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Response to Host Volatiles by Native and Introduced Populations of *Dendroctonus valens* (Coleoptera: Curculionidae, Scolytinae) in North America and China

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Abstract Bark beetles (Coleoptera: Curculionidae, Scolytinae) have specialized feeding habits, and commonly colonize only one or a few closely related host genera in their geographical ranges. The red turpentine beetle, *Dendroctonus valens* LeConte, has a broad geographic distribution in North America and exploits volatile cues from a wide variety of pines in selecting hosts. Semiochemicals have been investigated for *D. valens* in North America and in its introduced range in China, yielding apparent regional differences in response to various host volatiles. Testing volatiles as attractants for *D. valens* in its native and introduced ranges provides an opportunity to determine whether geographic separation

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promotes local adaptation to host compounds and to explore potential behavioral divergence in native and introduced regions. Furthermore, understanding the chemical ecology of host selection facilitates development of semiochemicals for monitoring and controlling bark beetles, especially during the process of expansion into new geographic ranges. We investigated the responses of *D. valens* to various monoterpenes across a wide range of sites across North America and one site in China, and used the resulting information to develop an optimal lure for monitoring populations of *D. valens* throughout its Holarctic range. Semiochemicals were selected based on previous work with *D. valens*: (*R*)-(+)-α-pinene, (*S*)-(−)-α-pinene, (*S*)-(−)-β-pinene, (*S*)-(+)-3-carene, a commercially available lure [1:1:1 ratio of (*R*)-(+)-α-pinene:(*S*)-(−)-β-pinene:(*S*)-(+)-3-carene], and a blank control. At the release rates used, (+)-3-carene was the most attractive monoterpene tested throughout the native range in North America and introduced range in China, confirming results from Chinese studies. In addition to reporting a more effective lure for *D. valens*, we present a straightforward statistical procedure for analysis of insect trap count data yielding cells with zero counts, an outcome that is common but makes the estimation of the variance with a Generalized Linear Model unreliable because of the variability/mean count dependency.

Keywords Bark beetles · Red turpentine beetle · Host attraction · Monoterpenes · Host–insect interactions · *Pinus* spp. · Invasive species · Upper confidence bound · Zero counts

Introduction

Olfactory cues play a crucial role in host location by insects, often mediating intra- and interspecific interactions among insects (Visser 1986; Bernays and Graham, 1988). Interactions between bark beetles (Coleoptera: Curculionidae, Scolytinae) and their conifer hosts are of interest because many species colonize and kill apparently healthy trees, resulting in significant economic losses (Wood 1982b). Many species have specialized feeding habits and attack one or a few closely related host genera (Sturgeon and Mitton, 1982; Kelley and Farrell, 1998). Volatiles released from trees can provide long- and/or short-range cues for beetle attraction and, in some cases, synergize beetle aggregation pheromones (Wood 1982a; Byers 1995; Miller and Borden, 2000; Erbilgin and Raffa, 2000).

The role of monoterpenes in the ecology of bark beetles is of interest because some, such as α-pinene and myrcene, are precursors of the aggregation pheromone components of several bark beetle species (Hughes 1974; Renwick et al., 1976; Hendry et al., 1980; Byers 1989). Other studies have demonstrated *de novo* synthesis of pheromones in some species (Seybold et al., 1995b; Ivarsson et al., 1997).

The red turpentine beetle, *Dendroctonus valens* LeConte, is widely distributed in North America, extending from Nova Scotia (Canada) west to California (USA) and south to Honduras, except for the southeastern United States where its sibling species, *D. terebrans* (Oliv.), occurs (Wood 1982b). Although it is a relatively minor pest within its native range, *D. valens* occasionally kills young ponderosa pines in plantations in the western USA (Eaton and Rodríguez Lara, 1967; Rappaport et al., 2001) and may predispose trees to subsequent lethal attacks by other species (Klepzig et al., 1996; Owen et al., 2005). Drought-stressed trees appear to be more susceptible to attack by *D. valens* (Smith 1961), and there is concern that global climate change may exacerbate damage caused by this insect. Larvae feed and develop in the phloem of host trees, and galleries often overlap spatially and temporally with larvae of other members of the subcortical-feeding guild of

insects (Wood 1982b; Erbilgin and Raffa, 2002). *D. valens* was introduced into China around 1985, and has caused extensive mortality of *Pinus tabuliformis* Carrière and other pine species in north central China (Li et al., 2001). Although *D. valens* has widely been considered a secondary pest in North America (Furniss and Carolin, 1977), it has been shown to act as a primary tree-killer in China. The reasons underlying the greater tree mortality in China are not known, but could include a lack of evolutionary adaptation with this new insect/host association (Sakai et al., 2001) and/or differences in host physiology, stand structure, or climate. Considering its wide host range and geographic distribution in North America and the abundance of suitable pine hosts throughout Eurasia (Critchfield and Little, 1966), the host range and distribution of *D. valens* in the Palearctic region are expected to increase.

