Verbenone interrupts attraction to host volatiles and reduces attack on *Pinus tabuliformis* (Pinaceae) by *Dendroctonus valens* (Coleoptera: Scolytidae) in the People's Republic of China

Jianghua Sun

Institute of Zoology, Chinese Academy of Sciences, Beijing, 100080
People's Republic of China

Nancy E Gillette[1]

USDA Forest Service, Pacific Southwest Research Station, Berkeley,
California 94701, United States of America

Zhengwan Miao

Pest Control Station, Forestry Department of Shanxi Province, Taiyuan, 030012
People's Republic of China

Le Kang, Zhongning Zhang

Institute of Zoology, Chinese Academy of Sciences, Beijing, 100080
People's Republic of China

Donald R Owen

California Department of Forestry and Fire Protection, Redding, California 96002,
United States of America

John D Stein

USDA Forest Service, Forest Health Technology, Morgantown, West Virginia 26505,
United States of America

The Canadian Entomologist **135**: 721 – 732 (2003)

Abstract—The introduced red turpentine beetle, *Dendroctonus valens* LeConte, is one of the most economically important forest pests in the People's Republic of China, having killed more than 6 million pines in recent years. There is an urgent need to develop effective behavioral chemicals to monitor and control *D. valens* in the People's Republic of China, as well as in its native range in North America. We tested host kairomones as a 1:1:1 blend of α-pinene, β-pinene, and Δ-3-carene (releasing in the same proportions) for monitoring *D. valens* populations in the People's Republic of China. We also tested two release systems of verbenone for protection of *Pinus tabuliformis* Lawson from *D. valens* attack: (1) polyethylene bubblecaps (BCs) filled with 800 mg of nearly pure verbenone (releasing 18 mg/tree per day) and (2) a sprayable water suspension of microencapsulated (MEC) verbenone (releasing about 100 mg/tree per day). The host-volatile blend trapped substantial numbers of both sexes of adult beetles, up to 15 beetles per day, proving its potential for monitoring. Both of the verbenone release systems significantly reduced *D. valens* trap catch, and there was no difference between the BC treatment and the MEC treatment. Both release systems also reduced beetle attack on trees to the same level as unbaited controls, from a mean of 5.1 per tree to a mean of 0.7 per

[1] Corresponding author (e-mail: ngillette@fs.fed.us).

tree (for both release systems), suggesting that the treatments may also reduce tree mortality.

Sun J, Gillette NE, Miao Z, Kang L, Zhang Z, Owen DR, Stein JD. 2003. Effets de la verbénone sur *Dendroctonus valens* (Coleoptera : Scolytidae), une espèce introduite en République populaire de Chine : réduction de l'attirance pour les substances volatiles de *Pinus tabuliformis* (Pinaceae) et diminution des attaques. *The Canadian Entomologist* **135** : 721–732.

Résumé—Le dendroctone rouge de l'épinette, *Dendroctonus valens* LeConte, une espèce introduite, est, sur le plan économique, l'un des plus importants ravageurs des forêts de République populaire de Chine; il a détruit plus de 6 millions de pins (Pinaceae) au cours des dernières années. Il y a donc un besoin urgent d'identifier les substances chimiques efficaces qui régissent son comportement, de façon à en faire la surveillance et le contrôle en République populaire de Chine, aussi bien qu'on le fait sur son aire de répartition originale en Amérique du Nord. Nous avons testé les kairomones de l'hôte en utilisant un mélange 1:1:1 de α-pinène, de β-pinène et de Δ-3-carène (libérées en proportions égales) pour l'évaluation des populations de *D. valens* en République populaire de Chine. Nous avons aussi testé deux systèmes de libération de verbénone pour protéger *Pinus tabuliformis* Lawson des attaques de *D. valens* : (1) des capsules émettrices de polythène (bubblecaps, BCs) contenant 800 mg de verbénone presque pure (libérant 18 mg/arbre par jour) et (2) une suspension aqueuse pulvérisable de verbénone microencapsulée (MEC; libérant 100 mg/arbre par jour). Le mélange volatile de l'hôte permet la capture d'un bon nombre d'adultes des deux sexes, jusqu'à 15 coléoptères par jour, et montre un bon potentiel comme outil de surveillance. Les deux systèmes de libération de verbénone réduisent significativement les captures de *D. valens* dans les pièges; il n'y a pas de différence entre les traitements BC et MEC. De plus, les deux systèmes d'émission réduisent les attaques des dendroctones sur les arbres au niveau des arbres témoins sans appât, soit de 5,1 par arbre en moyenne à 0,7 par arbre en moyenne, ce qui fait croire que les traitements peuvent aussi réduire la mortalité des arbres.

[Traduit par la Rédaction]

Introduction

The red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Scolytidae), is a widespread pest of pines in North America (Eaton and Rodriguez Lara 1967). Despite its abundance and wide distribution, outbreaks of *D. valens* have generally not been extensive or severe in North America (Smith 1961; Cibrian Tovar *et al.* 1995). Recently, however, Rappaport *et al.* (2001) reported that *D. valens* caused moderate mortality to ponderosa pine, *Pinus ponderosa* Lawson (Pinaceae), in a thinned, subsoiled plantation in northern California. *Dendroctonus valens* is also reported as a primary tree killer of *Pinus radiata* D. Don in stands in coastal California and of small-diameter plantation pines in Mexico (Eaton and Rodriguez Lara 1967). There is increasing concern that intensive forest management practices, such as precommercial thinning and prescribed fire, will exacerbate *D. valens* damage by creating stumps and stressed trees where the insects propagate. *Dendroctonus valens* vectors pathogenic fungi into its hosts (Owen *et al.* 1987; Klepzig *et al.* 1996), and tree mortality has been linked to insect – fungus – host interactions that result in declining stand health (Erbilgin and Raffa 2002), even though *D. valens* is not as aggressive a tree-killer as many of its congeners.

