

Field Evaluations of Potential Aggregation Inhibitors for the Southern Pine Beetle, *Dendroctonus frontalis* (Coleoptera: Curculionidae)¹

Brian T. Sullivan,² Mark J. Dalusky,³ David Wakarchuk⁴ and C. Wayne Berisford³

Southern Research Station, USDA Forest Service 2500 Shreveport Highway,
Pineville, Louisiana 71360 USA

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Abstract Semiochemicals that inhibit the response of the southern pine beetle, *Dendroctonus frontalis* Zimmermann, to its aggregation pheromone have been used with varying degrees of success to protect individual trees from attack and to stop infestation growth. However, semiochemical disruptants have not experienced wide use in management of *D. frontalis*, due in part to the normally prohibitive expense associated with treatments using verbenone and 4-allylnisole, the two EPA-registered semiochemicals for this species. Therefore, we conducted some initial trap-based screenings of candidate compounds with the aim of discovering alternative inhibitory semiochemicals for use in management of *D. frontalis*. In separate experiments in Mississippi and Georgia, baits containing either 2-phenylethanol or myrtenol significantly reduced attraction of one or both sexes of *D. frontalis* to traps baited with a standard attractant (i.e., the *D. frontalis* aggregation pheromone frontalin and the host monoterpene *alpha*-pinene). In combination, the two compounds caused a 92% decrease in total beetle response to the standard attractant, although this reduction was not significantly greater than that produced by 2-phenylethanol alone. In one test, a blend of nonhost volatiles (1-hexanol, *cis*-3-hexen-1-ol, hexanal, and nonanal) significantly reduced attraction of male *D. frontalis*, but these observations were not duplicated in a second test. Another combination of candidate inhibitors (the nonhost blend plus guaiacol and benzaldehyde) also significantly inhibited response of male beetles. At the specific doses used in our tests, we failed to observe reduction in *D. frontalis* attraction by the following compounds presented singly: benzaldehyde, guaiacol, 3-methylcyclohex-2-en-1-one (3,2-MCH), myrtenal, and verbenone.

Key Words southern pine beetle, semiochemical, pheromone, disruption, nonhost volatile, bioinsecticide, antiaggregation

Bark beetles in the genera *Dendroctonus* and *Ips* rely heavily upon olfactory cues for mediating sexual behavior, synchronizing mass attack on trees, selecting appropriate hosts, avoiding competing beetle species, and partitioning resources with conspecifics (Byers 1989). Beetle dependence on semiochemicals can be exploited through the deployment of synthetic baits that manipulate beetle behavior in beneficial ways, and successful semiochemical-based management techniques have been developed for a number of bark beetles species (Borden 1995, Skillen et al. 1997).

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²Address inquiries (email: briansullivan@fs.fed.us).

³Department of Entomology, University of Georgia, Athens, GA 30602.

⁴Synergy Semiochemical Inc., Box 50008, South Slope RPO, Burnaby, BC, Canada.

Table 1. Baits used in trapping tests of candidate attraction inhibitors for *D. frontalis*

Chemical name	Abbreviation	Test number	Source	Purity*	Chirality	Release device	Release rate (mg/d)**
Standard attractant	SA	all					
Frontalin			Phero Tech	99%	racemic	polyethylene centrifuge tube	5§
α -Pinene			Acros† Aldrich‡	>95%	(-)-†/racemic‡	2 polyethylene centrifuge tubes	18§
Test compound							
Guaiacol	G	1, 3	Phero Tech	>98%	non-chiral	polyethylene vial† bubble cap‡	30†, 5‡
Nonhost blend	NHB	1, 2					
1-Hexanol			Phero Tech	>98%	non-chiral	bubble cap	4
<i>cis</i> -3-Hexen-1-ol			Phero Tech	>98%	non-chiral	bubble cap	4
Hexanal			Phero Tech	>96%	non-chiral	urethane rope	50
Nonanal			Phero Tech	>93%	non-chiral	urethane rope	50

Table 1. Continued.

