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REPELLENT PROPERTIES OF THE HOST COMPOUND 4-ALLYLANISOLE TO THE SOUTHERN PINE BEETLE[1]

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Abstract—The phenylpropanoid 4-allylanisole is a compound produced by loblolly pines (*Pinus taeda* L.), an abundant species in southern pine forests and a preferred host of southern pine beetle (*Dendroctonus frontalis* Zimmermann). Repellency of individual beetles was demonstrated in laboratory behavioral assays of *D. frontalis* and other scolytids. Inhibition was demonstrated in natural populations of *D. frontalis* using baited traps. In both tests, response to the inhibitory pheromone verbenone was used for comparison. In the laboratory, a higher proportion of newly emerged and reemerged *D. frontalis* responded negatively to 4-allylanisole than to verbenone. However, fewer reemergent than newly emerged individuals responded to either compound. In all field trials, the response of *D. frontalis* to its attractant pheromone in funnel traps was significantly reduced by simultaneous release of 4-allylanisole. In most trials total reduction did not differ from verbenone; however, unlike verbenone, 4-allylanisole reduced male and female catches proportionally. Both compounds together did not significantly further reduce trap catch. The response of a major predator, *Thanasimus dubius* (F.), to the attractant pheromone of *D. frontalis*, did not differ with the simultaneous release of either verbenone or 4-allylanisole. The results of preliminary field applications are presented and discussed.

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Key Words—Coleoptera, Scolytidae, *Dendroctonus frontalis*, *Pinus*, host compound, 4-allylanisole, repellent, semiochemical, verbenone, inhibitor.

INTRODUCTION

Compounds originating in host trees are known to produce a wide range of behavioral responses in scolytids. Due to their importance in tree defense and insect olfaction, volatile components of the host's pre-formed resin system have received particular attention in research with aggressive, tree-killing bark beetles. Most studies have examined either pesticidal (e.g., Smith, 1965; Coyne and Lott, 1976; Bridges, 1987; Himejima et al., 1992) or semiochemical (see reviews by Wood, 1982; Raffa et al., 1993) effects of monoterpenes on beetles and their associated organisms. Studies usually focus on their role in mass-attack: either as primary attractants (e.g., Heikkenen and Hrutfiord, 1965; Rudinsky, 1966a,b; Werner, 1972a,b; Moeck et al., 1981; Moeck and Simmons, 1991) or as precursors (e.g., Byers and Wood, 1981; Francke and Vité, 1983; Hunt et al., 1989) or synergists (e.g., Renwick and Vité, 1969, 1970) in secondary attraction. In the southern pine beetle (*Dendroctonus frontalis* Zimmermann)–southern yellow pine (*Pinus taeda* L., *P. palustris* Mill., *P. elliotti* Engelm. var. *elliottii*, *P. echinata* Mill., *P. virginiana* Mill.) system, primary attraction has not been demonstrated, but α-pinene, a major host monoterpene in all of these pines, is well known to act synergistically with frontalin, the primary component of the aggregation pheromone, to stimulate mass attack by *D. frontalis* (Renwick and Vité, 1969, 1970).

Anti-aggregation pheromones are important components in terminating the attack process of many aggressive scolytids (Wood, 1982; Raffa et al., 1993), but less is known about the potential of host compounds as inhibitors or repellents. The host compound myrcene was shown to interrupt the response of *Dendroctonus brevicomis* LeConte to its pheromone blend, which also contains myrcene (Tilden et al., 1981). Both limonene, with *Scolytus ventralis* LeConte (Bordasch and Berryman, 1977), and β-pinene, with *D. pseudotsugae* Hopkins (Heikkenen and Hrutfiord, 1965), have repellent properties in walking olfactory assays. *D. pseudotsugae* is also repelled by volatile components of its host resin while walking (Rudinsky, 1966b; Jantz and Rudinsky, 1965), but these same compounds are attractants during flight (Rudinsky, 1966a). Nonhost, green-leaf volatiles are the only phytochemicals known to inhibit aggregation of *D. frontalis*, and these effects are mild compared to those of inhibitors produced by conspecifics (Dickens et al., 1992). Attempts to apply pine oil, a mixture of various host and other compounds (Nijholt, 1980), to unattacked pines as a repellent for *D. frontalis* have met with limited success (Berisford et al., 1986; O'Donnell et al., 1986). Presumably host compounds contained in the mixture

are responsible for its repellent properties, but further work is needed to identify active compounds and verify this assertion.

The phenylpropanoid, 4-allylanisole (also commonly known as methyl chavicol or estragole) is known from numerous pine and other conifer species (Mirov, 1961; Drew and Pylant, 1966). Although common, this compound usually exists in relatively minor quantities of the volatile component of the resin (Sutherland and Wells, 1956; Mirov, 1961; Drew and Pylant, 1966; Hayes et al., 1994); however, foliar quantities may be higher (Zavarin et al., 1971; Cobb et al., 1972). In the southern yellow pines, 4-allylanisole is found in roughly comparable amounts among species, usually making up 1–5% of the turpentine yields (Sutherland and Wells, 1956; Mirov, 1961; Drew and Pylant, 1966). While receiving less emphasis than individual monoterpenes, 4-allylanisole appears to have potentially important biological effects. A saturated 4-allylanisole environment retards the growth of fungi associated with *D. frontalis* including the blue-staining fungus *Ophiostoma minus* (Hedgc.) H. & P. Sydow and two mycangial fungi (Bridges, 1987). Although they erroneously claim to have been the first to identify 4-allylanisole from loblolly pine (a claim cited again by Nebeker et al., 1993), Gambliel et al. (1985) found higher levels in phloem inoculated with *O. minus*. In addition to a higher level of 4-allylanisole produced as part of a localized wound response to fungi, this compound has also been shown to increase more globally in *Pinus ponderosa* Laws. leaves exposed to high levels of air pollution (Cobb et al., 1972).

