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BIOASSAY OF PINE BARK EXTRACTS AS BITING STIMULANTS FOR THE SOUTHERN PINE BEETLE[1]

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ABSTRACT.—A bioassay was developed to compare pine outer-bark constituents extracted chemically from five species of southern pines. Extracts from inner and outer bark were also compared. Extracts from the outer bark of shortleaf pine elicited the greatest number of biting responses which significantly exceeded the controls. Beetles responded more often to extracts from the outer bark than to those from the inner bark.

Keywords: *Dendroctonus frontalis*, Coleoptera: Scolytidae, solvent extractives, *Pinus* spp.

Chemical substances are known to influence host finding, aggregating, and mating of southern pine beetles (SPB), *Dendroctonus frontalis* Zimmermann (Coster and Vité 1972; Renwick and Vité 1968, 1969; Vité and Renwick 1971), and in phytophagous insects, host substances are known to influence host selection (Thorsteinson 1960). How host substances influence host selection by SPB is not fully understood.

Scolytids initiate attack by first biting into the host or constructing galleries, releasing volatiles which induce subsequent mass attack. Bark constituents may be the key to continued attack or abandonment of a particular tree. Some host trees in heavily infested areas may survive by overcoming beetles through resinosis, by chance escape, or by their lack of chemical stimuli which induce pioneer beetles to attack. Furthermore, Coster and Vité (1972) found that pheromone production by the female SPB is related to feeding activity, and Hughes (1973) has shown that behaviorally important substances are produced by the beetles from the terpene α-pinene, which could be obtained during feeding.

The present study was undertaken to determine (1) whether substances in pine bark induce biting responses of the SPB, (2) whether these substances occur in the outer or the inner bark or differ among pine species, and (3) what general region in the bole contains active substances.

METHODS AND MATERIALS

We modified the elder (*Sambucus* sp.) pith bioassay used for *Scolytus multistriatus* (Marsham) by Norris and Baker (1967) and for *Hylobius pales* (Herbst) by Thomas (1969). A bioassay unit consisted of the bottom half of a disposable plastic petri dish, a moistened 9-cm-diameter disk of nonwoven polyester fiber[3] used as a floor, a 2-mm-thick elder pith wafer treated with pine bark extract or solvent,

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and a 3-mm-thick Plexiglas® disk cut to fit exactly inside the petri dish (fig. 1).[4] With the Plexiglas insert supported by the wafer, the beetles could walk about in the dish but could not climb. This arrangement limited the beetles' activity to the same plane as the wafers and encouraged them to feed. Notches were cut in the perimeter of the pith wafers to simulate bark crevices. Test solutions were applied to the wafers with a microsyringe at rates calculated to provide extracted substance in an amount equal to that found in the bark (dry-weight basis). After treatment, wafers were air-dried for 14 to 16 hours.

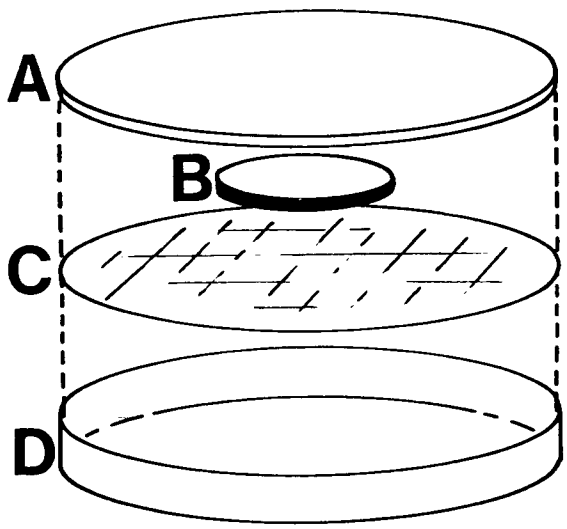

Figure 1.—Exploded view of bioassay unit: *A*, 3-cm-thick Plexiglas insert; *B*, elder pith wafer; *C*, 9-cm-diameter polyester nonwoven fiber disk; *D*, bottom half of plastic petri dish.

