Identification of a Host Compound and Its Practical Applications: 4-allylanisole as a Bark Beetle Repellent[1]

J.L. Hayes, L.L. Ingram, Jr., B.L. Strom, L.M. Roton, M.W. Boyette, and M.T. Walsh[2]

ABSTRACT

Gas chromatography/mass spectrometry analysis of resin collected before and after injection of loblolly pines (*Pinus taeda* L.) with a fungicide mixture known to make pines more "attractive" to southern pine beetle, *Dendroctonus frontalis* Zimm., resulted in the identification of 4-allylanisole as a likely candidate for repellent effects. The phenylpropanoid, 4-allylanisole (Chemical Abstract 140-67-0), is a compound produced by many conifers, including loblolly pine, an abundant species in southern pine forests and a preferred host of the southern pine beetle. The repellency of 4-allylanisole to southern pine beetle was demonstrated in laboratory behavioral assays and in natural populations by comparing its effects with those of the beetle-produced inhibitory pheromone, verbenone. Responses of other North American scolytids and associates were also determined. Additionally, responses of southern pine beetle to various chemical analogues of 4-allylanisole were tested. The response in the field of southern pine beetle to its attractant pheromone in funnel traps was significantly reduced by simultaneous release of either 4-allylanisole or verbenone, which did not differ from one another in repellency. Both compounds together did not significantly further reduce trap catch. The response of a major predator, *Thanasimus dubius* (F.), to the attractant pheromone of southern pine beetle did not differ with the simultaneous release of either compound. The results of preliminary field tests with 4-allylanisole, in which lightning-struck pines were protected from southern pine beetle attack, are presented and discussed in relation to implications for development of a practical tree protection tactic.

Keywords: Analogue, Coleoptera, *Dendroctonus frontalis,* 4-allylanisole, host compound, inhibitor, *Pinus*, repellent, Scolytidae, semiochemical, sodium-N-methyldithiocarbamate, verbenone.

INTRODUCTION

The southern pine beetle, *Dendroctonus frontalis* Zimmermann, is the most destructive insect to pine forests in the Southeastern United States. Since 1960, the southern pine beetle has been responsible for the loss of nearly a billion dollars of forest resources southwide (Price and others 1992). Management options for reducing southern pine beetle losses are limited, especially for individual tree protection and in areas with multiple management objectives.

Physical and chemical qualities of oleoresin are important in the ecological interactions between bark beetles and their coniferous hosts. It is well known that volatile components of the host resin can be important semiochemicals (i.e., message-bearing chemicals) for bark beetles (Raffa and others 1993, Wood 1982). In the southern pine beetle/southern yellow pine system, a-pinene is a predominant component of loblolly oleoresin and has been shown to act synergistically with the beetle's primary aggregation pheromone, frontalin, to enhance beetle aggregation. However, it has generally proven very difficult to quantitatively relate beetle behavior to host chemistry. For example, stressed trees, such as lightning-struck trees, are vulnerable to attack by bark beetles, but the reasons for preferential selection of these host trees are not well understood (Hodges and Pickard 1971). Previous work has also shown that trees treated with a formulation of the fungicide,

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sodium-N-methyldithiocarbamate (MS), and the carrier, dimethyl sulfoxide (DMSO), are very susceptible to attack by the southern pine beetle (Dalusky and others 1990, Miller and others 1994, Roton 1987).

In an effort to better understand the host selection process of the southern pine beetle, we utilized the apparent "attractiveness" of MS+DMSO-treated trees. Oleoresin, phloem, and wood were sampled before and after treatment, continuing until trees were successfully attacked by the southern pine beetle. Results were then related to the timing of southern pine beetle attack to determine whether there were quantitative or qualitative changes in chemical constituents that may be responsible for the observed change in host susceptibility.

IDENTIFICATION OF THE COMPOUND

In the spring of 1991, 30 loblolly pines (*Pinus taeda* L.) from a single site in Camp Beauregard, Rapides Parish, Louisiana were selected for study based on similar diameter and crown characteristics. Three treatments were randomly assigned to individual trees: (1) No treatment, (2) water treatment, and (3) MS+DMSO (4:1 v/v) treatment. Application of treatments was accomplished using a modified "hack and squirt" method (Dalusky and others 1990, Roton 1987). Hacks were made around the circumference of each tree, leaving 2 to 5 cm between them. Into each hack 8 to 10 ml of water or MS+DMSO were released and allowed to passively infuse. Thus, the number of hacks and total tree dosage were dependent on tree circumference.

Oleoresin was sampled prior to treatment and weekly thereafter following the methods of Lorio and others (1990). A 1.27-cm arch punch was used to remove the outer and inner bark and the injury was allowed to drain resin into a collection vial for 24 h. Phloem and wood were sampled in a like manner, with tissue being removed from the punch and placed immediately into vials. To minimize the potential influence of previous sampling, each new wound was offset horizontally and vertically by approximately 5 cm from the previous sample. Collected resin was stored in an ultracold freezer (-70 °C) until prepared for chemical analysis.

