significant differences among the attractant alone, the pooled verbenone or the pooled acetophenone in the mean number of *T. chlorodia* caught (Table 3; Fig. 1B). Pairwise comparisons of *T. chlorodia* response indicated that attractant alone had 2.1-fold more *T. chlorodia* as the pooled

Table 2 Pairwise comparisons of attraction of *Dendroctonus brevicomis* to pooled and unpooled semiochemical treatments in a field experiment in a ponderosa pine forest in northern California

		Total *Dendroctonus brevicomis*	
Hypothesis tested[a]	Comparison ratio[b]	Estimated ratio of means	*P*-value
1	Attractant versus Verbenone (pooled)	1.8	< 0.001[c]
1	Attractant versus Acetophenone (pooled)	2.5	< 0.001[c]
1	Verbenone versus Acetophenone (pooled)	1.4	< 0.001[c]
2	Verbenone dosages: 1 : 1 versus: 1 : 2	1.09	0.418
2	Verbenone dosages: 1 : 1 versus 1 : 5	1.12	0.348
2	Verbenone dosages: 1 : 2 versus1 : 5	1.02	0.830
3	Acetophenone dosages: 1 : 1 versus 1 : 2	0.87	0.200
3	Acetophenone dosages: 1 : 1 versus 1 : 5	1.3	0.096
3	Acetophenone dosages: 1 : 2 versus1 : 5	1.2	<0.001[c]

[a] Hypotheses tested: (i) there is no difference between attractant, verbenone and acetophenone; (ii) there is no difference between the verbenone doses; and (iii) there is no difference between the acetophenone doses.

[b] Estimated ratio of means indicates the ratio of the mean of the first treatment (such as attractant) to the mean of the second treatment (such as verbenone). The highest ratio produces the largest significance.

[c] Wald chi-square test with Bonferroni adjustment for an experiment-wise error rate of 0.05; significant at 0.05/3 = 0.01667 (comparisons made within each hypothesis).

