Field Response of *Dendroctonus frontalis* (Coleoptera: Scolytinae) to Synthetic Semiochemicals in Chiapas, Mexico

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ABSTRACT *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytinae) is the most serious pest of pines (*Pinus* spp.) in Mexico. Conspecifics are attracted to trees undergoing colonization by the aggregation pheromone frontalin, which is synergized by odors of pine oleoresin released from beetle-damaged host tissue. Synthetic racemic frontalin combined with turpentine has been the operational bait used in traps for monitoring populations of *D. frontalis* in Mexico as well as the United States. Recently, racemic *endo*-brevicomin has been reported to be a synergist of the frontalin/turpentine bait and as an important component of the aggregation pheromone for *D. frontalis* populations in the United States. To determine whether racemic *endo*-brevicomin also might function as an aggregation synergist for the geographically isolated *D. frontalis* populations of Central America and Mexico, we performed a field trapping trial in Lagunas de Montebello National Park, Chiapas, Mexico, during July and August 2007. The combination of *endo*-brevicomin (placed either directly on the trap or 4 m away) plus racemic frontalin and turpentine caught at least 5 times more *D. frontalis* of both sexes than did turpentine either alone or in combination with either frontalin or *endo*-brevicomin. The addition of *endo*-brevicomin to the frontalin/turpentine bait also increased the proportion of females trapped. We conclude that the addition of *endo*-brevicomin might substantially improve the efficiency of the frontalin/turpentine bait for monitoring of *D. frontalis* in Central America and Mexico. We discuss factors that reconcile our results with previous studies that reported *endo*-brevicomin to be an attractant antagonist for populations of *D. frontalis* in Mexico and Honduras.

KEY WORDS bark beetles, *endo*-brevicomin, monitoring, pheromone, attractant synergist

Bark beetles in the genus *Dendroctonus* Erichson (Coleoptera: Curculionidae: Scolytinae) are the most economically important killers of conifers in North and Central America (Wood 1982). Among the 11 species of *Dendroctonus* in Mexico, *D. frontalis* Zimmermann is the most serious pest of Mexican pine forests (Cibrián et al. 1995) due to its broad host range within the genus *Pinus,* and its preference for the widely planted, commercially important species of Ocote pine, *Pinus oocarpa* Schiede ex Schltdl. (Salinas et al. 2004). Successful reproduction by *D. frontalis* requires death of the host tree. Feeding adult beetles and their broods destroy the phloem tissue, thereby obstructing the flow of nutrients to the roots. In addition, growth of symbiotic fungi into the xylem tissue blocks water conduction to the crown, weakens host defenses, and hastens tree death (Paine et al. 1997).

Host finding and colonization by *D frontalis* are mediated by semiochemicals. Females, the sex that initiates attacks, produce the aggregation pheromone frontalin (1,5-dimethyl-6-8-dioxabicyclo [3.2.1.] octane) which is presumably synthesized de novo (Barkawi et al. 2003) and induces attraction and landing of both sexes (Hughes 1974). The combination of racemic frontalin and synergistic host odors in the form of turpentine (a distillate of pine oleoresin) has been the standard attractant used to monitor populations of *D. frontalis* in Mexico (Macías et al. 2006, Sánchez et al. 2007) as well as the United States (Billings et al. 1995, Clarke 2003). Recently, (+)-*endo*-brevicomin (*endo*-7-ethyl-5-methyl-6-8-dioxabicyclo [3.2.1.] octane), a compound produced predominantly by males after pairing (Sullivan et al. 2007), has been reported to be a synergist of frontalin/host odor combinations and likely an important component of the aggregation pheromone of *D. frontalis* (Vité et al. 1985, Sullivan et al. 2007). However, seemingly conflicting data exists concerning the function of *endo*-brevicomin for *D. frontalis*. In trapping experiments conducted in southern United States, *endo*-brevicomin alternatively either enhanced *D. frontalis* response to frontalin and host odors [tests using either racemic or pure (+)-*endo*-brevicomin; Vité et al. 1985, Sullivan et al. 2007] or was antagonistic to attractants [tests using either racemic or pure (−)-*endo*-brevicomin; Vité et al. 1985, Payne et al. 1977, 1978; Salom et al. 1992].