The potential semiochemical role of 4-allylanisole for scolytids has received little attention. Testing of 4-allylanisole as a semiochemical for *D. frontalis* has been limited to a single study in which synergistic activity with frontalin was not found (Renwick and Vité, 1969, 1970). In walking olfactory assays, using terpenoid compounds isolated from *P. taeda*, Werner (1972a,b) found that 4-allylanisole was attractive to (especially male, the pioneering sex) *Ips grandicollis* (Eichoff) at a 1% concentration. In a survey of volatiles associated with *D. frontalis* brood trees, Salom et al. (1991, 1992a) determined that 4-allylanisole elicited little in the way of electroantennogram responses of two *D. frontalis* parasitoids. Cobb et al. (1972) speculate that foliar 4-allylanisole levels of *P. ponderosa*, especially as influenced by photochemical air pollution, may influence attack behavior of *D. brevicomis* and *D. ponderosae* Hopkins, but the idea was not directly tested. The potential role of 4-allylanisole as an antiaggregant or repellent for *D. frontalis* and other scolytids is unknown.

In this paper, we describe and report the results of laboratory and field assays of the repellent properties of the host compound 4-allylanisole against *D. frontalis*, related bark beetles, and associated beetles, compared with the *D. frontalis* inhibitory pheromone, verbenone. Preliminary field applications of this potential repellent on lightning-struck trees are also described.

METHODS AND MATERIALS

Laboratory Assay. Individual beetle response to 4-allylanisole, versus verbenone, was determined in a simple assay. A strip (5 mm wide) of 4-allylanisole (Aldrich Chemical Co., Inc. Milwaukee, Wisconsin) or verbenone (34% + :66% −, Borregaard, Inc., Sarpsborg, Norway) was "painted" (neat) with a camel's-hair brush in a circle (≈ 17 cm diam) on a piece of uncoated (tablet backing) cardboard (28 × 21.5 cm). For comparison (controls), we observed the behavior of *D. frontalis* released in untreated circles (outlined in pencil) and in circles treated with the attractant frontalure (2 : 1 α-pinene–frontalin; Aldrich and BASF, Limbergerhoff, Germany, respectively). Three minutes after treatment, which allowed the materials to be absorbed into the cardboard, beetles (two to five individuals) were released in the center of the treated circle. Responses (≤ 30 sec exposure) were recorded as not repelled or repelled; scored beetles were then placed in separate containers by response and later sexed. A beetle was designated *not repelled* if it walked through the treated circle or stopped but proceeded across the circle within 30 sec of exposure. A beetle was designated *repelled* if it moved toward the circle but stopped abruptly and raised its antennae (some "rear-up" on hind legs), stood motionless, and/or moved away from the circle (some move abruptly in the opposite direction) without crossing out of the circle.

Testing was conducted at room temperature (22–25°C) with light supplied from an adjoining room. An object was used to cast a shadow over the test circle (< 1 lumen/m^2). Beetles were refrigerated briefly before testing to reduce their tendency to fly. Only beetles that were capable of walking up the sides of a collection container were used for this experiment. In each trial, including controls, 50 apparently healthy beetles were selected at random and tested. Another 50 beetles, previously unexposed, were then selected and tested with the other compound. The order of compound use was also random.

Trials were conducted with:

1. Newly emerged *D. frontalis* in three trials (N = 50 beetles per compound per trial) were collected on three different dates from the same source population (Colfax, Louisiana). Beetles were obtained from two infested loblolly pines (≈ 24 cm dbh), which were felled with brood in the pupal or callow adult stage. Four bolts (45 cm long) were cut from each tree and placed in two separate (by tree) rearing cans to collect adults as they emerged (Browne, 1972). Assays were conducted on the day of emergence; beetles tested on March 12, 1992, originated from one tree and those tested on April 3 originated from the other tree.

2. Newly emerged and reemerged *D. frontalis* from the same generation and source population (Colfax, Louisiana) were used. Newly emerged beetles (N = 100) were obtained as described above from an infested loblolly at the

front of an active infestation with brood in the callow adult stage. A nearby freshly attacked tree was felled in order to obtain parent adults ($N = 100$), which emerged ≈ 20 days later. Bolts from each tree were placed in rearing cans for collection.

3. Trials were also conducted with a clerid beetle, *Thanasimus dubius* (F.) and five other scolytid species. *T. dubius* ($N = 50$) for this study were obtained over a three-day period from five traps baited with *D. frontalis* attractant (frontalin + turpentine) (Catahoula Ranger District, Kisatchie National Forest, Louisiana). Newly emerged *Ips avulsus* (Eichhoff) ($N = 50$) and *I. calligraphus* (Germar) ($N = 9$), typical associates of *D. frontalis* were obtained from rearing cans containing bolts of a single loblolly tree removed from an infestation containing both *D. frontalis* and *Ips*. Mountain pine beetles ($N = 50$), *D. ponderosae*, a univoltine western species, were extracted prior to emergence from bolts of lodgepole pines (*P. contorta* Dougl. ex. Loud.) from a site (≈ 16 km north of LaPine, Oregon) in central Oregon and mailed overnight on ice to the Alexandria Forestry Center (AFC), Pineville, Louisiana, for testing. Similarly, western pine beetles ($N = 50$), *D. brevicomis*, another western species ecologically similar to *D. frontalis* were extracted prior to emergence from ponderosa pine (*P. ponderosa*) bolts from a site near MiWuk Village, California, in the Sierra foothills. These insects were sexed and then mailed overnight on ice to AFC for testing. Newly emerged *Ips pini* (Say), a transcontinental northern species, were obtained from a laboratory colony maintained on red pine (*Pinus resinosa* Ait.), housed at the University of Wisconsin, which was originated from Sauk County, Wisconsin, and replenished annually; specimens were mailed overnight to AFC. Newly emerged spruce beetles, *Dendroctonus rufipennis* (Kirby), from sitka spruce [*Picea sitchenis* (Bong.) Carr.] were mailed to AFC from Fairbanks, Alaska. In all cases, only apparently healthy beetles were used.

