The response of *Dendroctonus valens* and *Temnochila chlorodia* to *Ips paraconfusus* pheromone components and verbenone

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Abstract—The red turpentine beetle, *Dendroctonus valens* LeConte, 1860 (Coleoptera: Curculionidae, Scolytinae), is a common bark beetle found throughout much of North America and China. In 2004, we observed that attack densities of the California fivespined ips, *Ips paraconfusus* Lanier, 1970 (Coleoptera: Curculionidae, Scolytinae), in logging debris were inversely related to *D. valens* attacks on freshly cut stumps, which led to the demonstration that components of the aggregation pheromone of *I. paraconfusus* inhibited the response of *D. valens* to attractant-baited traps. In this study, we test the response of *D. valens* and *Temnochila chlorodia* (Mannerheim, 1843) (Coleoptera: Trogositidae), a common bark beetle predator, to racemic ipsenol, racemic ipsdienol, and (–)-*cis*-verbenol (IPSR) in the presence and absence of two release rates of (–)-verbenone. The addition of a relatively low release rate of (–)-verbenone (4 mg/24 h) to attractant-baited traps did not affect catch and had no significant effect on the response of *D. valens* to IPSR. IPSR significantly reduced *D. valens* attraction to baited traps. The addition of high release rates of (–)-verbenone (50 mg/24 h) to IPSR significantly increased inhibition; however, the effect was not significantly different from that observed with (–)-verbenone alone (50 mg/24 h). *Temnochila chlorodia* was attracted to traps baited with (–)-β-pinene, (+)-3-carene, and (+)-α-pinene. The addition of (–)-verbenone (50 mg/24 h) significantly increased attraction. Traps baited with IPSR caught significantly more *T. chlorodia* than those baited with (–)-verbenone. Few other beetles were collected. We are hopeful that these results will help facilitate the development of an effective tool for protecting *Pinus* spp. from *D. valens* infestations.

Résumé—Le dendroctone rouge de l'épinette, *Dendroctonus valens* LeConte, 1860 (Coleoptera : Curculionidae, Scolytinae), est un scolyte commun trouvé dans presque toute l'Amérique du Nord et la Chine. En 2004, nous avons observé que la densité des attaques du scolyte à cinq épines de Californie, *Ips paraconfusus* Lanier, 1970 (Coleoptera : Curculionidae, Scolytinae), dans les débris de coupe est inversement proportionnelle aux attaques de *D. valens* sur les souches nouvellement coupées; ceci nous a conduit à démontrer que la phéromone de rassemblement d'*I. paraconfusus* inhibe la réaction de *D. valens* à des pièges chargés de substances attractives. Dans ce travail, nous testons la réponse de *D. valens* et de *Temnochila chlorodia* (Mannerheim, 1843) (Coleoptera : Trogositidae), un prédateur commun des scolytes, à l'ipsénol racémique, à l'ipsdiénol racémique et au (–)-*cis*-verbenol (IPSR) en présence et en l'absence de libération de deux concentrations de (–)-verbénone. L'addition de (–)-verbénone à un taux relativement faible de libération (4 mg/24 h) dans les pièges munis de substances attractives n'affecte pas les captures et n'a pas d'effet significatif sur la réaction de *D. valens* à l'IPSR. L'IPSR réduit de façon significative l'attirance de *D. valens* pour les pièges appâtés. L'addition de (–)-verbénone à un fort taux de libération (50 mg/24 h) à l'IPSR réduit significativement l'inhibition; l'effet n'est cependant pas significativement différent de celui de la (–)-verbénone (50 mg/24 h). *Temnochila chlorodia* est attiré vers les pièges munis de (–)-β-pinène, de (+)-3-carène et de (+)-α-pinène. L'addition de (–)-verbénone (50 mg/24 h) augmente significativement l'attraction. Les pièges munis d'IPSR capturent significativement plus de *T. chlorodia* que ceux garnis de (–)-verbénone.

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Peu d'autres coléoptères ont été capturés. Nous croyons que ces résultats contribueront à faciliter la mise au point d'un outil efficace pour protéger les *Pinus* spp. des infestations à *D. valens*.