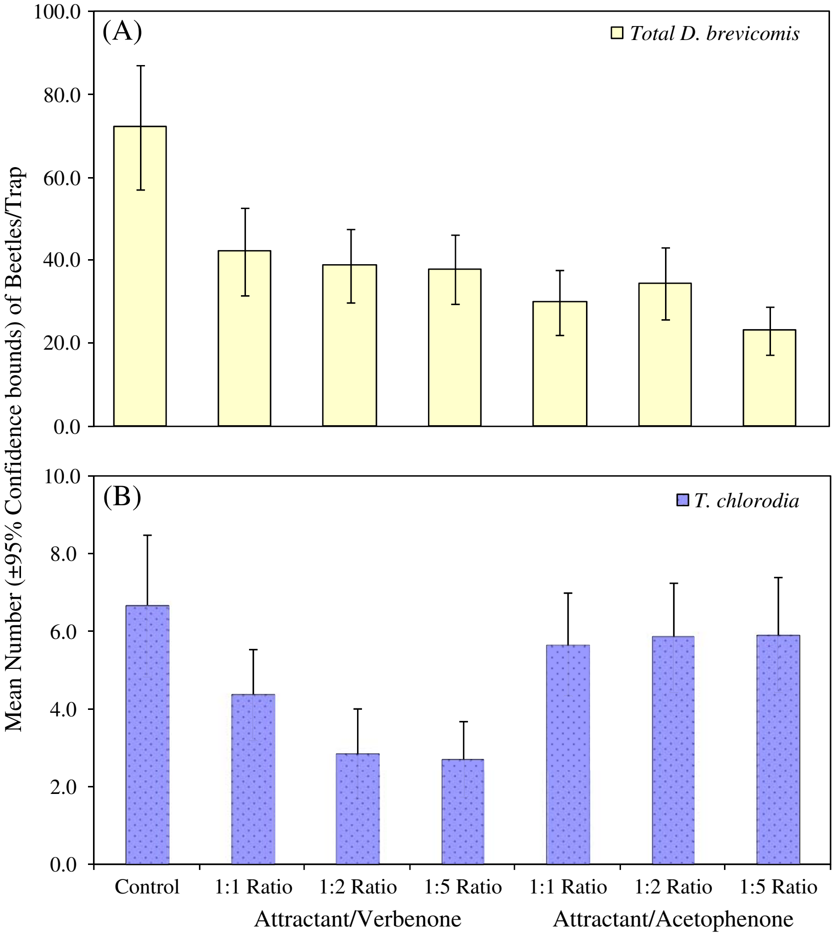

Figure 1 Field responses of *Dendroctonus brevicomis* and *Temnochila chlorodia* to flight intercept traps baited with aggregation pheromone of *D. brevicomis* alone or with the different release rates of verbenone or acetophenone in a ponderosa pine forest in Northern California. Data show mean numbers and their 95% confidence intervals for beetles per trap summed over the sampling period (seven sampling periods) for each treatment level from 17 May to 6 July 2007. There were a total of seven traps/treatment (49 counts/treatment). (A) Mean total numbers of *D. brevicomis*. (B) Mean numbers of *T. chlorodia*.

verbenone whereas the pooled verbenone treatments had only 0.6-fold as many *T. chlorodia* as the pooled acetophenone treatments. There was no significant difference among ratios within each anti-attractant compound (Table 3; Fig. 1B). The number of *C. angulicollis* responding to treatments did not vary among treatments (not shown).

The predator : prey ratio (*T. chlorodia* : *D. brevicomis*) also varied by the anti-attractant treatments (Table 3; Fig. 2). The ratio in traps containing the pooled acetophenone was 0.6-fold the ratio in attractant alone and it was two-fold greater than the ratio of the pooled verbenone treatments. Higher ratios of acetophenone treatments are a result of smaller *D. brevicomis* catches. There was no significant difference among ratios within each anti-attractant compound (Table 3; Fig. 2).

Discussion

The significant reduction in *D. brevicomis* catch and predator response to the simultaneous release of attractant and acetophenone are in agreement with an earlier study (Erbilgin *et al.*, 2007). Moreover, in the present study, we found that increasing the level of acetophenone, but not verbenone, provided even greater reduction in attraction of the aggregation pheromone for *D. brevicomis*, showing that increased release rates might provide enhanced efficacy for acetophenone in operational treatments. Conversely, the response of *T. chlorodia* to acetophenone treatments was constant across the range of release rates, whereas increasing the release rate of verbenone increased the inhibitory effect toward *T. chlorodia*. These two findings, when combined, suggest that higher application rates of acetophenone, unlike verbenone, may provide greater control of *D. brevicomis* without disrupting populations of its primary natural enemy; this finding may be important in implementing control strategies. Acetophenone also reduced attraction of the southern pine beetle *Dendroctonus frontalis* Zimmermann in southern U.S.A. (Sullivan, 2005), and the female Douglas-fir beetle *Dendroctonus pseudotsugae* Hopkins in British Columbia, Canada (Pureswaran & Borden, 2004) to their aggregation pheromones. On the other hand, Kohnle *et al.* (1987) found that acetophenone acted as a sex attractant for *Taphrorychus bicolor* (Coleoptera: Curculionidae: Scolytinae) and Conn *et al.* (1983) mention that acetophenone behaved as an attractant to *D. ponderosae* in laboratory assays. There remains the possibility that acetophenone may function as a multifunctional pheromone for species of *Dendroctonus*, and this further tests are warranted.

Table 3 Pairwise comparisons of attraction of *Temnochila chlorodia*, a predator of *Dendroctonus brevicomis* and predator/prey ratio among pooled and unpooled semiochemical treatments in a field experiment in a ponderosa pine forest in northern California