Chemical name	Abbreviation	Test number	Source	Purity*	Chirality	Release device	Release rate (mg/d)**
Benzaldehyde	B	1	Phero Tech	>98%	non-chiral	flex lure	5
3-Methylcyclo-hex-2-en-1-one	MCH	2, 3	Phero Tech	>98%	non-chiral	bubble cap	5
2-Phenylethanol	PE	4, 5	Phero Tech‡ Chemtica†	>96%	non-chiral	bubble cap	4†, 3§‡
Myrtenol	Mol	4, 5	Phero Tech‡ Chemtica†	>95%	(-)	bubble cap	3†, 1.5§‡
Myrtenal	Mal	4	Phero Tech	>98%	(-)	bubble cap	2
Verbenone	V	4	Phero Tech	>90%	(+)	bubble cap	5

* Provided by supplier (or measured by authors using GC when supplier data not available).

** Measured gravimetrically at 20°C by the supplier unless otherwise noted.

§ Measured gravimetrically by the authors in a fume hood at 22°C.

† Test 1, 2, and 5 (Chemtica International, San Jose, Costa Rica).

‡ Test 3 and 4 (Acros Organics, Geel, Belgium; Aldrich, Milwaukee, WI).

Table 2. Bait treatments, dates, and locations of individual trapping experiments for *D. frontalis*

	Trap treatments	Replicates	Dates	Site
Test 1	unbaited, SA, SA + B, SA + G, SA + NHB, SA + G + NHB + B	6	22 July-24 Aug. 2001	Putnam Co., GA
Test 2	unbaited, SA, SA + MCH, SA + NHB, SA + MCH + NHB	6	31 May-7 June 2002	Putnam Co., GA
Test 3	unbaited, SA, SA + MCH, SA + G	9	23-29 Aug. 2002	Franklin Co., MS
Test 4	unbaited, SA, SA + PE, SA + Mol, SA + Mal, SA + V	13	17 July-23 Aug. 2002	Franklin Co., MS
Test 5	SA, SA + PE, SA + Mol, SA + Mol + PE	7	5 Aug.-2 Sept. 2003	Stephens Co., GA