In contrast with its role as a secondary pest in North America, in the People's Republic of China, *D. valens* has spread rapidly since its first outbreak in 1999 in Shanxi Province to three adjacent provinces. It has since infested over 0.5 million ha of pine stands, killing more than 6 million *Pinus tabuliformis* Carr as well as other pines such as *Pinus bungeana* Bucc. (Li *et al.* 2001; Miao *et al.* 2001). *Dendroctonus valens* was introduced into the People's Republic of China in the early 1980s when unprocessed logs were imported from the west coast of the United States of America (report on file at the Chinese Academy of Sciences, Beijing). Several consecutive years of drought severely stressed the primary host of *D. valens* in the People's Republic of China, *P. tabuliformis*, probably contributing to the sudden outbreak seen in recent years (Li *et al.* 2001). *Pinus tabuliformis* is extremely important for reforestation in the People's Republic of China and is widely planted across a large portion of the country. Considering the wide host range of *D. valens* in North America (Eaton and Rodriguez Lara 1967), the majority of Chinese pines are probably at risk. The potential for damage from this exotic beetle is enormous, and effective means for monitoring and control in the People's Republic of China are desperately needed (Li *et al.* 2001).

Smith (1961) reported that *D. valens* is not a primary tree killer, and that attacks most frequently occur on injured or stressed trees in the United States of America. Attacks are initiated in the basal portion of the bole and then extend both up and down the bole and into the root collar (Smith 1961). Even though *D. valens* is known to initiate attacks on roots in North America, details of this behavior are lacking. In the People's Republic of China, *D. valens* has been found initiating extensive root attacks, and it overwinters in overlapping generations on the roots (Miao *et al.* 2001). Although insecticide applications to the basal portion of uninfested trees have been shown to reduce damage by *D. valens* in the United States of America (Hall 1984; Svirha 1995), the root colonization behavior seen in the People's Republic of China complicates chemical control because larvae are present on the roots year-round and are therefore less exposed to insecticide treatments. Semiochemicals for monitoring, detecting, and managing *D. valens* may be even more important in the People's Republic of China than in North America, because of its more cryptic habit in Chinese hosts. Bark beetles have long been considered good candidates for semiochemical-based management strategies (Borden 1997), and recent work suggests that such management programs may be feasible for *D. valens* (Rappaport *et al.* 2001).

Dendroctonus valens is known to use host odors or kairomones to locate and select its preferred host, *P. ponderosa* (Vité and Gara 1962; Owen 1985; Hobson *et al.* 1993). The principal monoterpene components of *P. ponderosa* are (-)-β-pinene, (+)-3-carene, and (+)-α-pinene, with (-)-β-pinene reported as the principal attractant (White and Hobson 1993; Hobson 1995). (-)-Verbenone (4,6,6-trimethylbicyclo[3.1.1.]hept-3-en-2-one) has been tested on several species in the genus *Dendroctonus* Erichson, including *D. valens*, and was shown to be effective as an inhibitor of attraction in most tests (Borden 1982, 1997; Livingston *et al.* 1983; Payne and Billings 1989; Phillips *et al.* 1989; Paine and Hanlon 1991; Bertram and Paine 1994; Miller *et al.* 1995; Paine *et al.* 1997; Rappaport *et al.* 2001). In the case of *Dendroctonus terebrans* (Olivier), however, verbenone repelled beetles in trap-based tests but increased beetle attack rate when applied as bubblecaps (BCs) to previously unattacked trees (Phillips *et al.* 1990). Although the response of *D. valens* to host volatiles and verbenone indicates that such semiochemicals could play a role in its management, the reported results for *D. terebrans* demonstrate the need for a careful approach in developing operational methods of semiochemical use for bark beetles in general.

The *D. terebrans* study was conducted using BC release devices stapled to tree trunks, which are typically deployed at the rate of about 40 point-sources per acre. We hypothesized that a microencapsulated (MEC) formulation, which could easily be formulated to release millions to billions of point-sources per acre, might more closely mimic the natural release of verbenone by individual beetles in a forest stand than do BCs or pouches, and might therefore be more effective for protecting stands from beetle attack. MECs also have the potential to be applied aerially to the crowns of trees, and thus might better deter beetles from entering a stand than do devices stapled to tree trunks.

The objectives of this study, therefore, were to (*i*) to evaluate the efficacy of *D. valens* kairomones from North American hosts for monitoring *D. valens* in *P. tabuliformis* stands in the People's Republic of China, (*ii*) assess the efficacy of verbenone in two release systems (polyethylene BCs and a sprayable water suspension of MEC verbenone) as inhibitors of host attraction for *D. valens* management in the People's Republic of China, and (*iii*) quantify *D. valens* flight period and relative population levels over time in the People's Republic of China.

Materials and methods

The study was conducted in a 30-year-old plantation of *P. tabuliformis* at the foot of the Tai Hang Mountains (37°26′N, 113°00′E, average elevation 1400 m), southeast of the city of Yuci, Shanxi Province. This site, dominated by *P. tabuliformis* plantations, is where the first large *D. valens* outbreak occurred in 1999. The stand was relatively uniform in age, DBH (about 15 cm), and height (10–15 m). The site had shallow, sandy soil overlaying rock. Trees had shortened internodes at the top of the crowns, indicating slow height growth. This slow growth rate may have resulted from high stocking levels and (or) poor site quality, and reflects suboptimal growing conditions. More than one third of the standing trees had *D. valens* attacks in the roots and boles at the time of the study, and a severe, 4-year drought may have contributed to the severity of the outbreak.