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Populations of *D. frontalis* in Central America and Mexico are isolated from those of the southeastern United States by >500 km of grassland and desert in southwestern Texas and northeastern Mexico (Payne 1980). These two populations differ in their production of *endo*-brevicomin: Vité et al. (1974) found levels of *endo*-brevicomin in newly emerged male *D. frontalis* from Honduras that were much higher than populations from Texas, and more recently Niño (2007) discovered large amounts of *endo*-brevicomin in some mining female *D. frontalis* collected in Chiapas, Mexico. Because *endo*-brevicomin is produced in relatively greater quantities by *D. frontalis* in Central America, we suspected that this pheromone might play a more important role in this species' communication system in this region.

We therefore performed a field trapping experiment to characterize the activity of racemic *endo*-brevicomin with *D. frontalis* populations in Chiapas, Mexico, and to determine whether this compound could enhance catches in this region as previously reported for populations in Texas and Mississippi in the United States (Vité et al. 1985, Sullivan et al. 2007). An enhanced bait for *D. frontalis* would be helpful for establishing a monitoring program for *D. frontalis* populations in southern Mexico, because the commercially available bait has not shown the activity reported in other regions under similar field conditions, where trap catch numbers are >100 times higher (J.M., unpublished data).

Materials and Methods

Experimental Site. The experiment was conducted in Lagunas de Montebello National Park, Chiapas, Mexico. Vegetation on the site was composed by a pine–oak (*Quercus* spp.) forest with uneven-aged mixed stands of *P. oocarpa* and *P. maximinoi* H.E. Moore, where several species of *Quercus* spp. and one *Liquidambar* sp. as the major broad leaf components. This national park is located in the municipality of La Trinitaria, Chiapas, Mexico (16° 06′45.7″ N and 91° 43′56.6″ W, at 1,494-m elevation).

Field Trapping Experiment. An experiment was performed to evaluate the behavioral response of *D. frontalis* to synthetic racemic *endo*-brevicomin, racemic frontalin, and/or turpentine baits. Two complete Latin squares (with trap site as rows and date as columns) were run on 3–8 July 2007 and again on 15–20 August 2007 that compared five trap bait treatments: turpentine alone; turpentine and frontalin; turpentine and *endo*-brevicomin; turpentine, frontalin, and *endo*-brevicomin; and turpentine and frontalin with *endo*-brevicomin released 4 m away from the trap. Studies with *D. frontalis* in southeastern United States indicated that placement of *endo*-brevicomin baits four to 16 m from traps could enhance their synergistic effect (B.T.S., unpublished data). Columns of each Latin square consisted of five 12-unit multiple funnel traps (Pherotech International Inc., Delta, BC, Canada; Lindgren, 1983) in a line with ≈100 m between traps within each square and ≈200 m between squares to avoid interference (Turchin and Odendaal 1996). Treatments were assigned to rows and columns of each square at random and rerandomized without repetition (Winer et al. 1991).

The frontalin bait consisted of a 400-μl capacity low-density polyethylene microcentrifuge tube containing ≈300 μl of racemic frontalin (Chemtica International, San Jose, Costa Rica). The *endo*-brevicomin bait was a 2- by 2-cm polyethylene pouch (Chemtica International) containing ≈30 mg racemic *endo*-brevicomin. The turpentine (steam-distilled from *P. oocarpa;* Pinosa S. de R.L. de C.V., Mexico) was released from a 150-ml capacity amber glass bottle with a piece of cotton wick (1 cm in diameter) immersed in the turpentine and protruding 3 mm from the cap. The release rate of the turpentine bait (5 g/d) was determined gravimetrically in a fume hood at ≈26°C for 5 d, whereas the release rates of the pheromones were provided by the supplier (frontalin, 2.5 mg/d; *endo*-brevicomin, 0.3 mg/d; both at 20°C). The 0.3 mg/d for the *endo*-brevicomin was taken from a similar release rate of 0.2 mg/d from Sullivan et al. (2007) and from some dose–response trials not yet published from the same authors.