Field Assay. A test of the response of local beetle populations to 4-allylanisole and verbenone (vs. the attractancy of frontalure) was conducted using baited multiple-funnel traps (Lindgren, 1983). Traps (16-unit; Phero Tech, Inc., Delta, British Columbia, Canada) were placed in active *D. frontalis* infestations in the spring (April–June) (six replicates in six sites) and fall (September) (seven replicates in four sites); site, elution device, and inclusive dates for each trial are given in Table 1. Treatments (two traps each) consisted of frontalure, frontalure + verbenone, and frontalure + 4-allylanisole. Traps were placed no less than 10 m from each other, from green trees, or from infested trees with emerging brood. Placement of treatments was initially randomly assigned and then changed daily in a sequential order for six days. In the spring replications, baits were moved daily among stationary traps, whereas in the fall whole traps with baits were moved. Although no contamination effects were detected, the protocol was changed to avoid the possibility of contaminating traps while mov-

TABLE 1. SOUTHERN PINE BEETLE INFESTATION LOCATION, ELUTION DEVICE, AND INCLUSIVE DATES OF EACH FIELD ASSAY WITH SEMIOCHEMICAL-BAITED TRAPS

Site	Location	Elution device	Dates
A. Spring			
GT1	Winn RD	bark	4/14–4/22
GT2	Winn RD	sponge	4/23–5/04
HUNT	Catahoula RD	sponge	5/03–5/20
EV2306	Evangeline RD	sponge	5/15–6/20
EV2273	Evangeline RD	sponge	5/31–6/07
EV2277	Evangeline RD	sponge	5/30–6/07
B. Fall			
EV2402	Evangeline RD	wick	9/12–9/17
EV2402	Evangeline RD	sponge	9/22–9/28
EV2403	Evangeline RD	wick	9/12–9/17
EV2403	Evangeline RD	sponge	9/22–9/28
CT	Catahoula RD	wick	9/12–9/18
CT	Catahoula RD	sponge	9/21–9/27
Winn	Winn RD	sponge	9/25–9/30
C. 4-Allylanisole + verbenone combination trial			
Winn	Winn RD	(4-allyl) wick (verb) sponge	10/08–10/15
D. Dose response (one, two, or four 4-allylanisole elution devices)			
GT2	Winn RD	sponge	5/09–5/17
EV2277	Evangeline RD	sponge	6/16–6/21
CT	Catahoula RD	wick	10/03–10/15
EV2402	Evangeline RD	wick	10/03–10/15
EV2403	Evangeline RD	wick	10/03–10/07
E. Dose–response (1:0, 3:1, 1:1, 1:3, 0:1 turpentine–4-allylanisole)			
GT	Winn RD	pipet	4/24–5/18

ing baits. To kill collected beetles, collection cups contained a 5.5 × 2.0-cm piece of 2,2-dichlorovinyldimethylphosphate (Pest Strip, Loveland Industries, Inc., Greeley, Colorado). *Dendroctonus frontalis* and *T. dubius* were collected and the number recorded daily.

Bait preparation differed slightly between seasons (Table 1): In spring, frontalure (≈3.5 ml) was eluted from a single polyethylene transfer pipette (Samco, St.-Amand Mfg. Co., Inc., San Fernando, California); verbenone (5 ml; 66% − : 34% +) was eluted from a 2.5 × 1.75 × 0.375-cm cellulose sponge in a 0.7-mil white plastic bag (low density polyethylene; United Plastic Films, Inc., Carterville, Georgia); and 4-allylanisole (5 ml) was prepared in bags the same way as verbenone. In fall, frontalure was unchanged; verbenone (10 ml; 66% − : 34% +) was eluted from a bag containing two cellulose sponges (Phero

Tech); and in four sites 4-allylanisole was eluted from a 20-ml polyethylene scintillation vial (Kimble Glass Inc., Vineland, New Jersey) with cotton wick (Fisher Scientific, Pittsburgh, Pennsylvania) in one trial, followed by a second trial in three of the four sites using the bag devise employed in the spring replicates.

Elution rates were determined gravimetrically at 24-hr intervals under typical field conditions in the spring and in the laboratory during the fall (Table 2).

To determine if there was a difference between sexes in response to the various baits, sex ratios were obtained from one spring replicate (GT1, see Table 1) based on subsampling up to 50 *D. frontalis* per trap from daily collections. Similarly, as part of another study, sex ratios were obtained for *D. frontalis* trapped per day in an assay conducted July 3–9, 1993, in an active infestation on the Catahoula RD (CA3034). In this test, traps were baited with frontalure alone and simultaneously with verbenone, 4-allylanisole, limonene, or 4-allylanisole + limonene (only the sex ratio data will be reported here, study results will be reported elsewhere). These data were analyzed for fit to 1:1 by chi-square and contingency-table analysis (Statistix 4.0; Analytical Software, 1992).