To determine whether MS-DMSO injection induced the formation of abnormal or unusual chemical compounds, sample extracts from the phloem and wood samples were analyzed with a Kratos MS80 gas chromatograph/mass spectrometer. Extraction was accomplished with a soxhlet-type apparatus using ethanol and benzene (1:1) as the solvent. A 0.5-ml aliquot was reacted with diazomethane/ether and the total volume adjusted to 1.0 ml. The gas chromatograph conditions were as follows: initial temperature 60 °C, hold time 2 min, program rate 6 °C per minute, final temperature 280 ° C, hold time 20 min. A representative total ion chromatogram of phloem tissue extract with a J & W DB-5 column is shown in figure 1.

For the quantitative determination of oleoresin components, 25 mg of oleoresin was diluted to 10 ml with 9 ml of benzene and 1 ml of 1.0 mg/ml diphenylmethane in benzene. A 0.5-ml aliquot of this solution was reacted with 0.5 ml of diazomethane/ether. Oleoresin samples were analyzed with a Hewlett-Packard HP5840A gas chromatograph equipped with a J & W DB-5 column and operated as described above. Average response factors were determined using five different standard levels in the concentration range of 10 to 250 µg/ml. The chemical identity of the oleoresin components was confirmed by gas chromatography/mass spectrometry using selected samples. Camphene, 4-allylanisole, and diphenylmethane were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI), and were utilized without further purification. Abietic acid was obtained from Aldrich and was recrystallized before use. Limonene, α-pinene, and myrcene were obtained from Sigma Chemical Company (St. Louis, MO), β-pinene was obtained form Pfaltz & Bauer, Inc. (Waterbury, CT). Pimaric acid, isopimaric acid, palustric acid, levopimaric, and neoabietic acid were obtained from Dwayne Zinkel, USDA Forest Service, Forest Products Laboratory, Madison, WI.

Results of the resin analysis showed that three volatile compounds--myrcene, β-pinene, and 4-allylanisole--had changed dramatically in MS-DMSO-treated trees by week 3 after treatment (table 1), a time coincident with southern pine beetle attack. Of these, the compound showing the highest degree of repellency to southern pine beetle in preliminary assays was 4-allylanisole. Further, the ratio of α-pinene (an important component in southern pine beetle aggregation) to 4-allylanisole rose dramatically (fig. 2), suggesting that 4-allylanisole may be involved as a **deterrent** in the beetle's host selection process. Therefore, laboratory and field assays were undertaken to determine the repellent properties of 4-allylanisole to southern pine beetle and related insects. Commonly known as methyl chavicol or estragole, 4-allylanisole (Chemical Abstract 140-67-0) (fig. 3), is known from numerous pine and other conifer species (Drew and Pylant 1966, Mirov 1961). Although there is considerable intra- and interspecific variation, 4-allylanisole is a consistent component in the oleoresin of southern yellow pines, usually making up 1 to 5 percent of the turpentine yields (Drew and Pylant 1966, Mirov 1961, Sutherland and Wells 1956).