D. valens exploits volatile pine monoterpenes as kairomones to locate host trees, and several monoterpenes have been tested for attraction of *D. valens* in both North America and China, revealing different responses in populations from different regions. Hobson et al. (1993) reported that traps baited with (+)-α-pinene, (+)-3-carene, and (−)-β-pinene were the most attractive monoterpenes tested in California, with (−)-β-pinene by far the most attractive for *D. valens*. They showed a positive dosage–response relationship in tests of (−)-β-pinene at high release rates. Fettig et al. (2004) showed positive response by *D. valens* to the standard three-component commercial lure consisting of a 1:1:1 ratio of (*R*)-(+)-α-pinene:(*S*)-(−)-β-pinene:(*S*)-(+)-3-carene (Phero Tech, Inc., Delta, BC, Canada) in California populations, but they did not test individual monoterpenes for relative attraction. Erbilgin and Raffa (2000) showed attraction of *D. valens* to (+)-3-carene and both enantiomers of α-pinene in Wisconsin. In China, (+)-3-carene was the most attractive monoterpene for *D. valens*, with beetles showing a dose-dependent increase in attraction to (+)-3-carene, but not to (−)-β-pinene (Sun et al., 2004).

These differences were puzzling, but it was not possible to make reasonable inferences about geographic variation in response because test conditions, notably release rates, varied widely among these earlier studies. Studies conducted in California, for example, used release rates several hundreds of times higher than those in China (Hobson et al., 1993; Sun et al., 2004). Other semiochemicals cause dose-dependent differences in insect behavior (Miller and Borden, 2000), so it is reasonable to question whether the behavioral differences reported in earlier studies resulted from differences in release rate rather than divergence in behavioral response of the Chinese populations from the indigenous North American populations. On the other hand, other widespread insect species use semiochemical "dialects" in disparate parts of their ranges (Wood 1982a; Seybold et al., 1995a; Erbilgin et al., 2001; Miller et al., 1997), so we could not rule out geographic variation in behavioral response to host monoterpenes by *D. valens*, especially considering the localized source of populations introduced into China from the Pacific Northwestern United States (Cognato et al., 2005). These apparent differences prompted us to conduct a systematic evaluation of *D. valens* response to host monoterpenes throughout its native range in North America and its introduced range in China, by using standardized (i.e., with equivalent release rate) lures containing individual host monoterpenes. These were compared with a three-component commercial lure used widely in North America.

Testing host volatiles as attractants for species with wide host and geographical ranges provides us with an opportunity to compare semiochemical-mediated host selection behavior in native and introduced ranges. Furthermore, understanding the ecological aspects of host selection is important for developing semiochemicals to monitor beetles (Wood 1982a; Schlyter and Birgersson, 1999). Our objective was to investigate the responses of *D. valens* to various monoterpenes across a wide range of sites across North

America and China and to assess the potential behavioral divergence of the populations. Our practical goal was to develop optimal blends or single-component lures, appropriate for each locality, for monitoring populations of *D. valens* throughout its Holarctic range.

The project, involving nine separate trapping studies, also provided an opportunity to describe an approach for estimating treatment differences in data sets with many "zero count" cells. Data sets with zero count cells have long been a problematic area for entomological researchers (Reeve and Strom, 2004) because it is not possible to estimate the standard errors of cells with no observations. We can, however, calculate theoretical confidence intervals for the expected means for these cells, allowing Bonferroni-adjusted comparisons (Miller 1991) between means from zero count cells and means from cells with nonzero observations. This problem has been addressed in the past by either recommending exclusion of zero-count cells from the analysis, adding a small constant to each count, or creating a "dummy variable" with a low mean and variance (Agresti 1996; Reeve and Strom, 2004). In contrast, our approach was to calculate Bonferroni-adjusted confidence intervals for means with zero observations to permit comparison with nonzero cells with a given level of certainty, thus using real data rather than "dummy" variables.

Methods and Materials

Study Sites The experiments were conducted in 2003, 2004, and 2005 at 6 sites spanning the range of *D. valens* in North America and at one site in China. These were in the following forests: a *P. resinosa* Ait. stand in Jackson Co. (Central WI, USA); an east side *P. ponderosae* Laws plantation in Siskiyou Co. (Northern CA, USA); a Sierra mixed conifer forest where *D. valens* colonizes *P. lambertiana* Dougl and *P. ponderosa*, in Blodgett Forest Research Station (Central CA, USA); a mixed conifer forest where *D. valens* colonizes *P. jeffreyi* Grev. & Balf. and *P. coulteri* D. Don, in the Laguna Mountains in San Diego Co. (Southern CA, USA); a mixed hardwood and conifer forest where *D. valens* colonizes *P. strobus* L. and *P. resinosa* at Raccoon Creek State Park in Beaver Co. (Western PA, USA), a natural *P. leiophylla* Schl. et Cham. stand in San Francisco Zentlalpan (Estado de México, México), and a *P. tabuliformis* plantation in Shanxi Province, China.