The dose–response of *D. frontalis* to 4-allylanisole was tested using two methods: (1) Response was tested to frontalure given an increasing number of 4-allylanisole elution devices (five replicates, May–October 1992; Table 1). Traps were baited (two traps per treatment) with frontalure alone or frontalure + one, two, or four, 4-allylanisole elution devices. Treatment position was initially randomly assigned and then changed daily in a sequential order for five to eight days. (2) Response was tested to frontalin given an increasing percentage of 4-allylanisole when mixed with turpentine (one replicate, one site; Table 1). Each trap was baited with one pipet containing frontalin and one with either

TABLE 2. GRAVIMETRICALLY DETERMINED ELUTION RATES ($\bar{X} \pm$ SE) FOR VARIOUS SEMIOCHEMICALS AND DEVICES USED IN BAITED-TRAP FIELD ASSAYS

Test	Compound	Device	*N*	T (°C)	Location	Elution rate (mg/24 hr)
Spring	frontalure	3.5 ml pipet	17	17–32	field	54.7 ± 2.4
Spring	4-allylanisole	2.0 ml bark	2	17–32	field	729.7 ± 59.9
	4-allylanisole	5.0 ml sponge	2	17–29	field	1149.7 ± 4.7
Spring	verbenone	5.0 ml sponge	4	17–32	field	94.0 ± 5.4
Fall	frontalure	3.5 ml pipette	3	22–27	lab	79.9 ± 5.5
Fall	4-allylanisole	5.0 ml sponge	3	22–27	lab	1236.6 ± 81.1
	4-allylanisole	20.0 ml wick	3	22–27	lab	159.5 ± 5.8
Fall	verbenone	10.0 ml sponge	3	22–27	lab	148.1 ± 8.4

100% turpentine, 75% turpentine and 25% 4-allylanisole, 50% turpentine and 50% 4-allylanisole, 25% turpentine and 75% 4-allylanisole, or 100% 4-allylanisole. In both dose–response experiments, the numbers of *D. frontalis* and *T. dubius* were recorded daily as described above.

In a single trial (Table 1), response of *D. frontalis* to 4-allylanisole in combination with verbenone was tested. Treatments (two traps per treatment) consisted of frontalure alone, frontalure + 4-allylanisole (two wicked 20 ml vials), frontalure + verbenone (two 10 ml bags), and frontalure + 4-allylanisole (one vial) + verbenone (one bag).

Data Analysis. In all field assays, mean values of *D. frontalis* and *T. dubius* were tested for normality, transformed by $\ln(Y + 1)$, and analyzed by analysis of variance (ANOVA) with separation of the transformed means performed by protected LSD and least-square mean where appropriate (PROC GLM; SAS Institute, 1988). Dose–response test data were analyzed by ANOVA and regression analysis (PROC GLM and PROC REG; SAS Institute, 1988). Mean values for dose–response data were transformed by $\ln(Y + 0.001)$ and separation of the transformed means was performed by protected LSD.

Preliminary Field Application. Two loblolly pines (*Pinus taeda*), one struck by lightning on June 1 and another on June 28, 1992, were treated with 4-allylanisole within two days of being struck and before attack by *D. frontalis*. A longleaf pine (*P. palustris*), struck by lightning on July 1 was treated with 4-allylanisole on July 2, 1992. The treatment consisted of placing nine 20-ml polyethylene vials with cotton wicks, evenly spaced from the ground to 8 m up the tree bole on the damaged side. Nearby pines of the same species, struck by lightning in the same storms, were located and served as controls. At day 30, numbers of *D. frontalis* attacks were counted in a 15.2-cm-wide band around the tree circumference at 2 and 4 m up the bole, and the general condition of the trees recorded.

RESULTS

Laboratory Assay. Male and female *D. frontalis*, newly emerged and to a lesser extent reemerged, were repelled when exposed to 4-allylanisole in laboratory assays. If the cardboard was untreated, all beetles walked immediately (<20 sec) out of the circle in all directions (i.e., 0% repelled). When placed in a frontalure-treated circle, 0% of the tested beetles were repelled: some beetles walked immediately (<20 sec) across the painted strip to the surrounding area out of the circle; others would stop at the strip, walk around, in, or back and forth across the painted strip before moving out to the surrounding area (<30 sec). Although the percentage varied from trial to trial, higher percentages of *D. frontalis* in all trials were repelled by 4-allylanisole than verbenone using

the same assay method, and generally beetles that were "repelled" by 4-allylanisole demonstrated a higher degree of alarm and more abrupt behavior than beetles "repelled" by verbenone. In trial 1, regardless of sex, 84% of the newly emerged beetles exposed to 4-allylanisole were repelled, while 11% of those exposed to verbenone were repelled (Table 3). In trial 2, regardless of sex, higher percentages of the newly or reemerged beetles exposed to 4-allylanisole were repelled than those exposed to verbenone (46 and 52% higher, respectively)

TABLE 3. RESPONSE OF NEWLY EMERGED *D. frontalis* TO 4-ALLYLANISOLE AND VERBENONE IN LABORATORY ASSAYS[a]

		4-Allylanisole		Verbenone	
Date	Sex	N	Repelled (%)	*N*	Repelled (%)
4/12/92	M	27	78	20	20
	F	23	78	30	7
	Subtotal	50	78	50	12
5/2/92	M	27	85	25	12
	F	23	83	25	16
	Subtotal	50	84	50	14
5/3/92	M	22	91	27	7
	F	28	89	23	4
	Subtotal	50	90	50	6
	Total	150	84	150	11

[a]Controls consisted of: untreated (N = 50; 0% repelled) and frontalure (N = 50; 0% repelled).