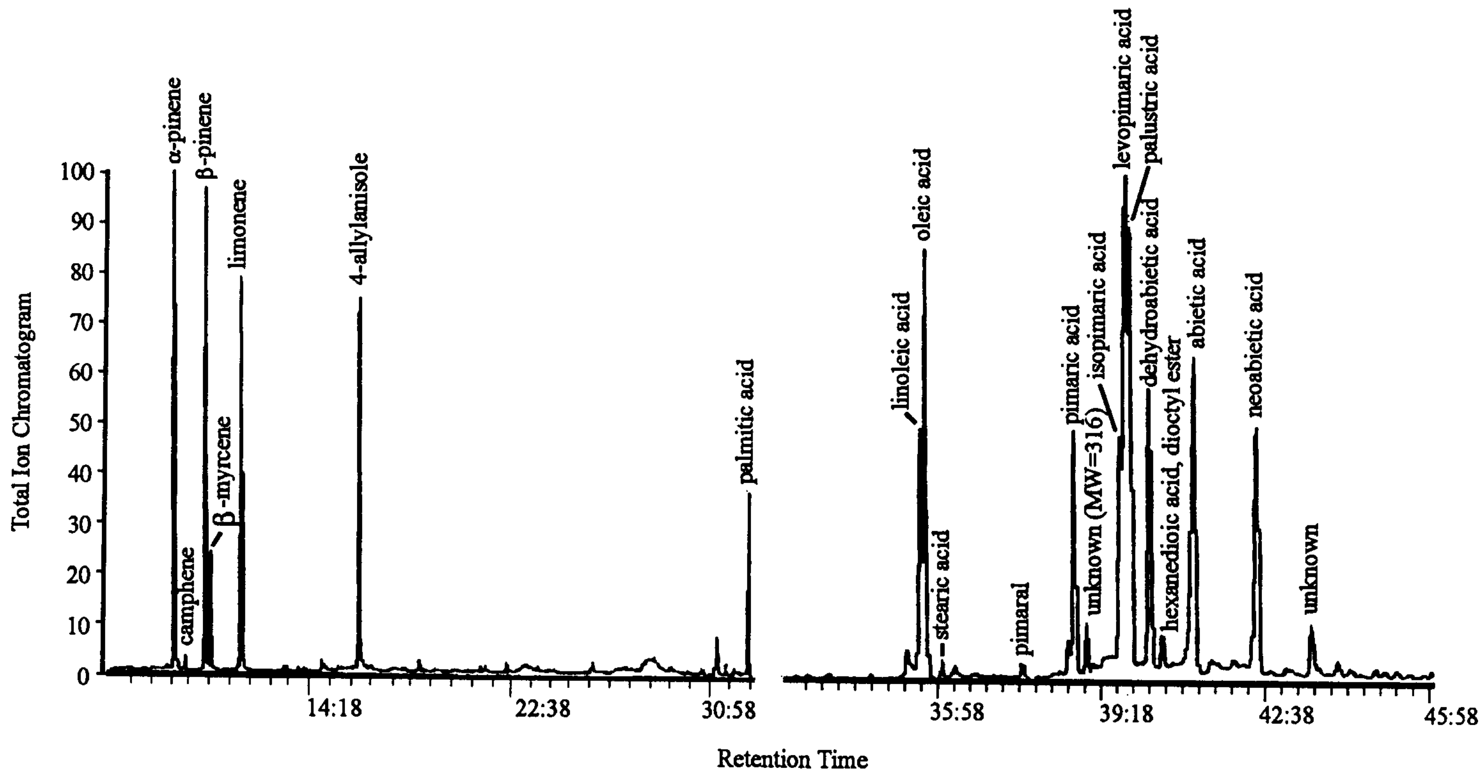

Fig. 1 A representative total ion chromatogram of phloem tissue from MS-DMSO injected trees.

Table 1. Concentration of chemical components of the oleoresin prior to treatment with MS-DMSO (week 0) and when trees were most likely to be attacked (3 weeks). Water-treated and untreated were combined for the control group. Percentage change is defined as: (week 3 - week 0)/week 0.

	Concentration					
	week 0		week 3		Percentage change	
Compound	Control	MS-DMSO	Control	MS-DMSO	Control	MS-DMSO
α-pinene	15.94	12.44	14.70	12.73	-7.8	2.3
Camphene	0.52	0.05	0.25	0.05	-51.9	0.0
β-pinene	7.74	6.54	7.38	4.67	-4.7	-28.6
Myrcene	0.63	1.03	0.77	0.34	22.2	-67.0
Limonene	1.64	0.81	1.74	0.90	6.1	11.1
4-allylanisole	**1.57**	**1.39**	**1.84**	**0.53**	**17.2**	**-61.9**
Pimaric acid	3.87	3.69	3.71	4.20	-4.1	13.8
Palustric & iso+levo Palmiric acids	34.39	35.43	33.96	36.06	-1.3	1.8
Dehydroabietic acid	6.85	6.48	6.83	10.15	-0.3	56.6
Abietic acid	14.10	15.41	14.26	17.54	1.1	13.8
Neoabietic acid	11.48	12.15	11.62	9.59	1.2	-21.1
Total	98.73	95.42	97.06	96.76		