We deployed an attractant semiochemical blend as a challenge to the two verbenone formulations that we wished to test for interruption of host attraction. That lure also served as a test of attractiveness of the standard North American *D. valens* lure to the Chinese population of *D. valens*. The attractive lure consisted of a 1:1:1 blend of three host monoterpenes: (-)-β-pinene, (+)-3-carene, and (+)-α-pinene (White and Hobson 1993), which is available commercially in a BC release device (Phero Tech Inc, Delta, British Columbia). The monoterpenes in the commercial lure, which have the same molecular weights and very similar structures, release at a 1:1:1 ratio (JP Lafontaine, personal communication). Release rates and purities of semiochemical lures are provided (Table 1). (-)-Verbenone was deployed both as a BC release system (Phero Tech Inc) and as a sprayable water suspension of MEC (3M of Canada, London, Ontario) verbenone.

We installed treatments on both real trees and surrogate trees (unpainted brown cardboard tubes, 24.0 cm diameter × 90.3 cm long (Pacific Paper Tube, Inc, Oakland, California)), for two reasons. First, the use of real trees results in higher variance (more "noise") because pine monoterpenes are variable (Smith 1977), beetle attack increases this variability (Raffa and Smalley 1995), and variations in monoterpene release can affect beetle response (Siegfried *et al.* 1986). Second, we wanted a consistent physical substrate for release of the MEC formulation, and the tubes were superior in this respect to real trees. These two treatments are hereinafter referred to as the tree and tube treatments. To standardize treatments and avoid bias in beetle response to a tree-shaped visual cue, we deployed the tubes in all treatments, including those where the treatment was a BC rather than a spray. Both the tree and the tube treatments were deployed in the same stand, with tubes randomly interspersed among trees using a computer-generated spatial grid but maintaining a distance of at least 50 m between tube and tree treatments.

Seven treatments (four tree treatments and three tube treatments) with 10 replicates each were applied in a completely randomized design (Table 2). We used an

TABLE 1. Description of semiochemical-releasing devices and their release rates assayed for responses by *Dendroctonus valens* in *Pinus tabuliformis* in Shanxi Province, People's Republic of China, in 2001.

Semiochemical*	Release device (rate)	Source	Release rate (mg/tree per day)
Dendroctonus valens lure†	15-mL vial (1 per tree)	Phero Tech	>100‡
(–)-Verbenone	BC (4 per tree)	Phero Tech	18
(–)-Verbenone	MEC (500 mL per tree)	3M Canada	>100‡

* Chemical purities >97% for all semiochemicals; enantiomeric purities as follows: α-pinene >75% plus, β-pinene >95% minus, and Δ-3-carene = 72% plus, verbenone bubblecap (BC) = 85% minus, and verbenone microencapsulated spray (MEC) = 97% minus.

† α-pinene, β-pinene, and Δ-3-carene in a 1:1:1 ratio.

‡ Averaged over 60 days; MEC value estimated from similar formulation.

TABLE 2. Description of treatments used in field experiments in Shanxi Province, People's Republic of China, in 2001.

Treated substrate	With or without *Dendroctonus valens* bait	Verbenone treatment*
Tree	Unbaited	None
	Baited	None
	Baited	MEC
	Baited	BC
Tube	Baited	None
	Baited	MEC
	Baited	BC

* MEC, microencapsulated spray; BC, bubblecap.

unbaited control tree treatment because trees could normally be expected to attract low numbers of *D. valens* even in the absence of lures (Rappaport *et al.* 2000). We used a baited control tree (with standard commercial lure) as a basis for comparing repellency of the verbenone applications (MEC and BC) to trees. We chose not to install an unbaited tube treatment because previous studies showed that beetles did not respond to unbaited cardboard tubes (NE Gillette, unpublished data). The baited tube treatment was included as a basis of comparison of the efficacy of the verbenone MEC and BC in repelling beetles from a baited tube. The experiment was similar to that of Rappaport *et al.* (2001), but in this experiment we used a higher release rate (4 times) for the BC treatment. In the tree treatments, 40 unattacked trees were randomly selected with trees at least 50 m apart. Selection of trees was done at random from the total population of suitable trees in the stand, and treatments were randomly assigned to each tree. In the tube treatments, 30 tubes were randomly placed, at least 50 m apart and 50 m from any treated tree, in a grid within the same stand. We installed traps to monitor beetle flight near each tree and tube to achieve a standardized frame of reference for comparison of the behavioral effects of the treated substrates; beetles can bore into real trees and thus leave evidence of the treatment effect, but that is clearly not the case with cardboard tubes. Beetles have been shown to be attracted to baited tubes (Rappaport *et al.* 2001), but traps are required to measure their attraction to the tubes because they do not bore into the tubes. An eight-funnel Lindgren trap baited with a *D. valens* kairomone BC (Phero Tech, Inc) was therefore suspended immediately adjacent to each tree or tube, with the bottom of the collection cup about 10 cm above ground level. Collection cups on the Lindgren traps were filled to 2.5 cm with propylene glycol as a killing agent. For the verbenone BC application, four BCs were stapled to each tree or tube 20 cm above the ground, with one BC at each cardinal direction. For the MEC treatments, the verbenone was prepared as a 20% water suspension of MEC verbenone in water and was sprayed to runoff onto trees and tubes to a height of 1 m (about 500 mL per tree or tube). All applications were done on 28 April 2001, and traps were checked every 3–5 days until 18 August 2001. Beetles captured in each trap on each date were counted, sexed, and recorded. Voucher specimens were deposited in the Institute of Zoology, Chinese Academy of Sciences, Beijing, People's Republic of China. Number of beetle attacks, as measured by pitch masses on each treated tree, were counted and recorded in mid-August 2001.