To initiate a bioassay, the polyester disks and treated elder pith wafers were put in place, beetles were added at the outside edge of the petri dishes, and the Plexiglas disks or regular covers were added.

Petri dishes in a complete bioassay were assigned to random positions on a table and received 1100 to 1400 lux from Daylight® fluorescent lamps. The testing room was kept at 24° C (∓2°) and 85 to 95 percent relative humidity and was without windows.

Bark was collected at breast height from two to five trees of five pine species: loblolly (*Pinus taeda* L.), shortleaf (*P. echinata* Mill.), Virginia (*P. virginiana* Mill.), slash (*P. elliottii* Engelm.), and pitch (*P. rigida* Mill.). Collections were made within 40 km of our North Carolina laboratory except for the pitch pine, which was collected approximately 400 km west. Sample trees were 20.3 to 35.5 cm in d.b.h. and 9.1 to 22.8 m in height. Loblolly, shortleaf, and Virginia pines are locally indigenous and susceptible to successful attack and colonization by the SPB. Slash and pitch pines are occasionally attacked but grow chiefly outside the range of the SPB. These latter two species were included to provide an indication of the sensitivity of the bioassay.

Bark samples were freeze-dried and ground to 60 mesh. Subsamples from each species were pooled and Soxhlet extracted simultaneously for 24 hours with six solvents: water, 95 percent ethanol, 70 percent ethanol, benzene, hexane, and diethyl ether. These solvents were selected to provide a complete range of polarity. The amount of material extracted from each source by each solvent was calculated.

Beetles were collected as they emerged in the laboratory from naturally infested pine bark collected within 121 km of the laboratory. Most infested bark came from shortleaf pine, but 10 to 20 percent came from loblolly pine. Beetles were collected within 6 hours after emergence and sexed by the method of Osgood and Clark (1963). We attempted to carry out each bioassay series with beetles which emerged from bark collected in one local area, usually from trees within a single stand.

The objective of the first series of bioassays was to investigate the solvent/species relationship; i.e., the question of which solvent extracts of the outer bark of each pine species elicited biting activity. The six solvents and the five species gave 30 treatments. Since each treatment was paired with a control (solvent alone, no extract), there were 30 controls. The extracts obtained with the six solvents from a single pine species were bioassayed together three times: once with male beetles, once with females, and once with unsexed beetles. Within a single bioassay, each of the six treatments was replicated five times; i.e., individual petri dish units containing six beetles each were paired with five units with beetles in which the pith wafers were treated with solvent alone. A diagram of the typical treatment array is given in figure 2.

The number of beetles at the wafer in each petri dish was counted at 30-minute intervals for 3 hours. The observations made at the end of the second hour were selected for ANOVA, because they were at the approximate midpoint of the test. Thirty

[4]Mention of commercial products in this paper is solely to identify materials used, and does not constitute endorsement by the U.S. Department of Agriculture.