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The red turpentine beetle, *Dendroctonus valens* LeConte, 1860 (Coleoptera: Curculionidae, Scolytinae), is a common bark beetle found throughout much of North America (Furniss and Carolin 1977), and it was recently introduced into China on imported unprocessed logs from western North America (Li *et al.* 2001; Sun *et al.* 2003; Cognato *et al.* 2005). In North America, *D. valens* colonizes all species of pine (*Pinus* spp.; Pinaceae) within its native range and occasionally spruce (*Abies* spp.) and larch (*Larix* spp.) (Furniss and Carolin 1977). Attacks are usually confined to the basal portions of stressed, weakened, or dead and dying trees. Significant tree mortality was attributed to *D. valens* in a 17-year-old ponderosa pine (*Pinus ponderosa* Dougl. *ex* Laws.) plantation (Rappaport *et al.* 2001). In China, *D. valens* infestations have spread to over 500 000 ha, killing > 6 million *Pinus tabuliformis* Carrière (Sun *et al.* 2003). The species has become one of the most economically important forest pests in China, and effective control measures are urgently needed (Li *et al.* 2001; Sun *et al.* 2003; Zhang *et al.* 2006).

Dendroctonus valens is attracted to newly harvested and burned areas, presumably by the resinous host volatiles released during such activity. Hobson *et al.* (1993) reported significant increases in *D. valens* trap catch attributable to (–)-β-pinene, (+)-3-carene, and (+)-α-pinene. Combinations of these three kairomones constitute a highly effective bait in traps (Sun *et al.* 2003; Fettig *et al.* 2004, 2005). Fettig *et al.* (2004, 2005) reported that *Temnochila chlorodia* (Mannerheim, 1843) (Coleoptera: Trogositidae), a common bark beetle predator, was not attracted to multiple-funnel traps baited with (–)-β-pinene, (+)-3-carene, and (+)-α-pinene (150 mg/24 h, 1:1:1 ratio) and that few other beetles were collected.

In 2004, we observed a unique interaction between *D. valens* and the California fivespined ips, *Ips paraconfusus* Lanier, 1970 (Coleoptera: Curculionidae, Scolytinae), that led to the demonstration that racemic ipsenol, racemic ipsdienol, and (–)-*cis*-verbenol (IPSR) inhibited the response of *D. valens* to multiple-funnel traps baited with (–)-β-pinene, (+)-3-carene, and (+)-α-pinene (Fettig *et al.* 2005). *Temnochila chlorodia* was attracted to the semiochemical blend. In that study, we concluded that the results could have important implications toward developing an effective semiochemical-based management tool for *D. valens* (Fettig *et al.* 2005).

Verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) is an anti-aggregation pheromone produced by several congenerics (Skillen *et al.* 1997). Although pheromones have yet to be identified for *D. valens*, studies have shown that verbenone is present in the frass (Grégoire *et al.* 1991) and hindgut (Yan *et al.* 2004) of this species. Rappaport *et al.* (2001) examined the effect of two formulations of verbenone, polyethylene bubblecaps and a sprayable microencapsulated formulation, applied to surrogate trees and measured efficacy based on captures of *D. valens* in baited traps. Both release systems significantly reduced trap catches; however, relatively few individuals were collected (all means < 0.22 *D. valens*/24 h). In China, applications of verbenone significantly reduced attacks on *P. tabuliformis* trees (Sun *et al.* 2003). Gillette *et al.* (2006) treated 20 individual lodgepole pine (*Pinus contorta* Dougl. *ex* Loud.) with a suspension of DISRUPT Micro-Flake® verbenone (Hercon Environmental, Emigsville, Pennsylvania) and reported significantly fewer *D. valens* attacks on treated trees. All trees were baited immediately after being sprayed with aggregation pheromones of mountain pine beetle, *Dendroctonus ponderosae* Hopkins. The authors stated that the increased rate of attack by *D. valens* in untreated control trees may have resulted from (*i*) an increase in host attractants released by *D. ponderosae* attacks in untreated control trees (Hobson *et al.* 1993), (*ii*) pheromones released by attacking *D. ponderosae*, or (*iii*) the anti-aggregation effects of the verbenone treatment itself. They suggested the outcome was most probably a result of both the verbenone treatment and the release of other semiochemicals by *D. ponderosae* during host colonization (Gillette *et al.* 2006). The addition of verbenone to IPSR (Fettig *et al.* 2005) would permit exploitation of a larger number of ecological interactions

(Borden 1997) and could increase the inhibitory effect observed for each semiochemical. The objectives of this study were to evaluate the responses of *D. valens* and *T. chlorodia* to two release rates of (–)-verbenone alone and in combination with IPSR in attractant-baited traps.

A trapping experiment was conducted 3–27 May 2005 on the Georgetown Ranger District, Eldorado National Forest, Eldorado Co., California, United States (38.91°N, 120.84°W, mean elevation = 914 m). The stand was dominated by *P. ponderosa* (mean diameter at 1.37 m (dbh) ± SE = 66.7 ± 2.2 cm) and had been prescribed burned in March 2005. Mean stand density was 32.3 m^2 of basal area per hectare, of which 99.7% was *P. ponderosa* and the remainder comprised sugar pine (*Pinus lambertiana* Dougl.). Mean crown cover was 40%.