Experimental Design We selected semiochemicals based on previous work with *D. valens* (Hobson et al., 1993; Erbilgin and Raffa, 2000; Sun et al., 2004). Four single-monoterpene baits [(*R*)-(+)-α-pinene, (*S*)-(−)-α-pinene, (*S*)-(−)-β-pinene, and (*S*)-(+)-3-carene], the standard commercial red turpentine beetle lure, hereafter referred to as the "RTB lure," which consists of a 1:1:1 ratio of [(*R*)-(+)-α-pinene:(*S*)-(−)-β-pinene:(*S*)-(+)-3-carene; Phero Tech Inc.], and a blank control were replicated at each site (Table 1). Trapped beetles were collected periodically, but the number of collections ("sampling periods" in Table 1) varied from site to site for reasons of local logistics. The number of replicates at each site was constant throughout the entire sampling period for each study. All of the analytical models accommodated these repeated measures, and in cases where the number of sampling periods was small (e.g., Pennsylvania), the number of replicates was increased to reduce the effect of overdispersion of counts.

Chemicals (all with purity >95%) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and from Phero Tech. Single-monoterpene release devices consisted of 15-ml polyethylene bottles (Phero Tech), each filled with 8 ml of the monoterpene. The high cost of enantiopure monoterpenes prohibited the use of the full measure of 15 ml per vial. To

Table 1 Site locations and sampling design for testing attraction of *Dendroctonus valens* to pine monoterpenes

Site	Number of Replications	No Sampling Periods
Northern California	5	5
Central California	7	6
Southern California		
2003	5	2
2004	5	5
Wisconsin	6	3
Pennsylvania	10	2
Mexico	8 (4 for control)	1
China		
2003	5	7
2004	10	19

ensure that the entire contents of each vial were exposed to the release surface, the bottles were squeezed until the liquid reached the shoulder, then were tightly capped leaving the bottles slightly collapsed. Each bottle also contained 100 mg BHT (butylhydroxytoluene) (Sigma-Aldrich) as an antioxidant. Each chemical maintained a sustained release of 103.4± 11.3 mg/d at 20±4.3°C with 6.2±2.3 km/hr wind velocity for at least 30 d in the field. Release rates of chemicals (determined by daily weight loss) under the same conditions did not vary significantly ($F_{4,3}$=1.23, P=0.45). The RTB lure contains 15 ml of the three-component blend (5 ml of each of the three monoterpenes) mixed with undefined stabilizers and antioxidants, and it releases at a slightly higher rate, 110 mg/d, under similar environmental conditions (personal communication, B. Thompson, Phero Tech). We concluded that this slight difference in release rate would have no significant effect on our overall inferences, especially considering the ambient release of monoterpenes from trees in a typical forest stand (Wilt et al., 1993; Schade and Goldstein, 2003).

Lures were attached to multiple-funnel traps (Phero Tech) or panel traps (Advanced Pheromone Technologies, Marylhurst, OR, USA) designed for trapping bark beetles. Propylene glycol antifreeze or a plastic insecticidal strip saturated with 2,2-dichlorovinyl dimethyl phosphate (Hercon Vaportape II Insecticidal strips; Hercon Environmental Co., Emigsville, PA, USA) was put in each collection cup to prevent predation. The experimental design was a completely randomized design with repeated measurements, with the exception of the Wisconsin site, which was a randomized complete block design. For some sites, traps were rerandomized at sampling time, and for other sites the trap was left at the same location for the entire time of the experiment. Statistical models for analysis were designed to account for these minor differences in design. The numbers of replications and sampling periods per treatment are shown in Table 1. 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), Universidad Autónoma Chapingo (Texcoco, México), or Chinese Academy of Sciences (Beijing, China) according to site.

Statistical Analyses Trap counts 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). The overdispersion error was assumed to be normally distributed for some sites according to sampling design.

The Maximum Likelihood Ratio test with the Bonferroni adjustment was used for multiple comparisons for an experimentwise error rate of 0.05. The SAS (2005) NLMIXED procedure was used to estimate parameters and their differences. Mean counts for treatments with nonzero observations were estimated the using the computing routines described above. For the treatments with zero observations, the mean counts were estimated as zero, and precision was estimated by a Bonferroni-adjusted confidence interval from 0.0% to 99.7% $(100 \times [1 - 0.05/\{15 = \text{number of comparisons}\}])$, where the upper confidence bound was calculated by using rules for multiplication and addition of probabilities (Hoel et al., 1971). We thereby created a way to determine, with a given level of certainty, the range of the treatment means with zero observations. We compared nonzero count treatment means by using the standard method of testing significance, but for zero-count treatment means we used the overlap between the calculated Bonferroni-adjusted confidence intervals for zero counts and the Bonferroni-adjusted confidence intervals of the nonzero treatment means. Even though this approach is occasionally conservative, it yields a more sensitive test of treatment differences. In general, it works best if the lower confidence bounds of the nonzero count treatment levels are not close to zero.

Procedure for Calculating the Upper Bound for Treatments with Observed Zero Counts Upper bounds are developed as follows. We present a $1 - \alpha$ upper bound for three count distributions: the Poisson distribution and two overdispersed Poisson distributions (Poisson-Gamma = Negative Binomial and Poisson-Normal).