TABLE 4. RESPONSE OF NEWLY AND REEMERGED *D. frontalis* TO 4-ALLYLANISOLE AND VERBENONE IN LABORATORY ASSAYS

	4-Allylanisole		Verbenone	
Sex	*N*	Repelled (%)	*N*	Repelled (%)
Newly emerged adults (10/1/92)				
M	63	95	58	47
F	37	95	42	52
Total	100	95	100	49
Reemerged parent adults (10/22/92)				
M	57	65	61	5
F	43	60	39	21
Total	100	63	100	11

(Table 4). In both treatments, fewer reemerged beetles were repelled (32% less for 4-allylanisole and 38% less for verbenone).

The *D. frontalis* predator, *T. dubius*, showed no repellent response when exposed to 4-allylanisole or verbenone in laboratory assays. Other scolytids tested, including local (*I. avulsus* and *I. calligraphis*) and nonresident (*I. pini*, *D. rufipennis*, *D. brevicomis*, and *D. ponderosae*) species, were also repelled when exposed to 4-allylanisole in laboratory assays (Table 5). Response to verbenone was assayed in species where verbenone is known to serve an inhibitory function; in all cases, equal or higher percentages were repelled by 4-allylanisole than verbenone.

Field Assay. In the spring trials, 4-allylanisole-baited traps captured significantly fewer *D. frontalis* than traps baited with frontalure alone or with verbenone ($P < 0.0001$ and $P < 0.0004$, respectively, ANOVA and LSD of transformed data) (Figure 1). The mean number of *D. frontalis* captured in frontalure alone and verbenone-baited traps did not differ ($P < 0.2179$). *T. dubius* captures did not differ significantly between traps baited with 4-allylanisole and frontalure alone, but both captured significantly more *T. dubius* than verbenone-baited traps ($P < 0.0391$ and $P < 0.0231$, respectively). Although statistically significant, the differences between treatments for *T. dubius* were small and were not apparent in any other trial.

In the fall trials, verbenone and 4-allylanisole-baited traps captured significantly fewer *D. frontalis* than traps baited with frontalure alone ($P < 0.0001$, ANOVA and LSD of transformed data) (Figure 2). There were no significant differences between *D. frontalis* captured in 4-allylanisole- and verbenone-baited traps. *T. dubius* captures did not differ significantly among treatments.

TABLE 5. RESPONSE OF OTHER INSECT SPECIES TO 4-ALLYLANISOLE AND VERBENONE IN LABORATORY ASSAYS

Insects	4-Allylanisole		Verbenone	
	N	Repelled (%)	*N*	Repelled (%)
T. dubius	50	0	50	0
Ips spp.[a]	59	88	50	70
I. pini	193	97		
D. brevicomis				
Males	16	88	16	75
Females	17	82	15	87
D. ponderosae	30	97	23	78
D. rufipennis	58	81		

[a]Comprised of *Ips* species from the southern pine bark beetle guild: *I. avulsus* ($N = 50$) and *I. calligraphus* ($N = 9$).

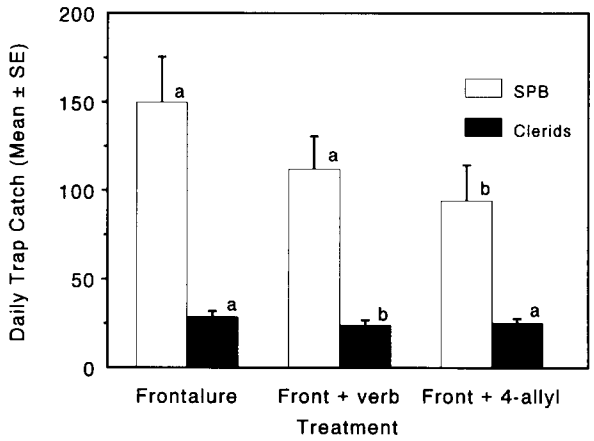

FIG. 1. Mean (± SE) daily capture in the spring of *D. frontalis* (SPB) and its predator, *T. dubius* (clerids), in traps (two per treatment) baited with the SPB attractant frontalure alone and in combination with the SPB antiaggregation pheromone, verbenone, or with the host compound, 4-allylanisole. Trapping was carried out in active SPB infestations ($N = 6$) in the spring and trap positions rotated daily for six days. For each species, bars with the same letter are not significantly different (based on ANOVA and LSD of transformed data).

Taken altogether, significantly fewer *D. frontalis* were captured in the spring and fall in traps baited with 4-allylanisole + frontalure than frontalure alone; trap captures differed between 4-allylanisole- and verbenone-baited traps in the spring but not in the fall trials (Figures 1 and 2; Table 6). No trial × treatment interaction was found with analysis of variance in either spring or fall trials ($F = 0.36$; $df = 5,2$; $P < 0.9614$ and $F = 0.19$; $df = 6,2$; $P < 0.9984$, respectively). However, that trap captures vary day to day, presumably influenced by weather conditions, is evident in significant results of a model in which day is treated as a nested component of trial (Table 6). *T. dubius* attraction was apparently unaffected by the addition of 4-allylanisole; however, in the spring trials significantly fewer *T. dubius* were captured in traps baited with verbenone + frontalure than 4-allylanisole + frontalure of frontalure alone. Although statistically different, it is not clear whether the difference is biologically meaningful. Based on mean captures in frontalure-alone traps, both *D. frontalis* and *T. dubius* showed seasonal differences in abundance, with significantly more *D. frontalis* captured in the fall than in the spring (245.4 ± 40.3 vs. 149.9 ± 25.6), in contrast significantly more *T. dubius* were captured in the spring than in the fall (28.5 ± 3.2 vs. 4.8 ± 0.6).