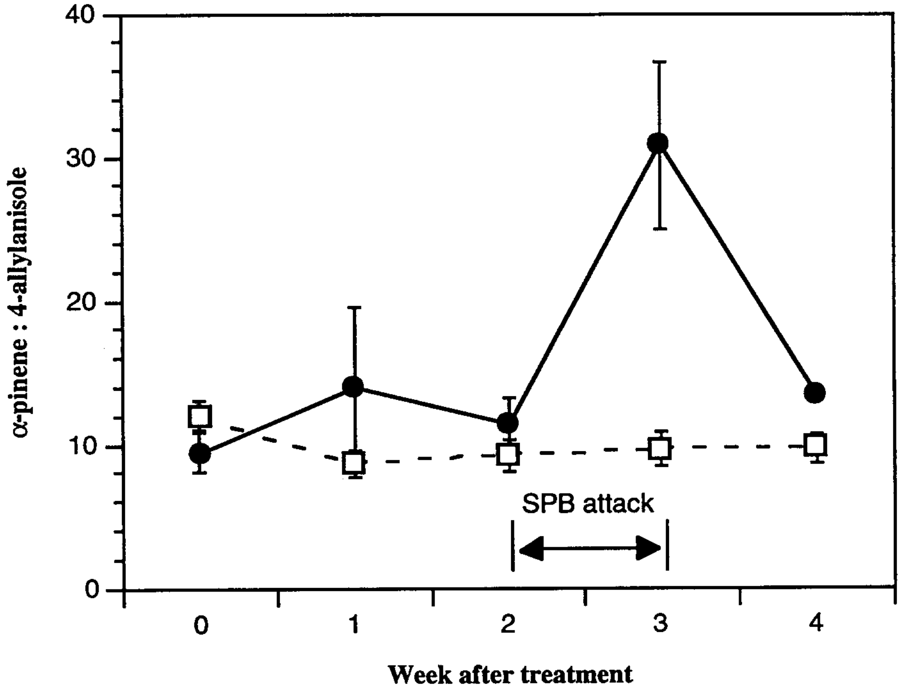

Figure 2. *The α-pinene: 4-allylanisole ratio changed significantly by week 3. Water-treated trees and untreated trees were not significantly different and were combined for the control treatment. Solid line is MS+DMSO; dashed line is control.*

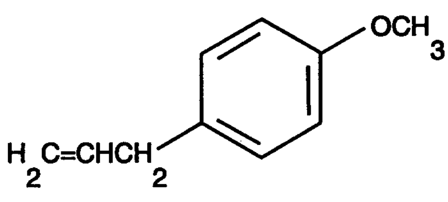

Figure 3. *Structure of 4-allylanisole.*

DETERMINATION OF REPELLENCY

With the exception of the analogue experiment, the experimental methods and results summarized below are described in greater detail elsewhere (Hayes and others 1994).

Lab Assay

A test of individual beetle response to 4-allylanisole vs. verbenone (a beetle-produced anti-aggregation pheromone) was conducted. A circle (17 cm diam by 5 mm wide) of 4-allylanisole or verbenone was "painted" with a camel' s-hair brush on a 28 by 21.5-cm piece of uncoated cardboard. After 3 min, beetles (2 to 5 individuals) were released in the center of the treated circle. Testing was conducted at room temperature with light supplied from an adjoining room. To prevent overwhelming photopositive responses, an object was used to cast a shadow over the test circle. Beetles were briefly refrigerated prior to testing to reduce their tendency to fly. Responses (<30 s exposure) were recorded as **not-repelled or repelled:** not-repelled beetles walked through the circle or stopped but proceeded across the circle within 30 s of exposure; and repelled beetles stopped abruptly, raised antennae (some "reared up" on hind legs), stood motionless and/or moved away from the circle (some moved abruptly in the opposite direction). Generally, beetles that were repelled by 4-allylanisole demonstrated a higher degree of alarm and more abrupt behavior than beetles repelled by verbenone.

Trials were conducted with newly emerged male and female southern pine beetles on three different dates from three different source populations; results of these trials (n = 300) were combined for presentation in figure 4. Trials were also conducted with a clerid beetle, *Thanasimus dubius* (F.), a common predator of southern pine beetle, and other scolytid species including: mountain pine beetle, *Dendroctonus ponderosae* Hopkins; western pine beetle, *Dendroctonus brevicomis* LeConte; spruce beetle, *Dendroctonus rufipennis* Kirby; pine engraver, *Ipspini Say*; the small southern pine engraver, *Ips avulsus* Eichhoff; and the six-spined ips, *Ips calligraphus* Germar, (fig. 4). In all trials, only apparently healthy beetles were used (n = 50).