Let α be the given level; therefore, a $1 - \alpha$ upper bound for expected count for a treatment is given by:

$$P(x > 0) = 1 - [P(x = 0)]^{n_{\text{reps}}} \leq 1 - \alpha,$$

where n_{reps} is the number of replications.

(A) Suppose x has the Poisson distribution with mean λ_T (not overdispersed) for a given treatment level T, then

$$P(x > 0) = 1 - \left[e^{-\lambda_T}\right]^{n_{\text{reps}}} \leq 1 - \alpha.$$

Therefore, the upper bound for expected mean count is given by

$$\lambda_T \leq -\frac{1}{n_{\text{reps}}} \log(\alpha), \tag{1}$$

where log indicates logarithm.

The next two techniques [Eqs. (2) and (3)], which assume the Poisson-Gamma or the Poisson-Normal distribution, can be problematic to calculate if the software is not available. If so, then a close approximation of the upper bound can be made by using just the Poisson distribution [Eq. (1)], since the α level and number of replicates are the only pieces of information required for that calculation.

(B) Suppose x has an overdispersed Poisson distribution with mean λ_T, and with a Gamma distributed overdispersion error (McCulloch and Searle, 2001). Therefore, x has the Negative Binomial distribution (k). In this case,

$$P(x > 0) = 1 - \left[(1 - k\lambda_T)^{-\frac{1}{k}}\right]^{n_{\text{reps}}} \leq 1 - \alpha,$$

and the upper bound is given by

$$\lambda_T \leq \frac{\alpha^{\frac{-k}{n_{\text{reps}}}} - 1}{k} \tag{2}$$

The parameter k can be estimated by SAS NLMixed or Glimmix procedures.

(C) Suppose, for a given treatment, x has an overdispersed Poisson distribution $P(\lambda_T)$ with mean $\lambda_T = e^{T+\sigma\varepsilon}$, and a normally distributed overdispersion error ε (McCulloch and Searle, 2001.). If ε is $N(0,1)$ (Standard Normal) distributed, and σ is the standard deviation of the overdispersion error, then

$$P(x>0) = 1 - \left[\int_{-\infty}^{+\infty} \frac{e^{-e^{T+\sigma\varepsilon}} e^{-\frac{\varepsilon^2}{2}}}{\sqrt{2\pi}} d\varepsilon\right]^{n_{\text{reps}}} \leq 1-\alpha,$$

where $\int$ is the integration symbol. In this case, the upper bound does not have a closed formula. However, it can be approximated by numerically calculating this probability for several values of T for the following function:

$$f(T) = \left[\int_{-\infty}^{+\infty} \frac{e^{-e^{T+\sigma\varepsilon}} e^{-\frac{\varepsilon^2}{2}}}{\sqrt{2\pi}} d\varepsilon\right]^{n_{\text{reps}}} \tag{3}$$

The numerical solution of the integral can be done with Mathematica (Wolfram 1999). Fig. 1 depicts the function $f(T)$ for possible upper bound values for several values of T. The value of T for a given $f(T)=0.05/15=0.0033$ is equal to 1.098 (the upper 99.7% confidence limit). Fig. 1 diagrams the method for the Central California site; the upper bound for the treatment levels with zero counts for this site can be compared with the lower bounds of other treatments at the same α level ($\alpha=0.0033$).

Panels A–D, F, and G of Fig. 2, each of which contains at least one zero-count cell, all illustrate this approach for making multiple comparisons with treatments with zero counts (the upper Bonferroni-adjusted confidence limit for multiple comparisons is indicated by a dotted line on those figures). The calculated upper bound for the treatment levels with zero counts can be compared to the lower bounds of other treatments at the same α level ($\alpha=$