Traps were suspended from metal poles with the bottom funnel ≈0.5 m above the ground. Traps were positioned >10 m from the closest pine (to avoid inducing "spillover" attacks) and >100 m from the nearest *D. frontalis*-infested tree. Trap cups were filled with a few centimeters of soapy water to prevent trapped insects from escaping. The frontalin and *endo*-brevicomin baits were hung separately with twist ties near the middle of the trap (sixth funnel from the bottom), and the turpentine bottle was placed within the upper funnel and beneath the trap top to protect the wick from rain. For treatments with the *endo*-brevicomin placed 4 m from the trap, the lures were hung at a height of ≈1 m from a branch of an adjacent hardwood shrub. Trapped *D. frontalis* were collected daily, preserved in 70% ethanol, counted, and sexed by the presence of the mycangial bulge in females (Barras 1967) and the deep frontal groove in males (Osgood and Clark 1963).

Statistical Analysis. We analyzed the experiment as a bifactorial in Latin square design (Winer et al. 1991), where factors in the analysis of variance (ANOVA) were bait treatment, month of trapping, row, and column (JMP 2000, version 4, SAS Institute 2000). To improve data normality and homoscedasticity, raw catches of *D. frontalis* were transformed with the Box–Cox transformation [males, $(X + 0.5)^{-0.4}$; females $(X + 0.5)^{-1.2}$]. All pairwise comparisons among treatments within significant factors were made with Tukey's test ($\alpha = 0.05$; JMP 2000, version 4, SAS Institute 2000. Additionally, sex ratios responding to bait treatments were compared for homogeneity of proportions ($\alpha = 0.05$, chi-square test; Zar 1984)).

Results

The numbers of trapped *D. frontalis* males ($F = 49.90$; df = 4, 82; $P < 0.001$) and females ($F = 17.39$,

Table 1. ANOVA of both male and female *D. frontalis* caught in multiple-funnel traps baited with synthetic semiochemicals during 3–8 July 2007 and 15–20 August 2007 in Lagunas de Montebello National Park, Chiapas, Mexico

Source	df	SS	MS	F	P
Males					
Trap position	4	1.472	0.368	5.890	0.000
Date	4	0.689	0.172	2.760	0.033
Bait	4	12.465	3.116	49.900	0.000
Month	1	0.646	0.646	10.350	0.002
Bait × month	4	0.264	0.066	1.060	0.382
Error	82	5.120	0.062		
Total	99	20.657			
Females					
Trap position	4	2.137	0.534	1.110	0.357
Date	4	3.902	0.976	2.030	0.098
Bait	4	33.444	8.361	17.390	0.000
Month	1	1.302	1.302	2.710	0.104
Bait × month	4	2.930	0.733	1.520	0.203
Error	82	39.424	0.481		
Total	99	83.137			

df = 4, 82; $P < 0.001$) were significantly influenced by bait treatment (Table 1; Fig. 1). Traps baited with the combination of turpentine, racemic frontalin, and racemic *endo*-brevicomin (either placed directly on the trap or 4 m away) caught more *D. frontalis* of either sex than turpentine either alone or in combination with either racemic frontalin or racemic *endo*-brevicomin. Turpentine/racemic frontalin baits trapped more males than turpentine either alone or combined with racemic *endo*-brevicomin but failed to attract females. The location of the racemic *endo*-brevicomin bait (i.e., either directly on the trap with the racemic frontalin and turpentine or 4 m away) did not affect the number of responding beetles of either sex. No interaction between bait treatment and month of trapping was found either in males ($P = 0.38$) or females ($P = 0.20$). The effect of trap position and month of trapping was significant only in males ($P < 0.001$ and $P < 0.01$, respectively; Table 1). The sex ratio (male:female) trapped by turpentine/racemic frontalin baits (13.4:1) was significantly reduced with the addition of racemic *endo*-brevicomin directly to the trap (4.3:1) [$\chi^2_{(1,0.05)} = 6.06$, $P = 0.013$] but did not change when this pheromone was displaced 4 m from the trap (6.5:1) [$\chi^2_{(1,0.05)} = 2.40$, $P = 0.12$], as shown in Fig. 1.