Sex ratios of *D. frontalis* captured in traps baited with aggregation pheromone alone were, on average, significantly male-biased (57% GT1 and 74%

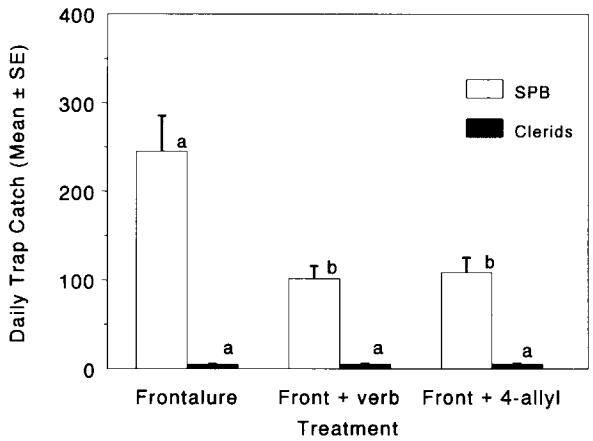

FIG. 2. Mean (± SE) daily capture in the fall of *D. frontalis* (SPB) and its predator, *T. dubius* (clerids), in traps (two per treatment) baited with the SPB attractant frontalure alone and in combination with the SPB antiaggregation pheromone, verbenone, or with the host compound, 4-allylanisole. Trapping was carried out in active SPB infestation ($N = 7$) in the fall and trap positions altered daily for six days. For each species, bars with the same letter are not significantly different (based on ANOVA and LSD of transformed data)

CA3034), as has been previously reported (e.g., Kinzer et al., 1969; Payne et al., 1978). The mean sex ratio (55% male GT1 and 71% CA3034) for *D. frontalis* captured in traps with frontalure + 4-allylanisole did not differ significantly from frontalure alone, whereas captures in traps baited with frontalure + verbenone differed significantly in sex ratio from frontalure alone (Pearson's chi square = 102.46, $P < 0.0001$ GT1; chi square = 23.94, $P < 0.0001$ CA3034). A significant female bias (66% female; chi square = 33.06, $P < 0.0236$) was observed in verbenone-baited traps at GT1 and a slight but not significant female bias was observed at CA3034 (52% female), suggesting that female *D. frontalis* are less inhibited by verbenone than males. A similar biased response was also apparent in trapping results presented by Salom et al. (1992b).

In a single trial in which 4-allylanisole, verbenone, and the combination were added to frontalure-baited traps, all three captured significantly fewer *D. frontalis* than frontalure alone ($P < 0.0004$; $P < 0.0011$; $P < 0.0016$; ANOVA and LSD of transformed data, respectively) but did not differ from each other. In rank order, 4-allylanisole caught the least (16.75 ± 4.02; $\bar{X}$ ± SE) followed by 4-allylanisole + verbenone (19.19 ± 4.37), verbenone (21.19 ± 5.95), and frontalure alone (45.88 ± 11.02). The mean number of *T. dubius* captured did not differ among treatments.

The repellent effect of 4-allylanisole on *D. frontalis* was not significantly

TABLE 6. ANALYSES OF VARIANCE OF FIELD ASSAYS WITH *D. frontalis* AND *T. dubius* USING BAITED TRAPS

Insect	Model	*df*	MS	*F*	*P*	RSQ
Spring trials						
D. frontalis	trial	5	27.88	20.34	0.0001	0.85
	date (trial)	36	1.37	3.28	0.0001	
	treatment	2	5.44	13.00	0.0001	
	error	82	0.42			
T. dubius	trial	5	3.22	1.90	0.1179	0.81
	date (trial)	36	1.69	7.43	0.0001	
	treatment	2	0.75	3.27	0.0431	
	error	82	0.23			
Fall trials						
D. frontalis	trial	6	8.39	3.83	0.0049	0.85
	date (trial)	35	2.19	6.58	0.0001	
	treatment	2	12.91	38.79	0.0001	
	error	82	0.33			
T. dubius	trial	6	0.88	1.19	0.3336	0.62
	date (trial)	35	0.74	3.15	0.0001	
	treatment	2	0.0008	0.00	0.9965	
	error	82	0.23			

enhanced by the addition of more than one 20-ml wicked elution device (Table 7, a). The dose–response of *D. frontalis* was defined by $Y = 26\ X^{-0.16}$ ($R^2 = 0.895$; $N = 5$; $P < 0.0001$) (Figure 3). Frontalure-baited traps captured significantly more *D. frontalis* than traps baited with frontalure + one, two, or four 4-allylanisole elution devices ($\bar{X} = 79.8 \pm 7.98$, $P < 0.0001$, ANOVA and LSD of transformed data). However, there were no differences in mean captures between the different number of devices ($\bar{X} \pm$ SE = 29.4 ± 0.87; 21.6 ± 0.75; 20.8 ± 2.76) (Figure 3). The addition of one or more 4-allylanisole elution device did not impact *T. dubius* attraction to frontalure (10.9 ± 0.98, 9.8 ± 0.80, 11.3 ± 0.89); however, all three captured significantly fewer clerids than frontalure alone (17.6 ± 1.61, $P < 0.01$ ANOVA and LSD of transformed data).