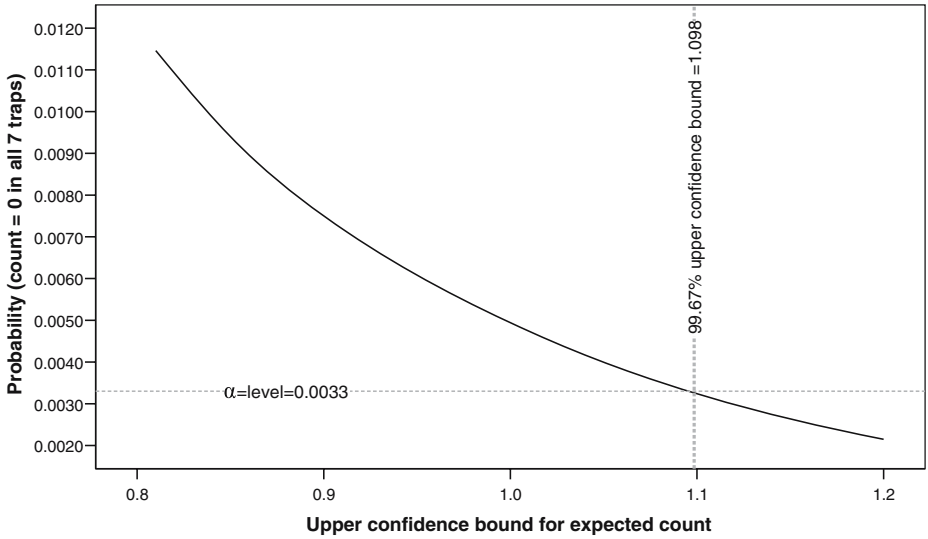
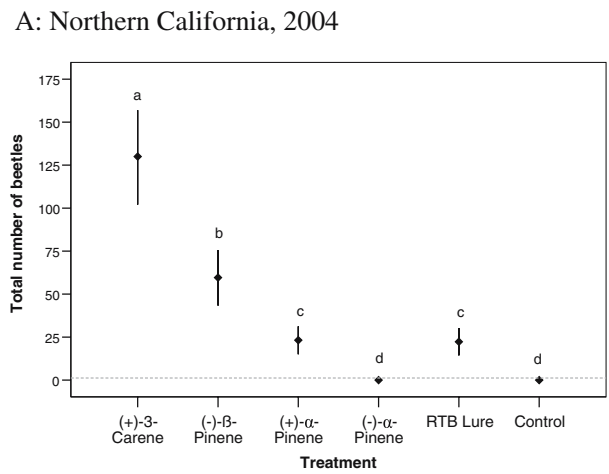
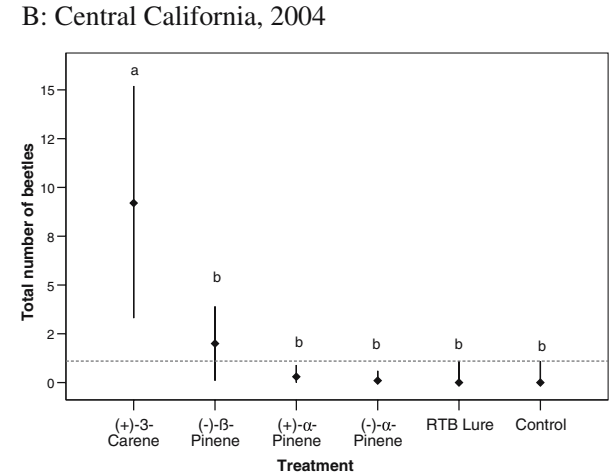


Fig. 1 Calculated upper Bonferonni-adjusted confidence limit for cells with zero counts (example using results from central California, 2004)





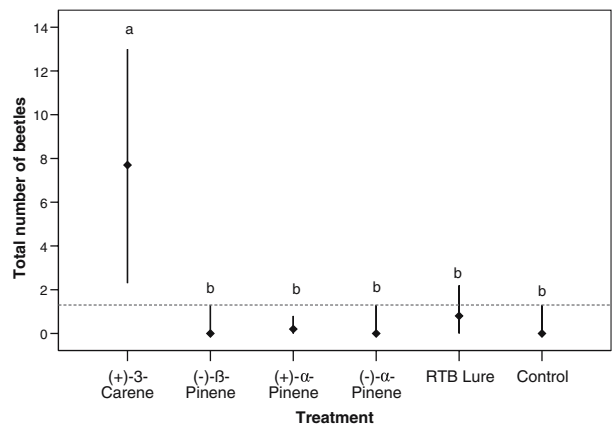
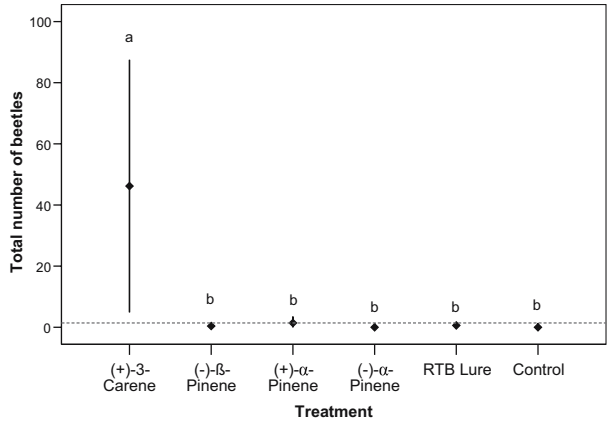


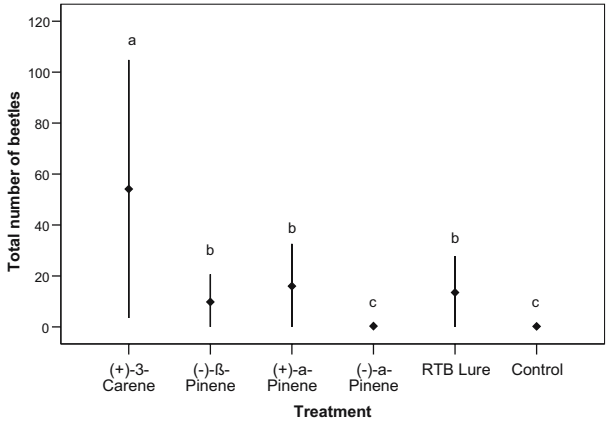
Fig. 2 (A-I) *Dendroctonus valens* response to pine monoterpenes at nine different localities and/or years in North America and China. Dates (location codes) for experiments: May 22–June 10 for Northern California (CA North); September 21–October 10 for Central California (CA Central); August 28–September 6 and May 20–August 26 for Southern California (CA South); May 3–May 18 for Wisconsin (WI); May 2–May 15 for Pennsylvania (PA); June 27 for Mexico (MX); May 2–June 20 for China 2003 (CH 2003) and May 4–August 2 for China 2004 (CH 2004). Data show mean numbers (lower=99.7% lower confidence bound, upper=99.7% upper confidence bound) of insects per trap summed over sampling period. Means followed by the same letter in a column are not significantly different from each other. Dotted lines indicate 99.7% calculated upper confidence bound for treatments with zero observations