Trap counts and number of attacks per tree were analyzed with generalized linear models for overdispersed Poisson distributed responses (counts) (McCullough and Nelder 1989). Results were graphed as estimated means, with 95% confidence intervals of the true means as a measure of spread around the estimates of the means. This approach provides a visual measure of the skewness of the response distributions, which unlike standard deviations and standard errors are rarely symmetrical. Multiple comparisons were based on the maximum likelihood ratio test with the Bonferroni approach (experimentwise $\alpha = 0.05$). SAS GENMOD procedures (SAS Institute Inc 1997) were used for the analysis.

Results and discussion

Effectiveness of *D. valens* lure and *D. valens* flight period in the People's Republic of China

The blend of host volatiles tested in this study was very effective in capturing substantial numbers of both male and female beetles (Fig. 1), over 15 beetles/trap per day at peak flight, and over 200 times the number caught near unbaited trees (Fig. 2). The lure consists of kairomones rather than pheromones, and pheromones, which normally elicit a stronger response than do kairomones, often attract greater numbers of insects. In comparison, Ross *et al.* (1995) trapped fewer than 20 *Dendroctonus pseudotsugae* Hopkins per trap per day in traps baited with an aggregation pheromone of *D. pseudotsugae*, a combination of frontalin and seudenol, in an area with high populations of *D. pseudotsugae*. Our results show comparable results using a kairomone rather than a pheromone. This result was not entirely surprising, however, because the monoterpene blend is effective for many populations of *D. valens* in its native range in North America. The *D. valens* / *P. tabuliformis* association, however, is new, and differences in host volatiles between North American and Chinese hosts might have affected the efficacy of the lure in the People's Republic of China. For example, synergistic or inhibitory effects of monoterpenes from the foliage of *P. tabuliformis* might have influenced trap catch in the People's Republic of China. In addition, enantiospecific effects of Scolytidae semiochemicals are well known (Wood *et al.* 1967), and it has been suggested that regional differences in *D. valens* responses to chiral semiochemicals may exist (Erbilgin and Raffa 2000). Blends shown to be effective in some parts of North America, therefore, may not have proven effective for the population introduced into the People's Republic of China. As a point of reference, the number of beetles trapped in the People's Republic of China was similar to that trapped with the same trap and lure in northern California (Rappaport *et al.* 2001; NE Gillette and DR Owen, unpublished data). Although more effective traps and lures may ultimately be found for *D. valens* in the People's Republic of China, the existing product clearly is useful for detecting and monitoring beetle populations in the People's Republic of China.

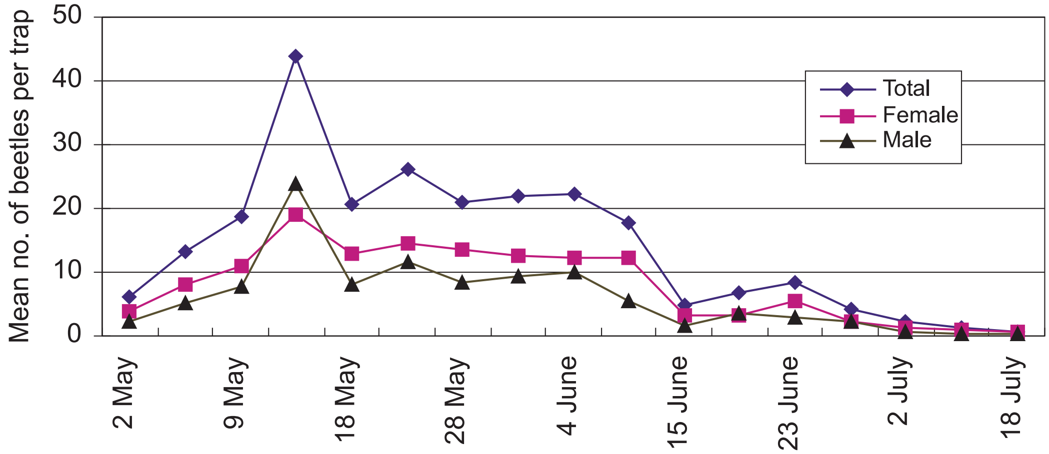

FIGURE 1. Flight period of *Dendroctonus valens* males and females measured by the numbers of beetles attracted to traps baited with a 1:1:1 ratio of (–)-β-pinene, (+)-3-carene, and (+)-α-pinene in a *Pinus tabuliformis* plantation in Shanxi Province, People's Republic of China, in 2001.