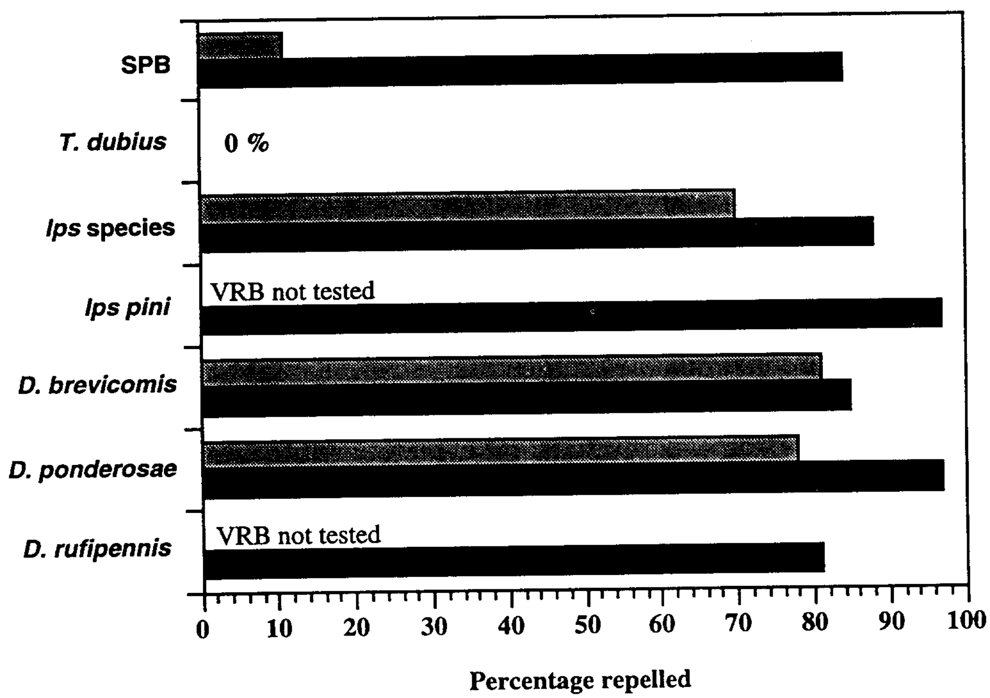

Figure 4. *The response of southern pine beetles, other scolytid species and the predatory clerid,* Thanasimus dubius *(F.), to 4-allylanisole (black bars) and verbenone (shaded bars) in laboratory assays. Response of southern pine beetles to frontalure was 0 percent.*

Behavioral assays with chemical analogues can provide information of ecological importance, as well as improve efficacy of control techniques. To determine the potential of analogues of 4-allylanisole for repelling the southern pine beetle, selected analogues were also tested using the assay described above (fig. 5).

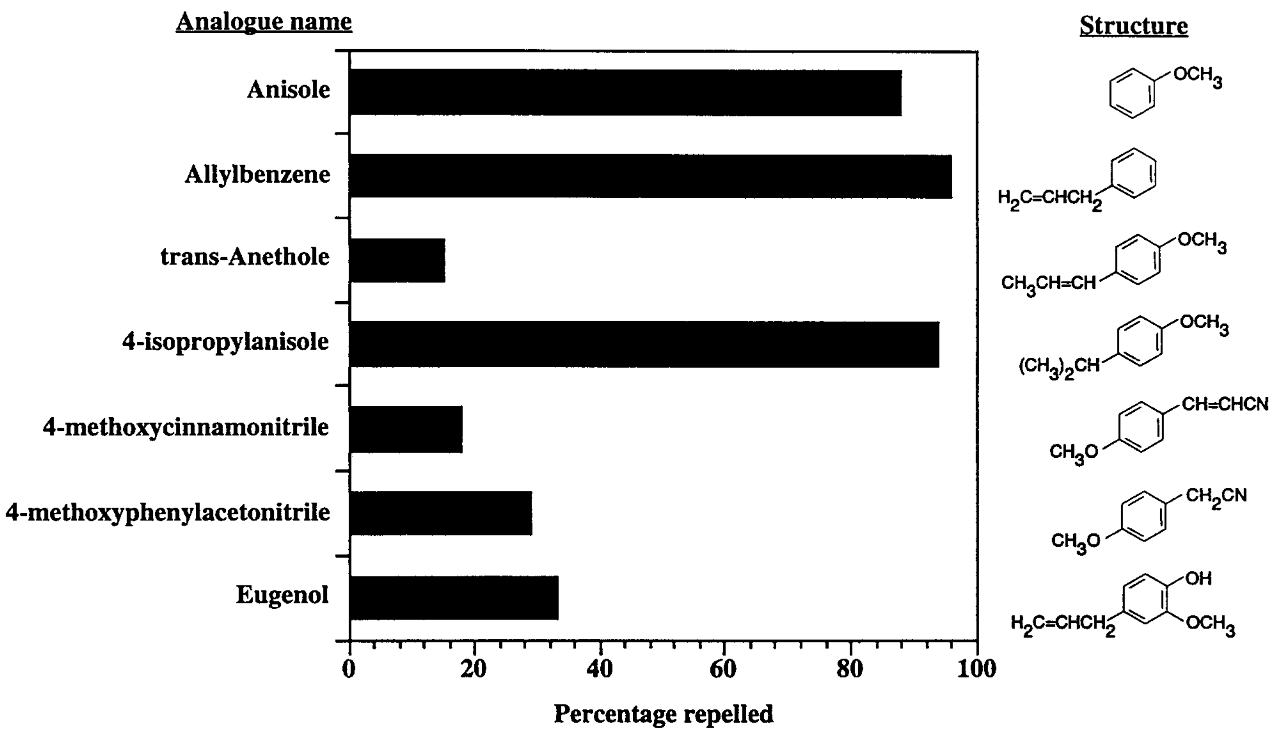

Figure 5. *Response of southern pine beetles to chemical analogues of 4-allylanisole. Analogue structures are also shown. Sample size for each analogue ranged from 45 to 53.*

Field Assay--A test of local southern pine beetle population's response to 4-allylanisole and to verbenone [vs. the attractancy of frontalure, the southern pine beetle aggregation pheromone frontalin + α-pinene (1:2)] was conducted using baited funnel traps (Lindgren 1983) placed in active southern pine beetle infestations in the spring (6 replications) and fall (7 replications) 1992. Traps were baited (2 traps/treatment) with frontalure, frontalure + verbenone, and frontalure + 4-allylanisole. Trap position was randomly assigned and changed daily in a sequential order for 6 days. The number of southern pine beetle and clerids were recorded daily (figs. 6, 7).