Fig. 2 (Continued)

D: Southern California, 2004



E: Wisconsin, 2004



F: Pennsylvania, 2004

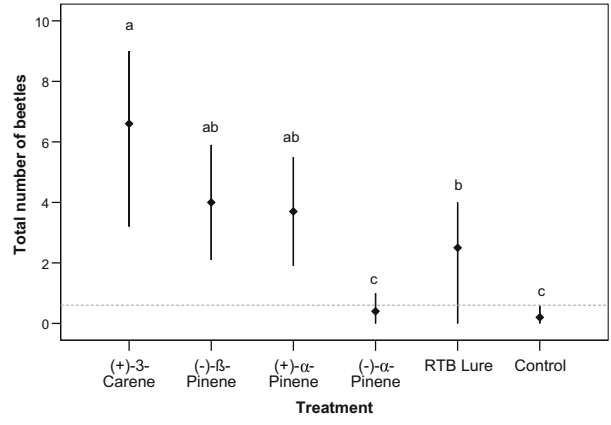
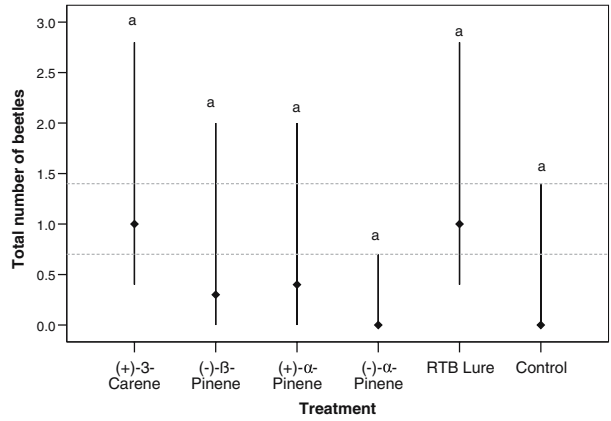
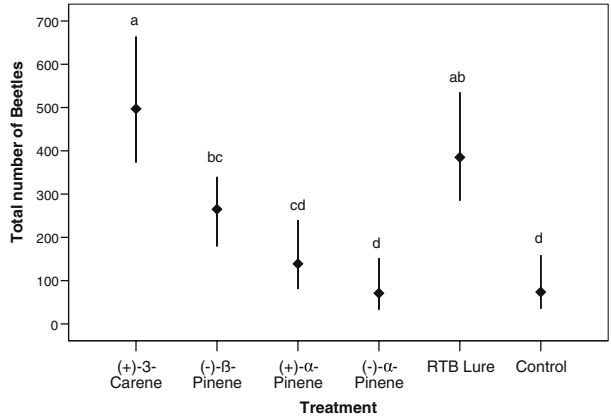


Fig. 2 (Continued)

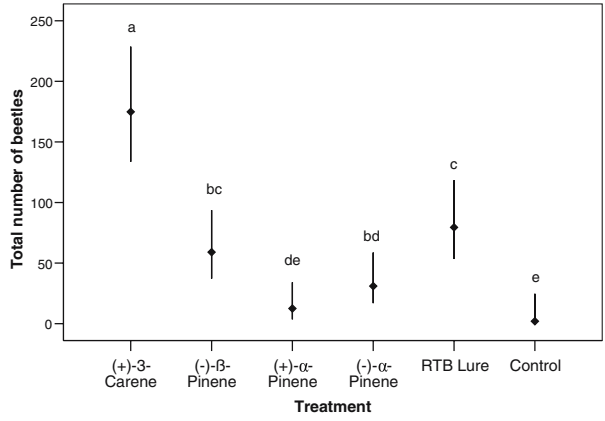
G: Mexico, 2005



H: China, 2003



I: China, 2004



0.0033). If they do not touch, then the treatment levels are significantly different. If they overlap, then there is not enough evidence to reject the null hypothesis. Mexico has two calculated upper bounds because each treatment level had eight replications except for the control, which had only four (and thus a greater upper bound). Fig. 2E, H, and I, which do not contain zero count cells, are handled in the traditional manner.

Results

Trap counts were estimated as mean numbers of beetles per trap summed over the sampling intervals (Table 1, Fig. 2A–I). The mean counts of beetles summed over sampling intervals are not comparable among sites because the sites had different sampling time ranges, from 1 wk for Mexico to nearly 3 mo for China and southern California in 2004 (Table 1).