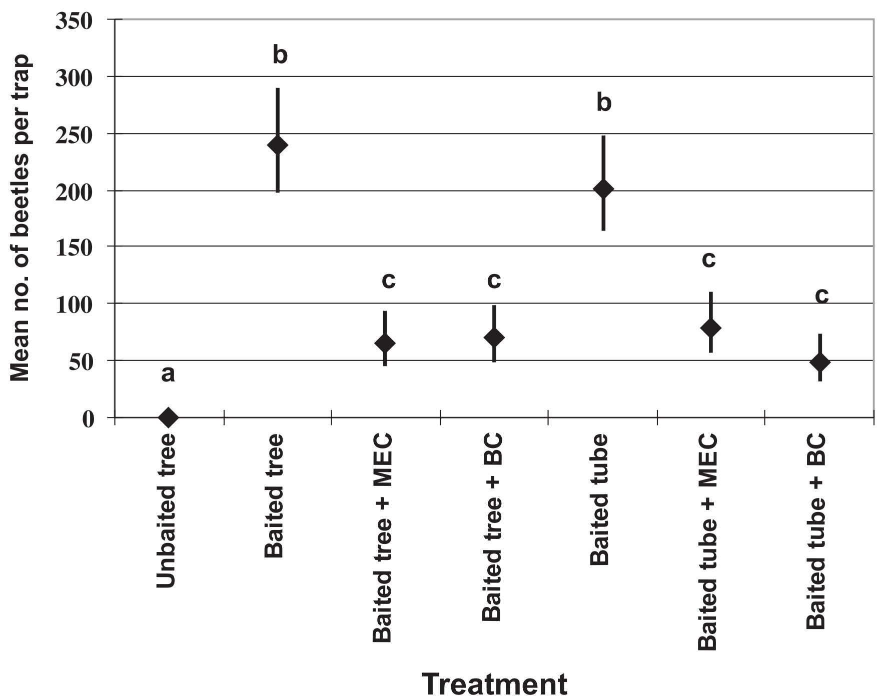

FIGURE 2. Mean number of *Dendroctonus valens* trapped in multiple-funnel traps by treatment over the entire flight period. Vertical bars show the 95% confidence intervals and means with the same letter are not different (likelihood ratio test followed by a Bonferroni test for multiple comparisons, P > 0.05). MEC, microencapsulated spray; BC, bubblecap.

Dendroctonus valens were trapped in Shanxi Province throughout the duration of the experiment, from the first of May until late July, with a clear peak in mid-May for both males and females (Fig. 1). Sampling of infested trees revealed overlapping generations (Owen 2001) and is consistent with the data on adult flight. There were some smaller subsequent peaks in adult flight, so in Shanxi Province there may be more than a single generation per year. The climate in the range of *D. valens* in the People's Republic of China, including Shanxi Province, is more arid than most of the range of

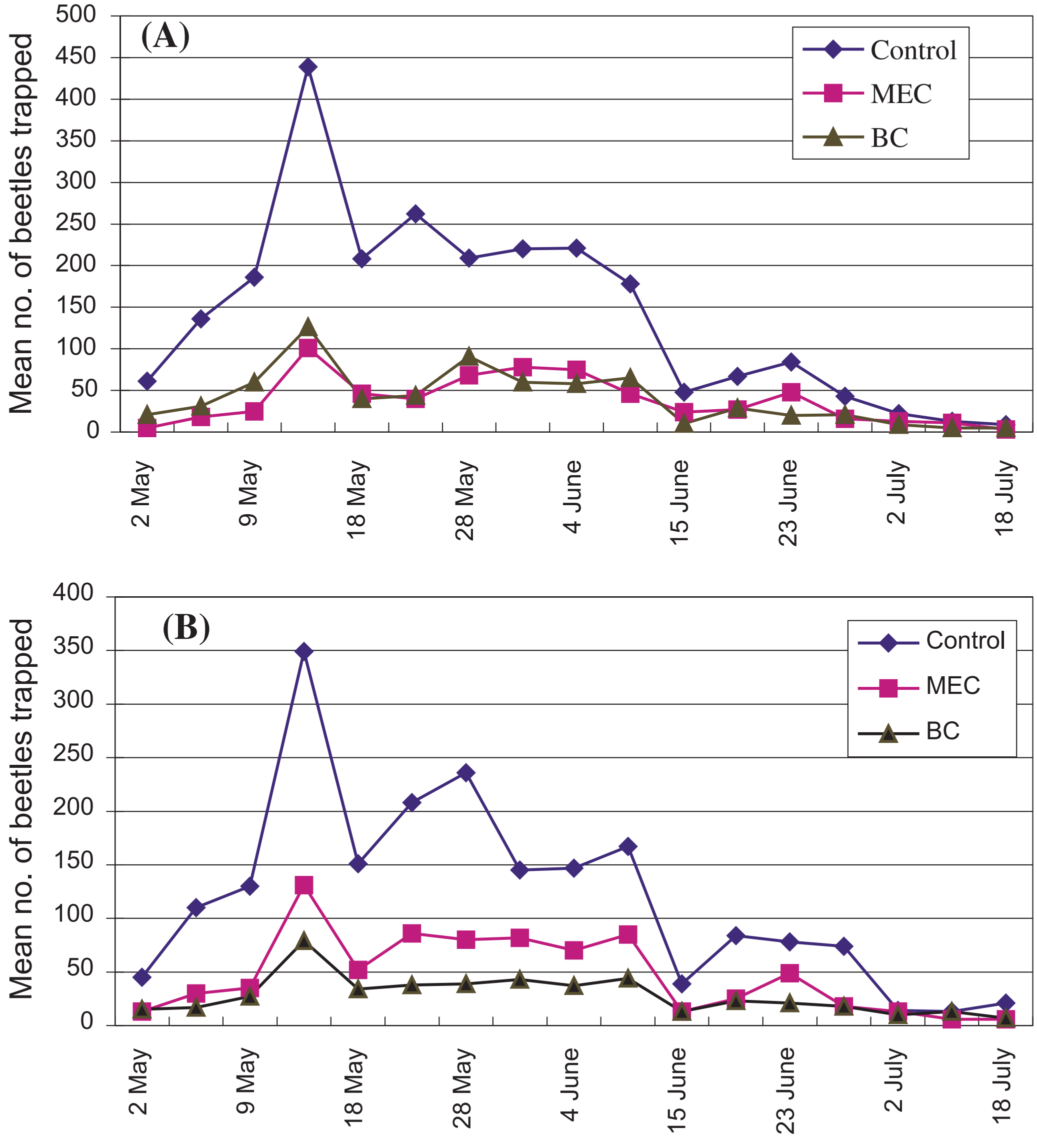

FIGURE 3. Mean number of *Dendroctonus valens* caught in multiple-funnel traps with host volatiles and placed next to real trees (A) and cardboard tubes (B) over time. MEC, microencapsulated spray; BC, bubblecap.