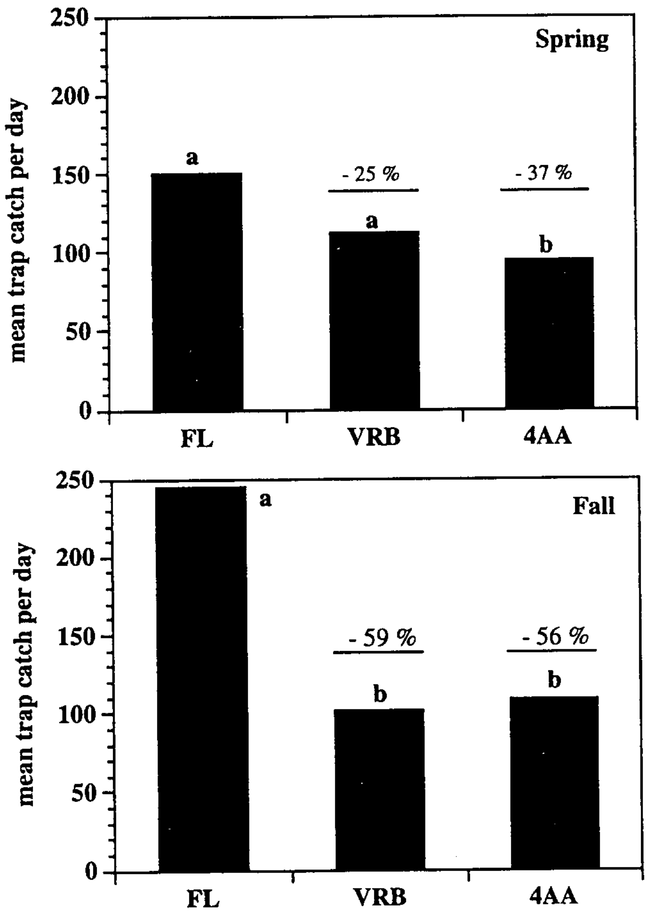

Figure 6. *Capture of southern pine beetles in the spring and fall using semiochemically baited* funneltraps. *Different letters near bar margins indicate significant difference in trap catch within a season (P<0.05 LSD of transformed data, SAS Institute, Inc. 1988). Percent change in catch relative to frontalure is shown above bars. FL = frontalure, VRB = verbenone, 4AA = 4-allylanisole.*

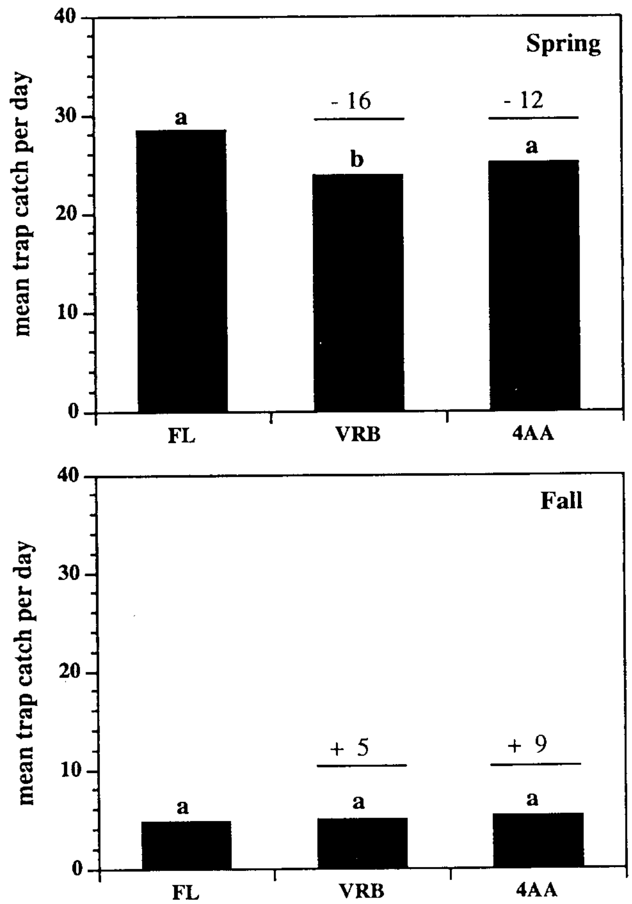

Figure 7. *Capture of the clerid,* Thanasimus dubius *(F.), in the spring and fall using semiochemically baited funnel traps. Different letters outside bar margins indicate significant difference in trap catch within a season (P<0.05 LSD of transformed data, SAS Institute, INC. 1988). Percent change in catch relative to frontalure is is shown above bars. FL = frontalure, VRB = verbenone, 4AA = a-allylanisole.*