California In northern California (Fig. 2A), (+)-3-carene was the most attractive compound tested, followed by (−)-β-pinene. The number of *D. valens* trapped was not different between (+)-α-pinene and the RTB lure, nor between (−)-α-pinene and the control. In central California (Fig. 2B), the numbers of *D. valens* were relatively lower than those in northern California, but the pattern of attraction was similar; only (+)-3-carene attracted significantly more *D. valens* than the control. In southern California (Fig. 2C and D), the results were identical to those of central California. In two consecutive years, only (+)-3-carene attracted a significantly higher number of *D. valens* than the control.

Wisconsin (+)-3-Carene was significantly more attractive than the remaining treatments, whereas (+)-α-pinene, (−)-β-pinene, and the RTB lure were more attractive than (−)-α-pinene and the control (Fig. 2E). There were no significant differences between mean numbers of *D. valens* caught in traps baited with (−)-α-pinene and the control, nor between (+)-α-pinene, (−)-β-pinene, and the RTB lure.

Pennsylvania (+)-3-Carene was the most attractive compound tested and was significantly different from the control, the (−)-α-pinene, and the RTB lures (Fig. 2F). Attraction of (−)-β-pinene and (+)-α-pinene were not different, and both were greater than the attraction of either (−)-α-pinene or the control.

Mexico None of the treatments in Mexico were significantly different from one another (Fig. 2G). However, there was only one sampling interval, and the number of beetles was low, with a median close to zero for all treatments except for (+)-3-carene and the RTB lure.

China (+)-3-Carene was the most attractive lure in both 2003 (Fig. 2H) and 2004 (Fig. 2I), but only in 2004 was it significantly more attractive than the RTB lure, which was the second most attractive lure in both years of testing. The mean number of *D. valens* caught by the RTB lure was not significantly different from that caught by (−)-β-pinene in both 2003 and 2004. The mean number of *D. valens* caught in (−)-β-pinene traps was significantly higher than for the control. (+)-α-Pinene, (−)-α-pinene, and the control were not significantly different from one another in 2003. Results for 2004 were identical except that (−)-α-pinene caught more insects than the control.

General Observations With the exception of the tests conducted in Wisconsin (Fig. 2E) and China (Fig. 2H and I), where beetle populations were among the highest, the total number of beetles trapped in unbaited traps was zero. There were significant differences among treatments in the mean numbers of *D. valens* caught in all sites and all years except for Mexico (2005), where beetle numbers were low and there was only one sampling interval (Fig. 2G). (+)-3-Carene was the most attractive treatment at all sites, and the difference between (+)-3-carene and the second most attractive treatment was significant in most cases, i.e., northern California (Fig. 2A), central California (Fig. 2B), southern California (both years) (Fig. 2C and D), Wisconsin (Fig. 2E), and China (both years) (Fig. 2H and I). In Pennsylvania (Fig. 2F), (+)-3-carene attracted 65% more beetles than did (−)-β-pinene, but this difference was not significant. In Mexico (Fig. 2G), (+)-3-carene tied with the RTB lure for most attractive treatment. The RTB lure, which also contains (+)-3-carene as a component, was generally the second most attractive lure, although in most cases it was not significantly more attractive than the third-ranking treatment. In three cases, however (northern California, central California, and Pennsylvania), (−)-β-pinene was the second most attractive compound tested, but only in northern California was this difference significant. In Wisconsin, (+)-α-pinene was the second most attractive treatment but it was not significantly different from (−)-β-pinene or the RTB lure. (−)-α-Pinene was not significantly different from the control in any case except for China in 2004.

Discussion

(+)-3-Carene was the most attractive monoterpene tested throughout the native range of *D. valens* and in China. These results confirm studies conducted recently in China, where (+)-3-carene has been shown to be the dominant attractant among pine host monoterpenes (Sun et al., 2004; J.H. Sun, personal communication). (+)-3-Carene is, thus, broadly applicable for monitoring *D. valens* in both China and North America. This uniform preference for the same signal over such a broad geographical range differs from responses by certain other bark beetle species to their pheromones, which vary geographically (Wood 1982a; Seybold et al., 1995a; Miller et al., 1997).

The practical, pest management implication of this finding is clear: a single-component lure will likely be the most cost-effective and biologically efficient one for *D. valens* across its range in the Holarctic, whether in its native range in North America or in its introduced range in Asia. Preferential attraction of *D. valens* to (+)-3-carene suggests that it is the key stimulus from its host trees, and plays role in host selection. Differences between this and earlier studies (Hobson et al., 1993; Erbilgin and Raffa, 2000) may result from differences in release rates of the compounds. For example, in the work of Hobson et al. (1993), where (+)-3-carene was less attractive than other monoterpenes, release rates were more than 200 times those used in this study. In other words, (+)-3-carene may become repellent or inhibitory at higher release rates relative to the other monoterpenes studied.