		Temnochila chlorodia		*Temnochila chlorodia*/ *Dendroctonus brevicomis* ratio	
Hypothesis tested[a]	Comparison ratio[b]	Estimated ratio of means	*P*-value	Estimated ratio of means	*P*-value
1	Attractant versus verbenone (pooled)	2.1	<0.001[c]	1.1	0.82
1	Attractant versus acetophenone (pooled)	1.1	0.4	0.6	0.014[c]
1	Verbenone versus acetophenone (pooled)	0.6	<0.001[c]	0.5	<0.001[c]
2	Verbenone dosages: 1 : 1 versus 1 : 2	1.5	0.16	1.4	0.26
2	Verbenone dosages: 1 : 1 versus 1 : 5	1.6	0.05	1.7	0.02
2	Verbenone dosages: 1 : 2 versus 1 : 5	1.0	0.89	1.2	0.56
3	Acetophenone dosages: 1 : 1 versus 1 : 2	1.0	0.86	1.0	0.99
3	Acetophenone dosages: 1 : 1 versus 1 : 5	1.0	0.79	0.8	0.35
3	Acetophenone dosages: 1 : 2 versus 1 : 5	1.0	0.95	0.8	0.38

[a] Hypotheses tested: (i) there is no difference between attractant, verbenone and acetophenone; (ii) there is no difference between the verbenone doses; and (iii) there is no difference between the acetophenone doses.

[b] Estimated ratio of means indicates the ratio of the mean of the first treatment (such as attractant) to the mean of the second treatment (such as verbenone). The highest ratio produces the largest significance.

[c] Wald chi-square test with Bonferroni adjustment for an experiment-wise error rate of 0.05; significant at $0.05/3 = 0.01667$ (comparisons made within each hypothesis).

In the present study, attractant : anti-attractant ratios higher than 1 : 1 were not tested, but Bertram and Paine (1994) reported that attraction of *D. brevicomis* to higher attractant : verbenone ratios was not significantly different from the beetle catch with attractant alone. They also found no difference in the ratios equal to or lower than 1 : 1. However, we demonstrated that increasing the release rate of acetophenone resulted in increased inhibition of *D. brevicomis*, although we did not test for a linear dose-response relationship *per se* because we tested a such small number of dosages. This result suggests that higher levels of inhibition of *D. brevicomis* could be achieved by increasing acetophenone dose and/or deploying multiple anti-attractants, such as acetophenone and verbenone together (Payne *et al.*, 1978; Bertram & Paine, 1994; Borden, 1996; Sullivan, 2005).

Although we do not know whether *D. brevicomis* produces acetophenone, it has been identified in volatiles from other scolytid species, female *D. pseudotsugae*, *D. frontalis*, *Dendroctonus rufipennis* (Kirby), *Dryocoetes confuses* Swaine, both males and females of *D. ponderosae*, and both male and female of *Ips pini* (Say) occurring in North America (Pureswaran *et al.*, 2000, 2004; Sullivan, 2005) and males of *Taphrorychus bicolor* in Europe (Kohnle *et al.*, 1987). If acetophenone is produced only by competitors of *D. brevicomis*, this olfactory cue could indicate unacceptable hosts and serve as an allomone (benefit the sender) for the first colonizer. Although aggregation pheromone-mediated inhibition of *D. brevicomis* by competing bark beetle species, including *Ips paraconfusus* or *D. ponderosae*, has been demonstrated (Byers & Wood, 1981; Paine & Hanlon, 1991;