Dose-response experiments (five replications) were conducted to test response to frontalure given increasing numbers of 4-allylanisole elution devices. Traps were baited (two traps per treatment) with frontalure alone or frontalure + one, two, or four 4-allylanisole elution devices. Trap position was randomly assigned and changed daily in a sequential order for 8 days. The numbers of southern pine beetles and clerids were recorded daily (fig. 8).

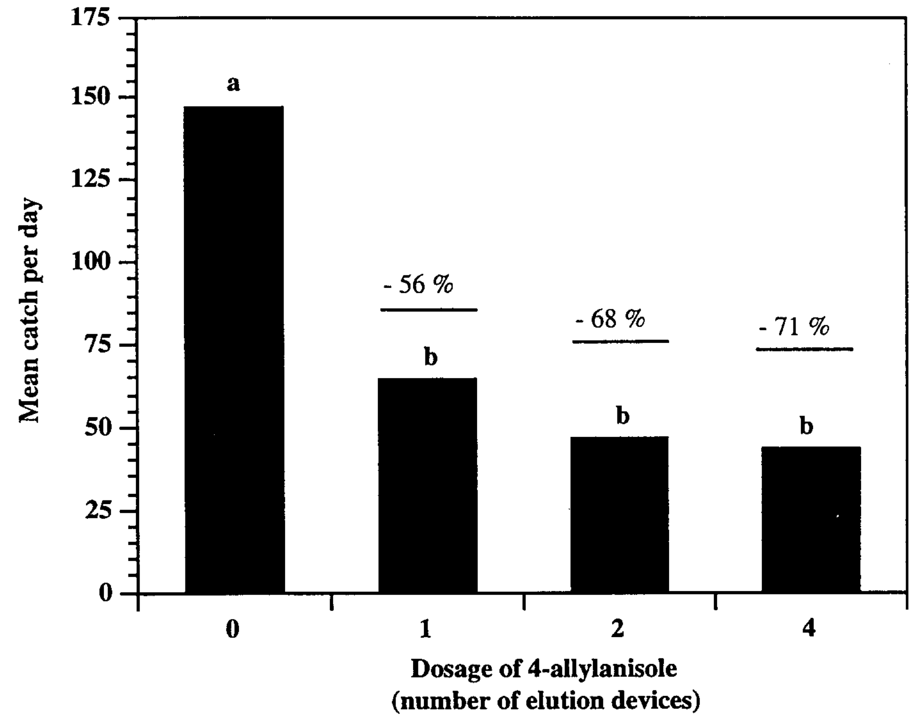

Figure 8. *Effect of 4-allylanisole dosage on the capture of southern pine beetles in funnel traps. Dosage is defined as the number of 4-allylanisole elution devices (wicked vials) in a trap. All traps also included the aggregation pheromone frontalure. Different letters near bar margins indicate significant difference in trap catch ($P<0.05$ LSD of transformed data, SAS Institute, Inc. 1988). Percentage change in catch relative to frontalure is shown above bars.*

RESULTS OF REPELLENCY TESTS

(1) Male and female southern pine beetles were repelled when exposed to 4-allylanisole in laboratory assays; higher percentages of all categories were repelled by 4-allylanisole than verbenone using the same assay method (fig. 4).

(2) Other scolytids, including local and nonresident species, were also repelled when exposed to 4-allylanisole in laboratory assays; for those species tested, equal or higher percentages were repelled by 4-allylanisole than verbenone (fig. 4).

(3) Significantly fewer southern pine beetles were captured in the spring and fall in traps baited with 4-allylanisole + frontalure than frontalure alone; trap captures did not differ between 4-allylanisole- vs verbenone-baited traps (fig. 6).

(4) Clerid beetles showed no repellent response when exposed to 4-allylanisole or verbenone in laboratory assays (fig.4) and were apparently unaffected by the addition of 4-allylanisole or verbenone to traps baited with frontalure (fig. 7).

(5) The repellent effect of 4-allylanisole on southern pine beetles was not significantly enhanced by the addition of more than one elution device (vial with 20 ml 4-allylanisole) (fig. 8); nor did one or more 4-allylanisole elution device impact clerid attraction to frontalure.