(+)-3-Carene constitutes varying proportions of the resins of *Pinus* species and other coniferous genera in North America (Mirov 1961; Hobson et al., 1993; Smith 2000; Erbilgin et al., 2001), but it is generally the predominant monoterpene in its most widely distributed host in North America, ponderosa pine (Smith 2000). *P. tabuliformis*, the primary host of *D. valens* in China, has a needle monoterpene composition that is similarly high in (+)-3-carene (Jin et al., 1994). Several other forest Coleoptera respond to 3-carene as an attractant or synergist. For example, the pine shoot beetle, *Tomicus piniperda*, utilizes

(+)-3-carene as a kairomone along with (±)-α-pinene and terpinolene to locate and assess the susceptibility of its host, *P. sylvestris* L. (Byers et al., 1985; Lanne et al., 1987; Byers 1992). Likewise, *Pityogenes chalcographus* (L.) utilizes α-pinene, β-pinene, and (+)-3-carene to locate and colonize hosts (Byers et al., 1988). In another study, (+)-3-carene synergized the attraction of *Conophthorus ponderosae* Hopkins to its sex pheromone in a ponderosa pine seed orchard in northern California (Gillette et al., unpublished data). (+)-3-Carene also influenced the attraction of bark beetles and their predators to bark beetle pheromones (Miller and Borden, 2003).

Although host monoterpenes are crucial in the *D. valens* host selection process, it is difficult to relate levels of emission from our lures to natural emissions from forest stands. For example, monoterpene concentrations in pines vary in response to drought (Lorio et al., 1995), fungal infection of roots (Klepzig et al., 1995), and defoliation (Wallin and Raffa, 1999). Emissions from foliage vary depending on state of decomposition (Wilt et al., 1993). Furthermore, boring activities initiated by beetles rapidly alter monoterpene concentrations and release rates of attacked trees (Paine and Stephen, 1987; Cook and Hain, 1988; Raffa and Smalley, 1995). On a broader scale, estimations of volatile emissions from forests are more difficult to make. For example, natural emission of α-pinene from ponderosa pine plantations at the Blodgett Forest Research Station is about 0.1 $mg/m^2/hr$ during the daytime in summer, and it changes as an exponential function of temperature (Schade and Goldstein, 2003). Monoterpene emissions from the same pine forest were enhanced about 40 times during a mechanical thinning operation. Previous reports indicate that bark beetle populations tend to increase following fire and tree thinning (Furniss and Carolin, 1977), and higher emissions of monoterpenes may explain such increases in populations of *D. valens* and other bark beetle species.

The broad host and geographical range (Kelley and Farrell, 1998), highly specialized olfactory responses (White and Hobson, 1993), and cryptic nature (Bernays and Graham, 1988) of *D. valens* may all have contributed to its successful establishment in China. Other factors, such as host susceptibility and a lack of competition and natural enemies (Sakai et al., 2001), undoubtedly also played a role. Although many species of *Dendroctonus* are restricted to one of four confamilial genera of conifers (Sturgeon and Mitton, 1982), *D. valens* can colonize almost three times as many hosts as its congeners (Kelley and Farrell, 1998), suggesting that it may have greater intrinsic capacity for adaptation to new hosts. Recently, Cognato et al. (2005) reported significant haplotype variation in *D. valens* in its native and introduced ranges. We initially expected that behavioral differences seen in earlier studies might be explained by genetic variability, but this variation was not reflected in behavioral differences in responses to host compounds.

The recent introduction of *D. valens* to China and its rapid establishment on pines indigenous to Asia demonstrate that *D. valens* can colonize and reproduce on new host species within the same genus as its ancestral hosts in North America. This new association may have resulted from adaptation to diverse secondary chemicals during colonization of ancestral hosts (Byers 1995; Kelley and Farrell, 1998), and/or close association with particular host volatiles that result in gaining access to chemically similar host species in new biogeographic areas (Futuyma et al., 1995; Becerra 1997). Recognition of common host volatiles, e.g., (+)-3-carene, may be the first phase of colonization of new host species by *D. valens*, since the dominant volatiles of its hosts are mainly monoterpenoids (Mirov 1961; Juuti et al., 1990; Smith 2000). However, the presence of the same attractive compounds in nonhost trees (Hobson et al., 1993) or the presence of inhibitory nonhost, e.g., green leaf, volatiles (Zhang and Schlyter, 2004) in the same forest suggests that host

location by this beetle species must rely on multiple factors, such as the relative and absolute release rates of host and nonhost compounds, timing of response elicitation, and other life history aspects. Indeed, Pureswaran et al. (2004a, b) presented extensive evidence for several beetle/host combinations supporting the hypothesis that scolytid beetles utilize quantitative, as well as qualitative, differences in host odor blends in the process of host selection. Such a strategy would optimize host selection and minimize the risks associated with response to nonhost cues in the process of host location. In China, *D. valens* is faced with many new pine hosts that provide a continuous corridor for its spread from its region of introduction in northeastern China across Eurasia to Europe (Critchfield and Little, 1966). For example, *D. valens* colonizes *P. sylvestris* planted in North America (Wood 1982b); thus, it could be predicted that *D. valens* will infest naturally occurring *P. sylvestris* throughout Eurasia. Effective semiochemical lures are of increasing importance as this introduced pest expands its range in the Palearctic. If, as predicted, *D. valens* becomes more of a primary tree killer as a result of the effects of global warming, such methods will also become more important in its native range in the Nearctic.

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