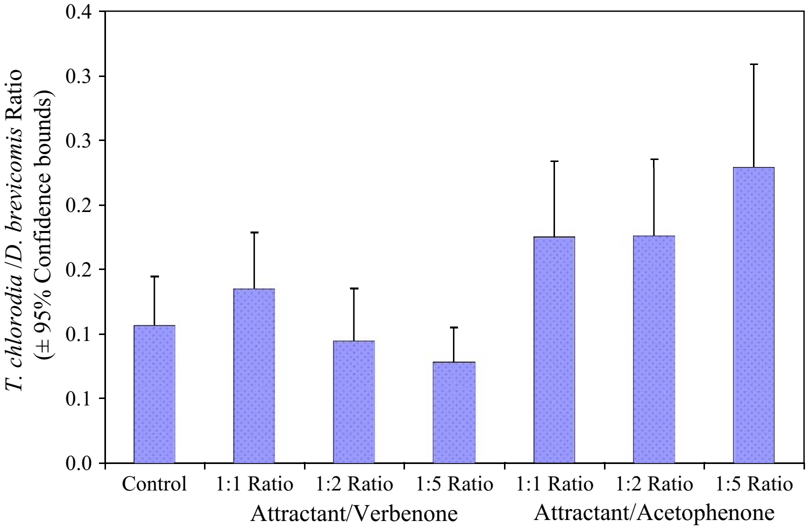

Figure 2 Estimated mean and 95% confidence intervals of *Temnochila chlorodia*/ *Dendroctonus brevicomis* ratio to flight intercept traps baited with aggregation pheromone of *D. brevicomis* alone or different release rates of acetophenone or verbenone in a ponderosa pine forest in Northern California. Data show mean numbers and their 95% confidence intervals for beetles per trap summed over the sampling period (seven sampling periods) for each treatment level from 17 May to 6 July 2007. There were a total of seven traps/ treatment (49 counts/treatment).

Bertram & Paine, 1994), the reduction in *D. brevicomis* attraction by anti-attractant compounds, such as acetophenone, may be another mechanism to reduce competition among colonizing beetles (Byers & Wood, 1980, 1981; Rankin & Borden, 1991; Safranyik *et al.*, 1996; Poland & Borden, 1998).

Alternatively, if acetophenone is produced by *D. brevicomis* (as is verbenone), then the anti-aggregation of *D. brevicomis* may be regulated by multi-component chemicals to maximize individual fitness (Byers *et al.*, 1984; Schlyter *et al.*, 1987, 1989), as suggested for *D. ponderosae* (Pureswaran *et al.*, 2000) and for *D. frontalis* (Salom *et al.*, 1992; Sullivan, 2005). For example, some studies speculated that verbenone acts as a short-range density regulator during host colonization (Byers & Wood, 1980; Byers *et al.*, 1984; Smith *et al.*, 1988; McPheron *et al.*, 1997; Miller, 2002). If the release of higher amounts of acetophenone relative to the aggregation pheromone conveys a risk of reduced fitness resulting from intraspecific competition, then we expect that the population of aggregating beetles will be reduced. This outcome would suggest that *D. brevicomis* response to behavioural chemicals is influenced by both the absolute and relative concentrations of pheromone component and anti-attractants (Bedard *et al.*, 1980a, b; Tilden *et al.*, 1981, 1983; Borden *et al.*, 1986; Bertram & Paine, 1994).

By contrast to acetophenone, verbenone reduced the response of *T. chlorodia* to the aggregation pheromone of *D. brevicomis*. It is not known how single-component versus multiple-component anti-attractants actually affect predator-prey relationships in nature; however multi-component blends clearly increase the potential for more complex trajectories of prey populations, leading to potentially quite different selection environments for both predators and prey. Prey survival is expected to be greatest in cases where host colonization mechanisms ensure limited contact by, or escape from, multiple species of predators aggregating on the host tree with the prey (Wood, 1982). Alternatively, predators can develop counter-adaptations by either responding differentially to the various chemicals produced by beetles, such as acetophenone versus verbenone, and/or by utilizing host tree chemicals (Erbilgin & Raffa, 2001).

The results obtained in the present study suggest that acetophenone may be useful as an anti-attractant to protect trees from attack by *D. brevicomis* for several reasons. First, the greater anti-attractant activity of acetophenone compared with verbenone was demonstrated in the present study and, thus, future studies should focus on increasing acetophenone dose, deploying multiple anti-attractants together, and/or improving both formulations and active ingredient blends to effectively protect pine stands (Payne *et al.*, 1978; Bertram & Paine, 1994; Borden, 1996; Sullivan, 2005). Second, unlike verbenone, acetophenone did not reduce attraction of *T. chlorodia*, and the highest predator/prey ratio was achieved by the release of acetophenone. This may provide better pest management strategies that utilize semiochemicals against *D. brevicomis*. Third, acetophenone is approximately one-tenth as expensive as verbenone (Sigma-Aldrich Chemical Co., St Louis, Missouri), which enhances the prospect of making repeated applications over large forested areas.

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