(6) Southern pine beetles were repelled by three of the chemical analogues of 4-allylanisole--allylbenzene, 4-isopropylanisole and anisole--to a degree at least equal to that of 4-allylanisole (fig. 5).

PROTECTION OF INDIVIDUAL TREES

For wildlife, cultural, and recreational resource management, as well as in suburban and urban settings, there is a need for the protection of individual trees threatened by bark beetles. Current control methods are based on stopping the spread of an infestation after it begins and usually requires the sacrifice of a large number of trees in the surrounding area (USDA 1987). Although two insecticides are registered as tree protectants for southern pine beetle, increasing environmental concerns may curtail their future use, indicating that additional tactics need to be developed. The results of our repellency assays suggest that 4-allylanisole is a candidate for a biologically-efficient tree protectant.

Preliminary Field Trials

Trees previously determined to be at high risk for southern pine beetle attack were selected to test the efficacy of 4-allylanisole for protecting individual trees.

Lightning Strikes

Loblolly and longleaf (*P. palustris* Mill.) pines struck by lightning were treated with 4-allylanisole within 48 h of being struck. 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. Trees of the same species and struck by lightning in the same storms were also located to serve as untreated controls. At day 30, numbers of southern pine beetle attacks were counted in a 15.2-cm- wide band and around the tree circumference at 2 and 4 m up the bole (table 2). In two other noteworthy instances, lightning-struck loblolly pines in residential settings were treated as described above. In both cases, the trees were protected from southern pine beetle attack for 30 days, until the 4-allylanisole was removed.

Table 2. *Paired lightning-struck trees treated with 4-allylanisole (4AA) or untreated. Total number of southern pine beetle attacks was measured at 2 m and 4 m up the tree bole. Tree fate is the apparent condition 30 days after treatment began, at which time, treatments were removed*

Lightning strike date	Pine species	D.b.h.	Treatment	Number of attacks/m^2		Tree fate
		—cm—		—2m—	—4m—	
6/1/92	Loblolly	49.3	4AA	0.0	0.0	Alive
6/1/92	Loblolly	40.9	Untreated	38.8	86.1	Dead
6/28/92	Loblolly	45.7	4AA	0.0	0.0	Alive
6/28/92	Loblolly	53.3	Untreated	86.1	150.7	Dead
7/1/92	Longleaf	51.3	4AA	16.1	6.9	Alive
7/1/92	Longleaf	43.2	Untreated	96.9	148.5	Dead

Red-Cockaded Woodpecker Trees

Currently 4-allylanisole is being tested for its operational efficacy as a bark beetle repellent on cavity trees of the endangered red-cockaded woodpecker (*Picoides borealis*). Mortality patterns of these trees suggest that they are susceptible to attack by southern pine beetles (Conner and others 1991, Mitchell and others 1991), and the importance of these trees for the management of this endangered species qualifies them for treatment with 4-allylanisole. A large study, funded by the National Center for Forest Health Management, involving over 30 red-cockaded woodpecker clans is underway on the Vernon Ranger District, Kisatchie National Forest, Louisiana.

CONCLUSIONS

Results presented in this paper indicate that 4-allylanisole may provide the basis of tactics for protection of high-value single trees and possibly stands from southern pine beetle attack. The results of the laboratory and field assays indicate the consistent repellent properties of 4-allylanisole to southern pine beetles (and other scolytid beetles) throughout the year. The fact that clerids are not repelled by 4-allylanisole provides further evidence for use of this semiochemical to protect trees in natural settings with minimal disturbance. Although additional studies are needed, preliminary natural field trials further support the prospect of using 4-allylanisole in single tree and possibly stand protection strategies. Based on the results obtained to date, a patent application entitled "Scolytid Repellent" has been submitted to the U.S. patent office (08/113,709).

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ABSTRACT

The phenylpropanoid, 4-allylanisole (CAS.# 140-67-0), is a compound produced by many conifers, including loblolly pine (*Pinus taeda* L.), an abundant species in southern pine forests and a preferred host of the southern pine beetle (SPB) *Dendroctonus frontalis* Zimm. Gas chromatography/mass spectrometry analysis of resin collected before and after injection of loblolly pines with a fungicide mixture known to make pines more "attractive" to SPB resulted in the identification of 4-allylanisole as a likely candidate for repellent effects. The repellency of 4-allylanisole to SPB was demonstrated in laboratory behavioral assays and in natural populations by comparing its effects with those of the beetle-produced inhibitory pheromone, verbenone. Responses of other North American scolytids and associates were also determined. The response in the field of *D. frontalis* to its attractant pheromone in funnel traps was significantly reduced by simultaneous release of either 4-allylanisole or verbenone, which did not differ from one another in repellency. 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 compound. The results of preliminary field tests with 4-allylanisole, in which lightning-struck pines were protected from SPB attack, are presented and discussed in relation to implications for development of a practical tree protection tactic.

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