Flight behavior was assayed using 28 sixteen-unit multiple-funnel traps (Phero Tech Inc., Delta, British Columbia, Canada) deployed along a trap line. Each of the 28 trap locations was separated by 25–30 m to avoid interference among adjacent treatments. Traps were hung on 2 m metal poles with collection cups 20 cm above the ground. Each trap location was randomly assigned one of seven treatments: (1) unbaited control, (2) red turpentine beetle lure (RTB; (–)-β-pinene, (+)-3-carene, and (+)-α-pinene), (3) RTB + racemic ipsenol (0.1–0.3 mg/24 h), racemic ipsdienol (0.1–0.3 mg/24 h), and 83% (–)-*cis*-verbenol (1.0–2.0 mg/24 h) (IPSR), (4) RTB + 80% (–)-verbenone (4.0 mg/24 h; VERB4), (5) RTB + 82% (–)-verbenone (50.0 mg/24 h; VERB50), (6) RTB + IPSR + VERB4, and (7) RTB + IPSR + VERB50. Monoterpenes were released from 15 mL polyethylene bottles (150 mg/24 h, 1:1:1 ratio, Phero Tech Inc.). Monoterpene alcohols were released from polyvinyl chloride bubblecap release devices (20 mg for ipsenol and ipsdienol, 150 mg for *cis*-verbenol; Phero Tech Inc.). Verbenone was released from polyvinyl chloride bubblecaps (150 mg) and pouches (2 g; Phero Tech Inc.). A 3 cm × 3 cm time-released insecticidal Prozap Pest Strip (2,2-dichlorovinyl dimethyl phosphate, Loveland Industries Inc., Greely, Colorado, United States) was placed in the collection cup to kill arriving insects and reduce loss by invertebrate predation. All *D. valens* and *T. chlorodia* were sexed according to Lyon (1958) and Struble and Carpelan (1941), respectively. Voucher specimens have been deposited in the USDA Forest Service Bark Beetle and Common Associates Collection housed in Placerville, California.

The experimental design was completely randomized. Each of four traps per treatment was re-randomized on a daily basis. However, we limited statistical analyses for both *D. valens* and *T. chlorodia* to days when ≥75 individuals were caught to maintain a data set of ecological significance. Drawing ecological inferences based on the responses of few individuals, such as those insufficient to influence overall tree health (Furniss and Carolin 1977), should be done with caution (Fettig *et al.* 2005). Owing to extended periods of rainy weather during May 2005, application of this criterion resulted in 20 and 24 replications for *D. valens* and *T. chlorodia*, respectively (*i.e.*, ≥75 individuals were caught on 20 and 24 days, respectively). A two-way analysis of variance (treatment and sex) was performed on the numbers of *D. valens* and *T. chlorodia* caught per trap per day using $\alpha = 0.05$ (SigmaStat Version 2.0, SPSS Inc., Chicago, Illinois, United States). For each species, a test of normality was performed and square-root transformations were used when data deviated significantly from a normal distribution. If a significant treatment effect was detected, Tukey's multiple comparison test (Tukey's HSD) was used for separation of treatment means.

A total of 1347 *D. valens* were captured during this experiment. There was no significant treatment × gender interaction ($F_{6,266} = 0.89$, $P = 0.50$), and therefore data were pooled and results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{6,133} = 41.46$, $P < 0.001$). Significantly more male than female *D. valens* were collected (paired *t* test, $P < 0.0002$; M:F = 1.29). The addition of low release rates of (–)-verbenone (4 mg/24 h) to attractant-baited traps did not influence *D. valens* attraction and had no significant effect on the response of *D. valens* to IPSR (Table 1). IPSR significantly reduced the response of *D. valens* to attractant-baited traps (Table 1), which agrees with previous reports (Fettig *et al.* 2005). The RTB + IPSR treatment resulted in a 43.1% reduction in trap catch compared with RTB alone. High release rates of (–)-verbenone (50 mg/24 h) significantly reduced attraction to baited traps to levels below that of IPSR (Table 1). The RTB + VERB50 treatment resulted in an 85.0% reduction in trap catch compared with RTB alone; however, inhibition was not complete, as significantly fewer beetles

Table 1. The response of *Dendroctonus valens* and *Temnochila chlorodia* to verbenone and *Ips paraconfusus* pheromone components in baited multiple-funnel traps, Eldorado National Forest (38.91°N, 120.84°W), California, 2005.