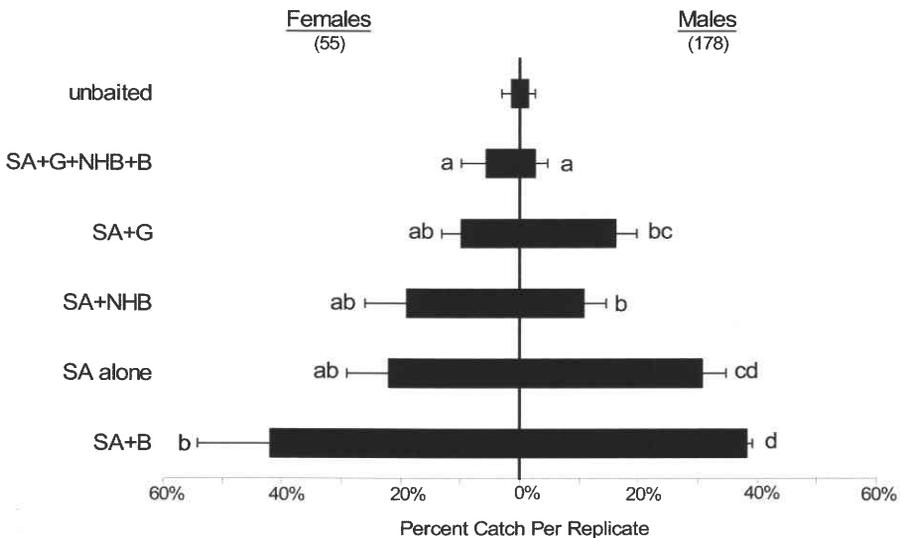


Fig. 1. Test 1. Responses of male and female *D. frontalis* to funnel traps baited with a standard attractant (SA) either alone or in combination with a blend of four nonhost volatiles (NHB), guaiacol (G), benzaldehyde (B), or all three. Additional details of baits are given in table 1. Bars represent mean (+SEM) percentage catch per replicate (i.e., the catch in each trap divided by catch for all traps in its replicate). Totals trapped for each sex are given in parentheses. Treatments associated with the same letter were not significantly different in mean numbers of beetles of each sex trapped per replicate (Tukey's test, $\alpha = 0.05$). Catches in unbaited control traps were excluded from statistical analyses.

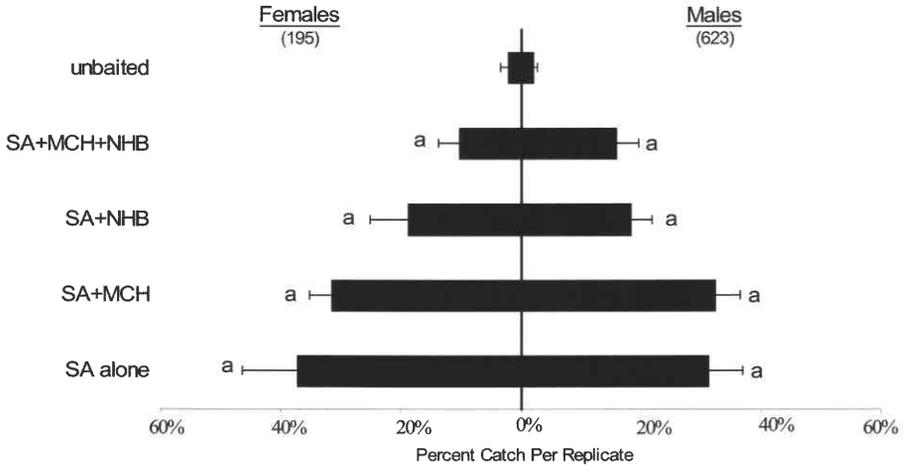


Fig. 2. Test 2. Responses of male and female *D. frontalis* to funnel traps baited with a standard attractant (SA) either alone or in combination with a blend of four nonhost volatiles (NHB), 3-methylcyclohex-2-en-1-one (MCH), or both. Additional details are identical to those given in the legend for figure 1.

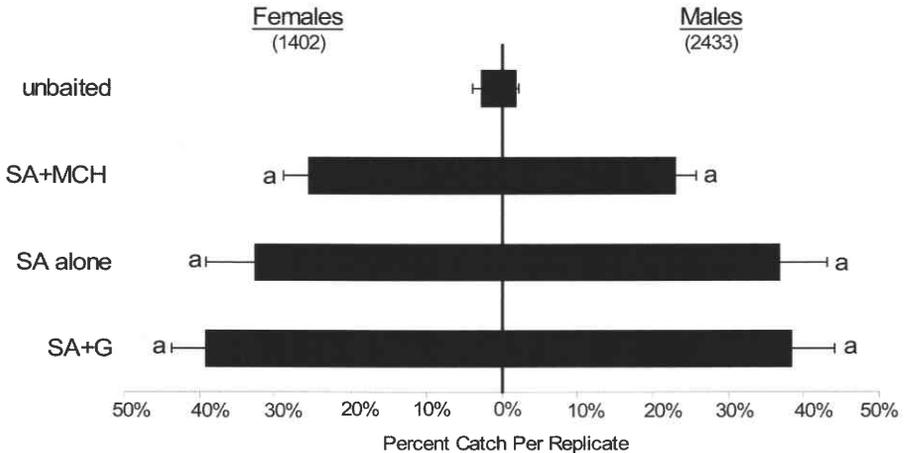


Fig. 3. Test 3. Responses of male and female *D. frontalis* to funnel traps baited with a standard attractant (SA) either alone or in combination with guaiacol (G) or 3-methylcyclohex-2-en-1-one (MCH). Additional details are identical to those given in the legend for figure 1.