Differing ratios of turpentine–4-allylanisole that contain more than 25% 4-allylanisole did not significantly influence the repellent effect of 4-allylanisole on *D. frontalis* (Table 7, b). Traps baited with frontalin + turpentine (in separate elution devices) captured significantly more *D. frontalis* than traps baited with frontalin (in one elution device) + 3:1, 1:1, 1:3, or 0:1 turpentine:4-allylanisole (in a separate elution device) ($\bar{X} = 87.6 \pm 21.0$; $P < 0.0001$, ANOVA and LSD of transformed data). There was little difference between 25, 50, or

TABLE 7. ANALYSES OF VARIANCE OF DOSE–RESPONSE TO 4-ALLYLANISOLE BY *D. frontalis* AND *T. dubius* IN FIELD ASSAYS

Insect	Model	*df*	MS	*F*	*P*	RSQ
a. Dose–response trials (one, two, and four 4-allylanisole elution devices)						
D. frontalis	site	4	39.33	0.36	0.8293	0.90
	treatment	3	3976.60	36.86	0.0001	
	error	12	107.89			
T. dubius	site	4	2.17	0.29	0.8788	0.68
	treatment	3	61.54	8.21	0.0031	
	error	12	7.49			
b. Dose–response trial (1:0, 3:1, 1:1, 1:3, 0:1 turpentine–4-allylanisole)						
D. frontalis	date	21	4.47	3.72	0.0001	0.58
	treatment	4	9.36	7.80	0.0001	
	error	78	1.20			
T. dubius	date	21	2.28	2.63	0.0011	0.42
	treatment	4	0.44	0.51	0.7285	
	error	78	0.87			

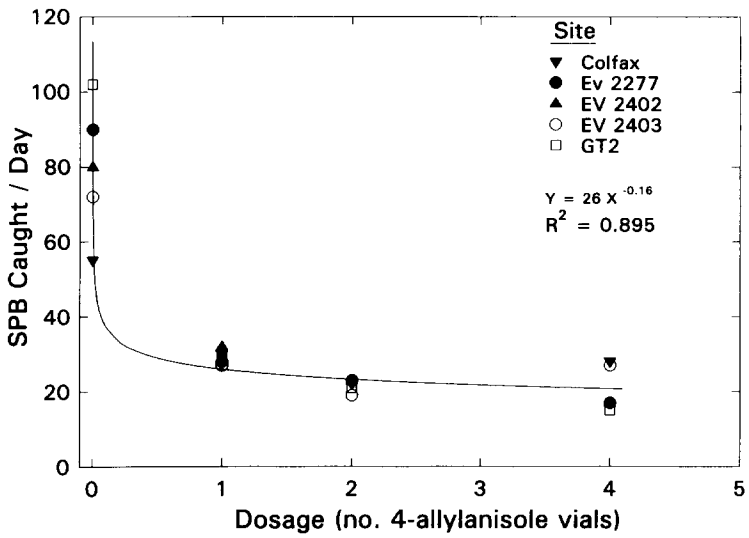

FIG. 3. Dose–response of *D. frontalis* (SPB) [mean daily trap catch transformed by ln(Y + 0.001)] to increasing numbers of 4-allylanisole elution devices (0–4) per trap baited with frontalure (trap captures were normalized across sites; see text for detailed methodological description).

TABLE 8. PAIRED TREATMENTS WITH 4-ALLYLANISOLE OF LIGHTNING-STRUCK TREES

Lightning strike date	Pine spp.	dbh (cm)	Treatment type	Attacks (N/m^2) 2 m	Attacks (N/m^2) 4 m	Tree fate
6/1/92	loblolly	49.3	treated	0	0	alive
6/1/92	loblolly	40.9	control	38.8	86.1	dead
6/28/92	loblolly	45.7	treated	0	0	alive
6/28/92	loblolly	53.3	control	86.1	150.7	dead
7/1/92	longleaf	51.3	treated	16.1	6.9	alive
7/1/92	longleaf	43.2	control	96.9	148.5	dead

75% 4-allylanisole, but 100% 4-allylanisole caught significantly fewer *D. frontalis* than 25 and 50% ($P < 0.0347$ and $P < 0.0439$) ($\bar{X} = 29.5 \pm 8.5$; 22.2 ± 4.9; 39.4 ± 11.8; 15.0 ± 6.0). The addition of 4-allylanisole in differing concentrations with turpentine did not impact *T. dubius* attraction to frontalin ($\bar{X} = 34.1 \pm 5.5$; 33.5 ± 7.8; 42.4 ± 10.5; 28.1 ± 5.8; 39.5 ± 8.9).

Preliminary Field Application. Individual tree attributes and results of 30-day treatment of lightning-struck pairs of pines are given in Table 8. The 4-allylanisole-treated member of each lightning-struck pair suffered considerably fewer attacks than the untreated control and were apparently still alive after the test period, while controls were obviously dead. In two other noteworthy instances, large lightning-struck loblolly pines in residential settings were treated within 48 hr as described above. In one case the tree was protected even though a few pitch tubes were evident and an adjacent (untreated) lightning-struck tree was attacked by *D. frontalis* and removed. In the other case, the tree was protected from significant attack even though a few pitch tubes were present at the time of treatment. After treatment was stopped at day 30, the tree was attacked by *Ips* spp. and *D. frontalis*.

DISCUSSION

4-Allylanisole was identified in loblolly pine as early as 1956 (Sutherland and Wells, 1956) and reported as a component of needle oils (Zavarin et al., 1971; von Rudloff, 1975; Adams and Edmunds, 1989; von Rudloff and Lapp, 1992) and resins from many species since (Mirov, 1961; Drew and Pylant, 1966; Renwick and Vité, 1969, 1970; Werner, 1972a; Gambliel et al., 1985; Pierce et al., 1987). 4-Allylanisole also has been recognized and used as an attractant in other insect–host systems, e.g., western corn rootworm (*Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae) (Lampman et al., 1987).