	Mean daily catch per trap ± SE	
Treatment	*D. valens*	*T. chlorodia*
RTB	16.7±2.5*a*	2.0±0.6*d*
RTB + VERB4	10.8±1.3*ab*	3.8±0.7*cd*
RTB + IPSR	9.5±1.3*b*	9.8±1.2*a*
RTB + IPSR + VERB4	9.4±1.3*b*	6.2±0.8*abc*
RTB + IPSR + VERB50	4.2±0.7*c*	8.3±1.1*ab*
RTB + VERB50	2.5±0.4*c*	5.2±0.9*bc*
Untreated control	0.2±0.1*d*	0.0±0.0*e*

Note: RTB = (–)-β-pinene, (+)-3-carene, and (+)-α-pinene (150 mg/24 h, 1:1:1 ratio, 98% purity); IPSR = racemic ipsenol (0.1–0.3 mg/24 h, 97% purity), racemic ipsdienol (0.1–0.3 mg/24 h, 93% purity), and 83% (–)-*cis*-verbenol (1–2 mg/24 h, 94% purity); VERB4 = 80% (–)-verbenone (4 mg/24 h, 97% purity); and VERB50 = 82% (–)-verbenone (50 mg/24 h, 97% purity). Means followed by the same letter are not significantly different ($P > 0.05$; Tukey's HSD).

were collected in the untreated control (Table 1). The addition of VERB50 to RTB + IPSR significantly increased inhibition; however, the effect was not significantly different from that of RTB + VERB50 (Table 1). In China, Zhang *et al.* (2006) reported that (–)-verbenone inhibited the response of *D. valens* to attractant-baited traps at several release rates (*i.e.*, 3.4, 5.2, 8.2, and 24.8 mg/24 h). Reductions in trap catch ranged from 31% to 54.4%. Catches in traps with (–)-verbenone eluted at 24.8 mg/24 h were significantly lower than those in traps with the two lowest release rates. (+)-Verbenone also significantly reduced *D. valens* attraction, with a 36.8% reduction in trap catch observed. No significant differences between the two enantiomers of verbenone were observed. In the laboratory, electroantennogram assays showed no significant differences between antennal responses by sex, and only one significant difference between responses to equivalent doses of (–)- and (+)-verbenone (Zhang *et al.* 2006). Few other Scolytinae were collected in our study. Racemic ipsdienol, which was present in our semiochemical blend (IPSR), is not attractive to *I. paraconfusus* (Fettig *et al.* 2005).

A total of 858 *T. chlorodia* were captured during this experiment. There was no significant treatment × gender interaction ($F_{6,322}$ = 0.18, P = 0.98), and therefore the data were pooled and results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{6,161} = 34.77$, $P < 0.001$). Significantly more female than male *T. chlorodia* were collected (paired *t* test, $P <$ 0.02; M:F = 0.86). *Temnochila chlorodia* was attracted to traps baited with (–)-β-pinene, (+)-3-carene, and (+)-α-pinene, which differs from results obtained in two other studies (Fettig *et al.* 2004, 2005). The addition of (–)-verbenone (50 mg/24 h) significantly increased attraction. Traps baited with IPSR caught significantly more *T. chlorodia* than those baited with (–)-verbenone (Table 1). The addition of lower release rates of (–)-verbenone (4 mg/24 h) to attractant-baited traps had no effect on catch (Table 1). Paine and Hanlon (1991) did not find any difference in the response of *T. chlorodia* to several different release rates of verbenone (all <20 mg/24 h).

In regard to pest management, working in the area of allomonal inhibition and competition (*i.e.*, presence of aggregation pheromone of one species reduces aggregation in another) can be risky in terms of trading one potential source of tree mortality for another. Fettig *et al.* (2005) and others have reported that racemic ipsdienol is not attractive to *I. paraconfusus*, which agrees with the current study, as no individuals were collected. Natural enemies play an important role in regulating bark beetle populations (Furniss and Carolin 1997). A negative response to semiochemical-based management techniques could adversely affect long-term management of the target species. In this study, we demonstrated that *T. chlorodia* is attracted to high release rates of (–)-verbenone (Table 1), which is encouraging from the standpoint of operational use of this semiochemical for *D. valens* and other congenerics (Skillen *et al.* 1997). IPSR was not as effective as VERB50 for disrupting *D. valens* attraction to baited traps, and a synergistic effect was not observed when the two semiochemicals were combined. However, IPSR was more attractive to *T. chlorodia* than VERB50. The combination of IPSR and VERB50 may be most useful for disrupting *D. valens* and enhancing *T. chlorodia* attraction in areas where these species co-occur. Zhang *et al.* (2006) suggested that the use of verbenone for management of *D. valens* should be viewed with caution for a number of reasons, and indicated that a potential mechanism for improvement was to combine verbenone with other repellents.

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