Discussion

Our findings are in concordance with those of Vité et al. (1985) and Sullivan et al. (2007), who found synergistic effects when either racemic or (+)-*endo*-brevicomin was added to turpentine/frontalin baits. However, they contrast with other studies that reported that racemic *endo*-brevicomin produced a significant decrease in *D. frontalis* responses to attractant (Vité and Renwick 1971; Vité et al. 1974; Payne et al. 1977, 1978; Salom et al. 1992). Vité et al. (1985) discovered that (−)-*endo*-brevicomin inhibited attraction of *D. frontalis,* and they speculated that the inhibitory activity reported for racemic *endo*-brevicomin was due to the inhibitory (−)-enantiomer overriding the synergistic activity of the (+)-enantiomer when the racemic mixture was released at a relatively high rate. Our data seem to conform to this hypothesis, because our release rate of racemic *endo*-brevicomin (0.3 mg/d) was substantially less than that reported in published studies in which racemic *endo*-brevicomin inhibited responses to attractant-baited traps (12 mg/d; Payne et al. 1978, Salom et al. 1992; rate not reported in other studies). Additionally, our observation of synergistic attractive activity for *endo*-brevicomin may have been influenced by our use of atypically large spacing both among traps as well as between traps and natural sources of *D. frontalis* semiochemicals. Studies in the southeastern United States have shown that insufficient trap spacing and competing sources of semiochemicals can conceal the attractive activity of *endo*-brevicomin baits with *D. frontalis* (B.T.S., unpublished data). In the only previous investigation of behavioral responses of Mexican and Central American populations of *D. frontalis* to *endo*-brevicomin, this semiochemical inhibited re-

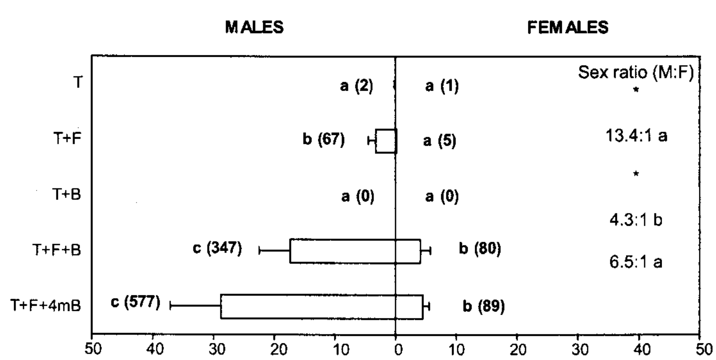

Fig. 1. Mean (±SE) catch of *D. frontalis* in multiple-funnel traps baited with turpentine (T), racemic frontalin (F), and/or racemic *endo*-brevicomin on trap (B) or 4 m away (4mB), during 3–8 July 2007 and 15–20 August 2007, in Lagunas de Montebello National Park, Chiapas, Mexico. Within sex, bars associated with the same letter were not statistically different ($\alpha = 0.05$; Tukey's test). Total numbers of beetles caught are given in parentheses. * Sex ratio of treatment excluded from the analysis of proportions ($\alpha = 0.05$; chi-square test) due to low catches.

sponses to traps baited with frontalin and α-pinene; however, neither the dose and chirality of the *endo*-brevicomin nor the trap spacing were reported (Vité et al. 1974).

In our study, moving the racemic *endo*-brevicomin bait 4 m away from a frontalin/turpentine-baited trap caused no significant change in its synergistic effect. Hence, *D. frontalis* in Chiapas, Mexico, resemble those of the southern United States in that the source of *endo*-brevicomin need not be collocated with a source of frontalin/turpentine for synergistic activity to be evident (B.T.S., unpublished data). Thus in both regions *endo*-brevicomin seems to mediate long-range attraction but not landing of *D. frontalis.*

The sex ratio (male:female) trapped by the turpentine/frontalin baits in our experiment (13.4:1) differed greatly from those in similar field trapping tests conducted in the United States (3.4:1, Sullivan et al. 2007; 4.6:1, Vité et al. 1985; 1.5:1, Salom et al. 1992; and 1:0.46, Payne et al. 1978). Strongly male-skewed sex ratios of *D. frontalis* are typical for catch in traps baited with turpentine/frontalin in Chiapas (J.M., unpublished data.). The novel ability of the three-component bait to trap significant numbers of females may enhance the management potential of *D. frontalis* traps in this region, because trap-out schemes and population predictions may be more successful if they address both sexes as is the case in other *Dendroctonus* spp. where the addition of other pheromone components got better attraction (Rudinsky and Ryker, 1980, Borden et al., 1996).

In summary, our data suggest that the addition of a 0.3 mg/d racemic *endo*-brevicomin commercially available bait placed either on or a short distance from the trap might substantially improve the efficiency of frontalin and turpentine as the operational bait for detection and monitoring of *D. frontalis* in Central America and Mexico (Macías et al. 2006, Sánchez et al. 2007).

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