D. valens in North America, which nearly spans the North American continent (Furniss and Carolin 1977). In southern North America, two to three generations may develop per year, but in the northern extreme of the *D. valens* range 2 years are required for development of a single generation (Eaton and Rodriguez Lara 1967). Fluctuations in temperature can produce variations in beetle emergence and flight, so our results are not conclusive evidence of multiple generations per year. Further investigation will be required to resolve the voltinism of *D. valens* in the People's Republic of China.

Interruption of *D. valens* attraction to host volatiles by MEC and BCs

Efficacy of verbenone as an interruptor of attraction to hosts was measured by the reduction in numbers of beetles captured in baited traps and numbers of beetle attacks in treated trees. Both release systems significantly reduced trap catch in both trees and

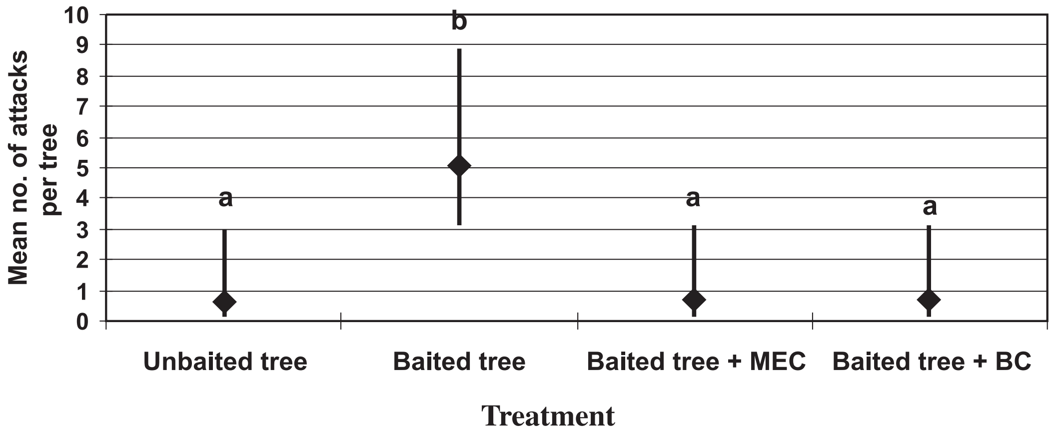

FIGURE 4. Mean number of *Dendroctonus valens* attacks per tree by treatment. Vertical bars show the 95% confidence intervals and means with the same letter are not different (likelihood ratio test followed by a Bonferroni test for multiple comparisons, *P* > 0.05). MEC, microencapsulated spray; BC, bubblecap.

tubes, and there was no significant difference between the BC treatment and the MEC treatment (Fig. 2).

Time-series plots of verbenone repellency on both trees and tubes (Figs. 3A and 3B, respectively) show that both release systems, applied a single time at the beginning of the season, have sustained efficacy through most of the *D. valens* flight period. Holsten *et al.* (2002) report consistent and sustained release rates of verbenone from BC releasers, a finding that is in good agreement with our results. Although our results use a bioassay rather than a chemical assay to demonstrate longevity of release, the results are promising for both the BC and the MEC systems for at least 1 month following deployment. The effect of both formulations of verbenone was more than adequate to reduce attack rate during the heavy attack period in May, so early deployment of treatments is advisable.

The two release systems appear equally effective for operational purposes, and both systems reduced numbers of *D. valens* attack to the level found on unbaited control trees. When applied to real trees, both release systems reduced numbers of attacks per tree from a mean of 5.1 to a mean of 0.7 (Fig. 4) (*F* test for overall treatment effect, $P = 0.0018$). Multiple comparisons using the Bonferroni approach to maintain an experimentwise error rate of $\alpha = 0.05$ showed no significant differences between treatments, although both were significantly different from the baited control. This reduction in attack rate has positive implications for management in plantation conditions; rates of attack as low as 4–5 per tree can be sufficient to kill small plantation trees, but a single attack per tree is unlikely to cause tree mortality (NE Gillette, unpublished data). We did not assess tree mortality in this experiment because *D. valens*, unlike most of its congeners, does not mass-attack and rapidly kill its hosts (Smith 1961). Future studies should be designed to include long-term tree mortality as a response variable, however, because a reduction in the level of *D. valens* attack could be expected to enhance tree survival. Previous attempts to protect trees from attack by related bark beetle species, however, have proven to be problematic (Gibson *et al.* 1991, Shea *et al.* 1992). Gibson *et al.* (1991), using BC release devices, were able to reduce attack on *Pinus contorta* Douglas *ex* Loudon but not on *P. ponderosa* by *Dendroctonus ponderosae* Hopkins. The authors of that study speculated that stand characteristics, including canopy closure and tree density, might have affected semiochemical dispersal within the stands, resulting in variable efficacy. Shea *et al.* (1992) demonstrated successful reduction in attack by

D. ponderosae on *P. contorta* using an aerial application of verbenone-impregnated 5-mm-diameter polyethylene beads, but were unable to replicate this success in a subsequent study. Kostyk *et al.* (1993) hypothesized that some of these failures may have resulted from photo-isomerization of verbenone to chrysanthenone (Erdman 1967), a chemical that is behaviorally neutral for *D. ponderosae*.