Dendroctonus frontalis and several other species of *Dendroctonus* and *Ips* bark beetles have been found to produce 2-phenylethanol in small amounts (Renwick et al. 1976, Pureswaran et al. 2000, Sullivan 2005). 2-phenylethanol has been isolated also from cultures of yeast associates of *D. frontalis* (Brand et al. 1977). Bioassays in a

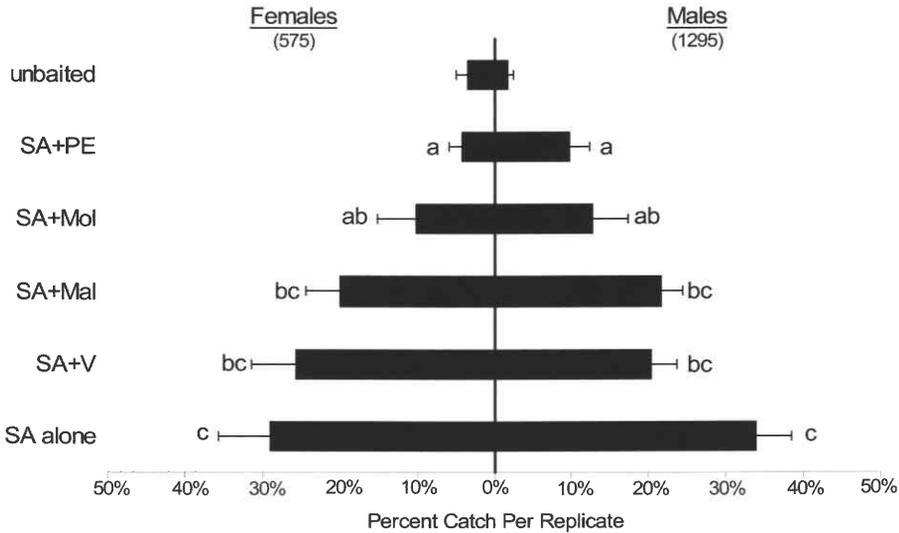


Fig. 4. Test 4. Responses of male and female *D. frontalis* to funnel traps baited with a standard attractant (SA) either alone or in combination with 2-phenylethanol (PE), myrtenol (Mol), myrtenal (Mal), or verbenone (V). Additional details are identical to those given in the legend for figure 1.

platform olfactometer showed that this compound could inhibit response of walking *D. frontalis* to attractant (Brand et al. 1977). Additionally, response of male *D. frontalis* to traps baited with frontalin and *alpha*-pinene was significantly reduced when baits releasing 2-phenylethanol at either 8 or 80 mg/d (measured at 22°C) were added, whereas no significant reduction occurred at 0.8 mg/d (Sullivan 2005). 2-phenylethanol similarly has been shown to reduce response by *Dendroctonus ponderosae* Hopkins to attractant-baited traps (Pureswaran et al. 2000). The variety of possible origins for 2-phenylethanol in the environment of *D. frontalis* (including conspecifics, heterospecific bark beetles, and associated fungi) suggests that this compound could have multiple functions in their biology, including avoidance of intra/interspecific competition and fungi-degraded host tissue.

Guaiacol, benzaldehyde, and compounds in the nonhost blend belong to a class of volatile chemicals that are associated with foliage and/or bark of angiosperm trees but not conifers, and evidence indicates that many coniferophagous bark beetle species are repelled by such "nonhost volatiles" either singly or blended (Zhang and Schlyter 2004). Two of the compounds in our nonhost blend (1-hexanol and hexanal) were previously shown to reduce *D. frontalis* responses to its aggregation pheromone (Dickens et al. 1992). Additionally, blends incorporating guaiacol, benzaldehyde, or two of the compounds in our nonhost blend (*cis*-3-hexen-1-ol and nonanal) have been shown to disrupt responses by certain other *Dendroctonus* spp. to aggregation pheromones (Zhang and Schlyter 2004). Although not inhibitory singly, guaiacol and benzaldehyde enhanced the inhibitory properties of the nonhost blend in test 1. By itself, the nonhost blend was significantly inhibitory in test 1, but not in test 2 (although the