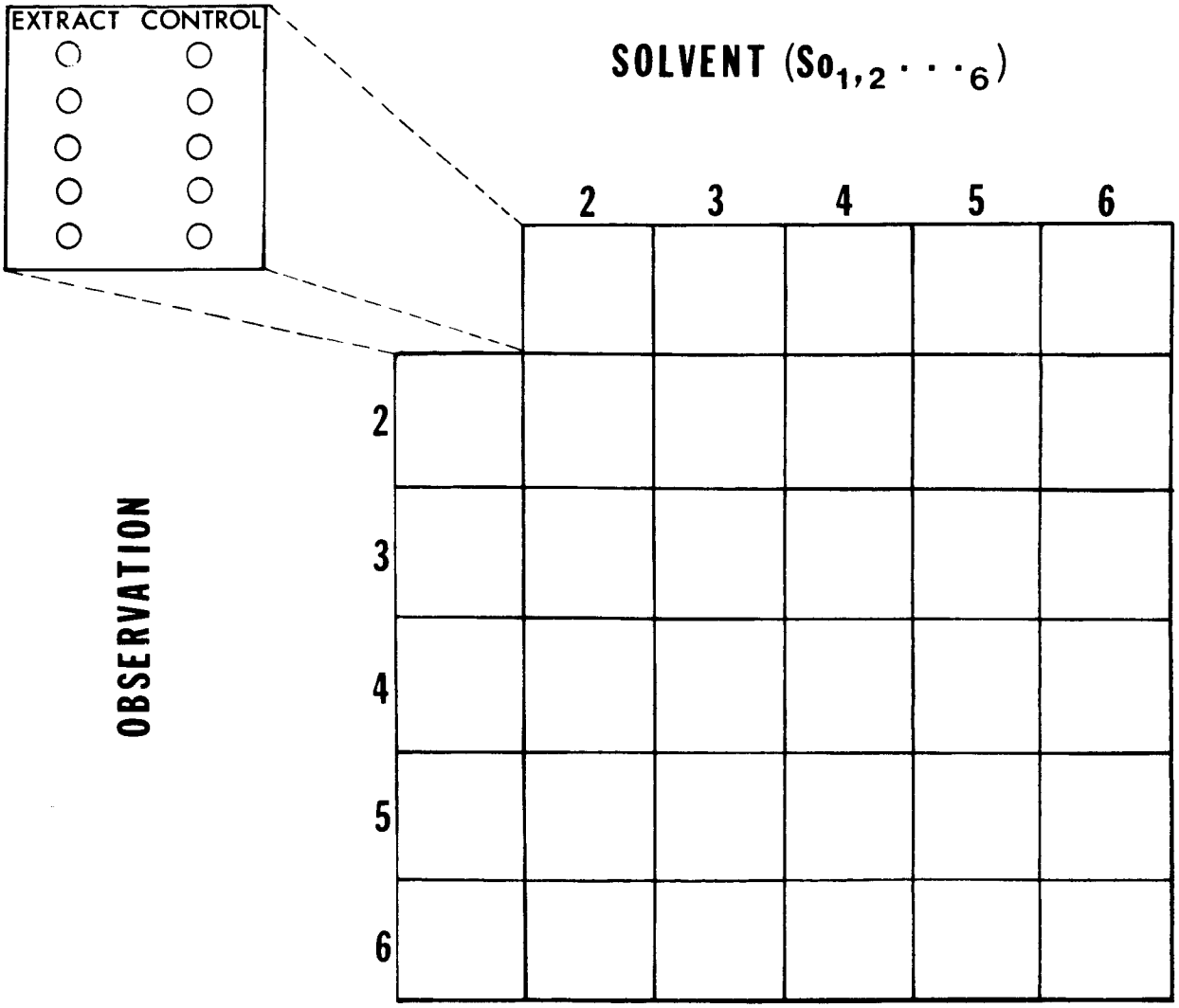

Figure 2.—Experimental design of biting bioassay for southern pine beetles.

individual tests of significance were performed to compare the biting response for each treatment combination (solvent containing extract) with its own control (solvent only).

In the next series of bioassays, beetle biting responses to methanolic extracts of phloem (inner bark) were compared with responses to methanolic extracts of outer bark (periderm) from shortleaf and loblolly pines, two preferred hosts of the SPB. Bark was collected at breast height and at the base of the live crown in both species. Five trees of each species were sampled. Samples from the same species and height were pooled, freeze-dried, ground, and extracted in a Soxhlet apparatus for 24 hours with methanol. In preliminary studies, methanol had been found to remove a wide range of substances from bark. Comparisons of the methanol extracts were replicated daily for 8 days in a balanced, complete-block design with separate bioassays for male, female, and unsexed beetles. Again, the number of beetles at the wafer in each petri dish was counted at 30-minute intervals for 3 hours. A mean biting response was calculated by pooling the data from similar replications, finding the mean and standard deviation of the number of beetles at the extract and control wafers at the end of the 3-hour period, and finally obtaining the difference between the mean biting response to the various extracts and corresponding controls (solvent only). Note that this arrangement used fewer control units than did the previously described series.