In summary, our results show that the *D. valens* lure used in stands of native pine hosts in North America is also very effective for monitoring *D. valens* populations in stands of *P. tabuliformis* in the People's Republic of China. We also demonstrated the efficacy of two release systems of verbenone in reducing attack on individual trees by *D. valens*, and these results were consistent with studies conducted in North America (Rappaport *et al.* 2001). One of the release systems, the MEC, is a novel technology with promise for situations where BCs might be problematic because of wildlife or recreation concerns. A formulation such as the MEC, which can be applied as a spray to the lower bole, root collar, and soil might also provide greater protection in situations such as that in the People's Republic of China where beetles infest roots as well as the lower trunk.

Acknowledgments

This project was funded by the Natural Science Foundation of the People's Republic of China (project 3023006) and the Chinese Academy of Sciences Innovation Program (KSCX3-IOZ-04). S Burke, formerly of Phero Tech Inc, provided valuable assistance with selection of pheromone blends and graciously lent research materials. LN Eden, Canadian Agritech Development Corporation, Vancouver, British Columbia, and Aolida Pharmaceutical Company, Heilongjiang Province, People's Republic of China, provided assistance in transporting experimental materials into the People's Republic of China. Mention of a product does not constitute endorsement of it use by the authors. The Division of Pest Control, State Forestry Administration of the People's Republic of China, and the USDA Forest Service International Forestry, Forest Health Enterprise Team all provided financial and logistical support for the study. Helpful reviews by N Erbilgin (University of California, Berkeley, California), RF Powers (USDA Forest Service, Pacific Southwest Research Station), D Miller (USDA Forest Service, Southern Research Station, Athens, Georgia), and CW Berisford (University of Georgia, Athens, Georgia) greatly improved the manuscript. S Mori, mathematical statistician with USDA Forest Service, Pacific Southwest Research Station, provided statistical guidance and review of the manuscript. The US Government is authorized to produce and distribute reprints for government purposes notwithstanding any copyright notation that may appear hereon.

References

Bertram SL, Paine TD. 1994. Influence of aggregation inhibitors (verbenone and ipsdienol) on landing and attack behavior of *Dendroctonus brevicomis* (Coleoptera: Scolytidae). *Journal of Chemical Ecology* **20**: 1617–29

Borden JH. 1982. Aggregation pheromones. pp 74–139 *in* JB Mitton, KB Sturgeon (Eds), *Bark beetles in North American conifers: a system for the study of evolutionary biology.* Austin, Texas: University of Texas Press

——— 1997. Disruption of semiochemical mediated aggregation in bark beetles. pp 421–38 *in* RT Cardé, AK Minks (Eds), *Insect pheromone research, new directions*. New York: Chapman and Hall

Cibrian Tovar DE, Mendez Montiel JT, Campos Bolaños R, Yates HO III, Flores Lara J. 1995. *Forest Insects of Mexico.* Asheville, North Carolina: USDA Forest Service Southeastern Station

Eaton B, Rodriguez Lara RR. 1967. Red turpentine beetle, *Dendroctonus valens* LeConte. pp 21–4 *in* AG Davidson, RM Prentice (Eds), *Important forest insects and diseases of mutual concern to Canada, the United States and Mexico*. Ottawa, Ontario: Canadian Department of Forestry and Rural Development

Erbilgin N, Raffa, KF. 2000. Opposing effects of host monoterpenes on responses by two sympatric species of bark beetles to their aggregation pheromones. *Journal of Chemical Ecology* **26**: 2527–48

——— 2002. Association of declining red pine stands with reduced populations of bark beetle predators, seasonal increases in root colonizing insects, and incidence of root pathogens. *Forest Ecology and Management* **164**: 221–36

Erbilgin N, Szele A, Klepzig KD, Raffa KF. 2001. Trap type, chirality of α-pinene, and geographic region affect sampling efficiency of root and lower stem insects in pine. *Journal of Economic Entomology* **9**: 1113–21

Erman WF. 1967. Photochemical transformations of unsaturated bicyclic ketones. Verbenone and its photodynamic products of irradiation. *Journal of the American Chemical Society* **89**: 3828–41

Furniss RL, Carolin VM. 1977. Western forest insects. *USDA Forest Service Miscellaneous Publication* **1339**

Gibson KE, Billings RF, Amman GD, Oakes RD. 1991. Mountain pine beetle response to different verbenone dosages in pine stands of western Montana. *US Forest Service Research Paper* **INT-444**

Hall RW. 1984. Effectiveness of insecticides for protecting ponderosa pines from attack by red turpentine beetle (Coleoptera: Scolytidae). *Journal of Economic Entomology* **77**: 446–8

Hobson KR. 1995. Host compounds as semiochemicals for bark beetles. *US Forest Service General Technical Report* **INT-GRT-318**. pp 48–51

Hobson KR, Wood DL, Cool LG, White PR, Ohtsuka T, Kubo I, Zavarin E. 1993. Chiral specificity in responses by the bark beetle *Dendroctonus valens* to host kairomones. *Journal of Chemical Ecology* **19**: 1837–46

Holsten EH, Web W, Shea PJ, Werner RA. 2002. Release rates of methylcyclohexenone and verbenone from bubble cap and bead releasers under field conditions suitable for the management of bark beetles in California, Oregon, and Alaska. *US Forest Service Pacific Northwest Research Paper* **544**: 1–21

Klepzig KD, Smalley EB, Raffa KF. 1996. Combined chemical defenses against an insect–fungal complex. *Journal of Chemical Ecology* **22**: 1367–88