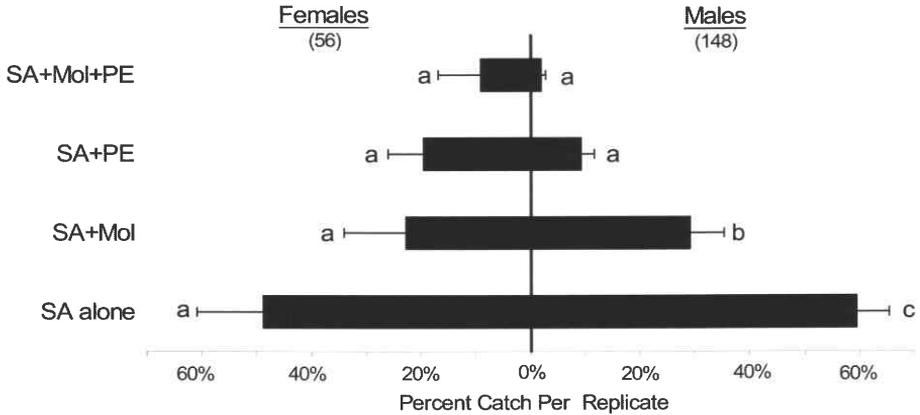


Fig. 5. Test 5. Responses of male and female *D. frontalis* to funnel traps baited with a standard attractant (SA) either alone or in combination with 2-phenylethanol (PE), myrtenol (Mol), or both. Additional details are identical to those given in the legend for figure 1.

mean catch was reduced by approx. 40%). The relatively small number of replicates (six) executed in test 2 could have contributed to our failure to observe statistically significant catch reductions by the nonhost blend.

3,2-MCH alone failed to disrupt attraction of *D. frontalis* in separate tests in Georgia and Mississippi. 3,2-MCH is an antiaggregation pheromone for both *Dendroctonus pseudotsugae* Hopkins and *Dendroctonus rufipennis* (Kirby) (Borden 1996). It is currently in operational use for protecting individual trees and stands from attacks by *D. pseudotsugae* (Ross et al. 2002), and it has shown promise for similar uses with *D. rufipennis* (Borden 1996, Holsten et al. 2003). We chose this particular compound for bioassay against *D. frontalis* because closely related bark beetle species sometimes have been found to respond to the same antiaggregation pheromones (Borden 1996), and coupled gas chromatograph-electroantennographic detection tests indicated that *D. frontalis* possesses olfactory sensitivity for this compound (B.T. Sullivan, unpublished data).

Neither myrtenal nor verbenone inhibited *D. frontalis* response to attractant at the concentrations assayed in test 4. Myrtenal, like verbenone and myrtenol, is an oxidation product of the host terpene *alpha*-pinene, and is produced almost exclusively by male beetles (Renwick et al. 1973). Sullivan (2005) found that myrtenal reduced *D. frontalis* response to attractant when released at 66 mg/d (measured at 22°C) but not at rates one and two orders of magnitude below this. This finding is consistent with the results of test 4 in which myrtenal was released at 2 mg/d (measured at 20°C). The ability of (+)-enriched verbenone to inhibit *D. frontalis* responses to frontalinal/host terpene mixtures was documented by Salom et al. (1992). However, the release rate of our (+)-verbenone baits in test 4 (5 mg/d measured at 20°C) was possibly substantially less than the lowest active rate described in this earlier study (24 mg/d, temperature not reported; Salom et al. 1992), and this might explain the discrepancy in observed activities.

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