RESULTS AND DISCUSSION

The results of the bioassay series to compare the responses with the species X solvent combinations are shown in table 1.

There appeared to be no relationship between biting activity and species of pine from which the samples were obtained. Female biting activity on extracts prepared with 95 and 70 percent ethanol showed more significant 'F' values than on extracts prepared with other solvents. When the solvent means were subject to Duncan's multiple range test, however, no indication could be found of grouping along any point in the range of polarity represented.

Again, when methanol extracts were prepared from periderm and phloem of shortleaf and loblolly pine, there were considerable variations in mean biting response among three bioassays (table 2). In this series of tests, only the shortleaf pine periderm extract consistently elicited a response greater than the control. This extract also caused an equal or greater response than that of extracts from shortleaf pine phloem. The biting response was highly variable among the extracts from the other sources.

Table 1.—'F' values solvent X species

Solvent	Loblolly	Pitch	Shortleaf	Slash	Virginia
			FEMALES ONLY		
Water	0.16	2.50	7.20*	0.29	0.0
95% ethanol	2.02	.33	2.25	4.90*	.08
70% ethanol	7.72*	5.76*	3.20*	.07	12.72*
Benzene	.09	1.00	1.20	0	.80
Hexane	1.91	.57	.29	1.52	.23
Diethyl ether	.93	2.67	2.67	3.20	0
			MALES ONLY		
Water	.03	1.38	.05	.55	0
95% ethanol	4.92*	.20	8.06*	.64	.91
70% ethanol	.02	.71	2.93*	3.66*	1.11
Benzene	1.67	10.29*	1.00	.24	6.45*
Hexane	1.02	.35	1.06	.60	.13
Diethyl ether	1.66	6.40*	.18	.57	.44

*Significantly different from control (P = 0.10).

Table 2.—Biting response of southern pine beetles to methanolic extractives of loblolly and shortleaf pine phloem and periderm during three bioassays

Sources	Biting response per bioassay ($\bar{x} \pm$ SD)[a]		
	I	II	III
Loblolly phloem	7.97 ± 2.40	7.10 ± 2.93	5.66 ± 2.66
Shortleaf phloem	6.12 ± 3.39	5.06 ± 2.15	4.08 ± 2.25
Loblolly periderm	10.50 ± 2.90	3.50 ± 3.08	5.85 ± 2.88
Shortleaf periderm	9.54 ± 5.57	5.04 ± 3.21	7.75 ± 3.31
Control (solvent only)	7.33 ± 3.26	4.37 ± 3.00	5.13 ± 2.03

[a]Means were calculated from the number of beetles responding during a 3-hour period. Bioassays for each source consisted of beetles from the same population (area) and tested in groups of 30 male and 30 female beetles.

Our findings indicate that methanol extracts from the periderm of shortleaf pine contain substances that induce more biting initiation by the SPB than do those from the phloem of shortleaf, the periderm and phloem of loblolly, or the periderm of Virginia, pitch, and slash pines. There was no significant difference in the biting response to samples collected at breast height or at the base of the live crown. Although the biting response of male and female beetles to all sources was similar, tests with unsorted beetles often produced relatively low responses or none. Data from these tests were eliminated because of the possibility of interaction between the sexes.

The results show that positive biting responses can be obtained in the laboratory to substances extracted from the bark of hosts of the SPB, but these responses are relatively weak and variable. Unpublished research at this laboratory has shown the importance of volatile substances in initiating feeding by the SPB. Those results show that virtually all of the substances affecting this behavior are removed during ovendrying but not during freeze-drying. Nevertheless, it is probable that some important volatiles (or at least critical amounts of them) are lost even during freeze-drying. Other work on pine bark composition and its effect on initiation of beetle tunneling (White, this Laboratory, unpublished) suggests that composition varies significantly between trees. Because bark samples from several trees were pooled in this study, this variation may have been obscured.

Our work suggests that other factors are more important than biting stimuli in governing host selection by the SPB.

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