Kostyk BC, Borden JH, Gries G. 1993. Photoisomerization of antiaggregation pheromone verbenone: biological and practical implications with respect to the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). *Journal of Chemical Ecology* **19**: 1749–59

Li JS, Chang GB, Song YS, Wang YW, Chang BS. 2001. Control project on red turpentine beetle (*Dendroctonus valens*). [In Chinese.] *Forest Pest and Disease* **4**: 41–4

Livingston WH, Bedard WD, Mangini AC, Kinzer HG. 1983. Verbenone interrupts attraction of roundheaded pine beetle, *Dendroctonus adjunctus* (Coleoptera: Scolytidae) to sources of natural attractant. *Journal of Economic Entomology* **76**: 1041–3

McCullough P, Nelder JA. 1989. Generalized linear models. *In Monographs on statistics and applied probability* **37**. New York: Chapman and Hall

Miao ZW, Chou WM, Huo FY, Wang XL, Fang JX, Zhao MM. 2001. Biology of *Dendroctonus valens* in Shanxi Province. [In Chinese.] *Shanxi Forest Science and Technology* **23**: 34–7

Miller DR, Borden JH, Lindgren BS. 1995. Verbenone: dose-dependent interruption of pheromone-based attraction of three sympatric pine bark beetle species. *Environmental Entomology* **24**: 692–6

Owen DR. 1985. The role of *Dendroctonus valens* and its vectored fungi in the mortality of ponderosa pine. PhD thesis, University of California, Berkeley

——— 2001. Consultation on the red turpentine beetle, *Dendroctonus valens,* in Shanxi Province, P. R. China, 1–7 July 2001. *USDA Forest Service International Programs Report*

Owen DR, Lindahl KQ Jr, Wood DL, Parmeter JR Jr. 1987. Pathogenicity of fungi isolated from *Dendroctonus valens*, *D. brevicomis*, and *D. ponderosae* to ponderosa pine seedlings. *Phytopathology* **77**: 631–6

Paine TD, Hanlon CC. 1991. Response of *Dendroctonus brevicomis* and *Ips paraconfusus* (Coleoptera: Scolytidae) to combinations of synthetic pheromone attractants and inhibitors verbenone and ipsdienol. *Journal of Chemical Ecology* **17**: 2163–76

Paine TD, Raffa KF, Harrington TC. 1997. Interactions among scolytid bark beetles, their associated fungi, and host conifers. *Annual Review of Entomology* **42**: 179–206

Payne TL, Billings RF. 1989. Evaluation of (*S*)-verbenone applications for suppressing southern pine beetle (Coleoptera: Scolytidae) infestations. *Journal of Economic Entomology* **82**: 1702–8

Phillips TW, Nation JL, Wilkinson RC, Foltz JL. 1989. Secondary attraction and field activity of beetle-produced volatiles in *Dendroctonus terebrans*. *Journal of Chemical Ecology* **15**: 1513–33

——— 1990. Protecting individual pine trees from bark beetle attack using inhibitory behavioral chemicals. Final report of the Georgia Forestry Commission, Project 89-08. Stillwater, Oklahoma: Oklahoma State University

Raffa KF, Smalley EB. 1995. Interactions of pre-attack and induced monoterpene concentrations in host conifer defense against bark beetle – fungal complexes. *Oecologia* **102**: 285–95

Rappaport NG, Owen DR, Stein JD. 2001. Interruption of semiochemical-mediated attraction of *Dendroctonus valens* (Coleoptera: Scolytidae) and selected nontarget insects by verbenone. *Environmental Entomology* **30**: 837–41

Ross DW, Birgersson G, Espelie KE, Berisford CW. 1995. Monoterpene emissions and cuticular lipids of loblolly and slash pines — potential bases for oviposition preference of the Nantucket pine Tip Moth. *Canadian Journal of Botany* **73**: 21–5

SAS Institute Inc. 1997. *SAS/STAT software: changes and enhancements through release 6.12*. Cary, North Carolina: SAS Institute Inc

Shea PJ, McGregor MD, Daterman GE. 1992. Aerial application of verbenone reduces attack of lodgepole pine by mountain pine beetle. *Canadian Journal of Forest Research* **22**: 436–41

Siegfried BD, Fatzinger CW, Wilkinson RC, Nation JL. 1986. In-flight responses of the black turpentine beetle (Coleoptera: Scolytidae) to individual monoterpenes, turpentine, and Paraquat-treated slash pines. *Environmental Entomology* **15**: 710–4

Smith RH. 1961. Red turpentine beetle. *US Department of Agriculture Forest Pest Leaflet* **55**: 8

——— 1977. Monoterpenes of ponderosa pine xylem resin in western United States. *US Forest Service Technical Bulletin* **1532**

Svirha P. 1995. Prevention of red turpentine beetle attack by Sevimol and Dragnet. *Journal of Arboriculture* **21**: 221–4

Vité JP, Gara R. 1962. Volatile attractants from ponderosa pine attacked by bark beetles (Coleoptera: Scolytidae). *Contributions of the Boyce Thompson Institute* **21**: 251–73

White PR, Hobson KR. 1993. Stereospecific antennal response by the red turpentine beetle, *Dendroctonus valens*, to chiral monoterpenes from ponderosa pine resin. *Journal of Chemical Ecology* **19**: 2193–202

Wood DL, Browne LE, Ewing B, Lindahl K, Bedard WD, Tilden PE, Mori K, Pitman GB, Hughes R. 1967. Western pine beetle: specificity among enantiomers of male and female components of an attractive pheromone. *Science (Washington, DC)* **192**: 896–8

(Received: 20 February 2003; accepted: 6 June 2003)