Speculations about the role(s) of this phenylpropanoid compound in pine-bark beetle interactions have varied from parasitoid (Salom et al., 1991, 1992a) and bark beetle attractant (Renwick and Vité, 1969, 1970; Werner, 1972a,b) to microbial growth inhibitor (Gambliel et al., 1985; Bridges, 1987). Bridges (1987) demonstrated reduced fungal growth, although others have dismissed 4-allylanisole as unimportant (Himejima et al., 1992). Cobb et al. (1972) hypothesized but did not directly test a connection between a drop in 4-allylanisole in leaves of *P. ponderosa*, air pollution, and susceptibility to bark beetles.

In previous publications, 4-allylanisole has been identified from *D. frontalis*-infested tree parts and female frass (R.M. Silverstein and J.R. West, personal communication, as cited in Thatcher et al., 1980), and although no reference is given, 4-allylanisole is cited by Salom et al. (1991, 1992a) as being one of numerous volatiles arising from trees containing heavily parasitized *D. frontalis* brood. In surveys of potential semiochemicals and electroantennogram (EAG) responses of two *D. frontalis* parasitoids, Salom et al. (1991, 1992a), found that 4-allylanisole elicited little activity at the sensory level of either of these natural enemies. Pierce et al. (1987) found 4-allylanisole in *P. ponderosa* phloem oil, but not in the abdominal or frass volatiles of *D. ponderosae*.

Our laboratory bioassay appears to provide a simple, reliable method for screening compounds that elicit repellent or inhibitory responses from bark beetles. The results of exposure to 4-allylanisole are consistent with our baited-trap experiments, which is the most commonly used field technique. The relative difference in repellency of 4-allylanisole and verbenone evident in the laboratory assays was not consistently apparent in the baited-trap experiments, but may reflect the difference between the beetle's response to a single stimulus versus the multiple stimuli (both visual and chemical) emanating from a trap. In contrast to our laboratory assay results, in which we found no differential response between the sexes, McCarty et al. (1980) reported that only males responded to verbenone using a walking olfactometer. Our trap results are in agreement with findings of other researchers (Renwick and Vité, 1969; Salom et al., 1992b), who found that the presence of verbenone in traps significantly reduces total catch but has little or no influence on female *D. frontalis*. With the exception of the spring trials, our results are in agreement with Richerson and Payne (1979), Billings and Cameron (1984), and Salom et al. (1992b), who found that the addition of *D. frontalis* inhibitor to attractant did not influence the response of *T. dubius*. Generally, there were no differences between the number of *T. dubius* captured in traps baited with frontalure alone versus 4-allylanisole. The exception was the first dose-response assay, and we have no explanation for this apparent deviation.

Our laboratory assays suggest that 4-allylanisole would be an effective repellent of *D. frontalis*, its associated scolytids (e.g., *Ips* spp.), and in other

scolytid–conifer systems. The strong reactions to verbenone of the species tested is consistent with other reports in which this inhibitor is known to play a role. Specific antiaggregation pheromones have not been identified from *Ips* spp. (Borden, 1986); however, a negative reaction to the *D. frontalis* inhibitor is in agreement with the findings of others (Richerson and Payne, 1979; Byers and Wood, 1980, 1981). Both *D. brevicomis* and *D. ponderosae* are known to produce and be responsive to verbenone as an inhibitory pheromone, but apparently in slightly different enantiomeric blends and concentrations than *D. frontalis* (Amman et al., 1989; Paine and Hanlon, 1991; Shea et al., 1992). Renwick and Vité (1970) found *D. brevicomis'* reaction to verbenone was more pronounced than that of *D. frontalis*.

The results of the laboratory and field assays indicate the consistent repellent properties of 4-allylanisole to *D. frontalis* throughout the times of year when dispersal is considered highest (Thatcher and Pickard, 1964; Turchin and Thoeny, 1993). Additionally, unlike verbenone, which elicits a male-biased response, there is no apparent differential response between the sexes to this host compound; females and males are repelled proportional to their attraction to frontalure, a desirable finding given that the female is considered the pioneering sex (i.e., the sex that initiates attack on a new host) in *D. frontalis*. The combination of the two semiochemicals does not appear to further suppress beetle response than either alone. The differential response we observed between newly emerged and reemerged beetles to 4-allylanisole and verbenone should be further explored and may be a contributing factor in the inconsistencies seen with verbenone studies (e.g., Payne and Billings, 1989; Payne et al., 1992).

Among compounds that are inhibitory or repellent to scolytids, those originating from the host are especially important because of their potential olfactory role in host selection by pioneering individuals. Due to a variety of evidence, Smith (1977) speculated that *P. ponderosa* high in limonene may be resistant to *D. brevicomis*. A considerable amount of research has been devoted to finding primary attractants of tree-killing bark beetles (Tunset et al., 1993, and references therein) and the predominant hypotheses are that beetles either land randomly on potential hosts or that host selection is the result of chemical and/or visual cues. The latter hypothesis implies that beetles are able to detect a suitable host in flight, but does not preclude the possibility that the information perceived is alternatively about the unsuitability of a potential host. Although additional replicates are necessary, protection of lightning-struck trees, highly susceptible hosts for *D. frontalis* (Hodges and Pickard, 1971), suggests that in contrast to (or in addition to) the primary attractant hypothesis, host selection may be dependent on a lack of repellency (see similar discussions by Heikkenen and Hrutfiord, 1965; Cobb et al., 1968). The attractancy or synergistic natures of many oleoresin compounds (e.g., α-pinene) have been well documented, but

the repellent or inhibitory effects of others, such as 4-allylanisole, have been largely overlooked and warrant further exploration.

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