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OVIPOSITION STIMULANTS FOR THE BEETLE, *Monochamus alternatus* HOPE, IN INNER BARK OF PINE

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Abstract—Field and laboratory ovipositional responses of *Monochamus alternatus* Hope, respectively, to methanol and water extracts from pine inner bark were examined in comparison with those to pine inner bark, especially using a laboratory-built apparatus for the latter bioassay. Irrespective of the existence of volatiles from paraquat-induced lightwood, pine inner bark and its methanol and water extracts stimulated ovipositional response only in the presence of free moisture.

Key Words—*Monochamus alternatus* Hope, Coleoptera, Cerambycidae, pine inner bark, methanol extracts, water extracts, oviposition stimulants, lightwood.

INTRODUCTION

It has been well known that the cerambycid beetle, *Monochamus alternatus* Hope, is a vector of the pine wood nematode, *Bursaphelenchus xylophilus* Steiner et Buhrer, which causes severe mortality of pines (Morimoto and Iwasaki, 1972; Mamiya and Enda, 1972).

Recently, attractiveness of pines, treated with the herbicide paraquat, to *M. alternatus* has been confirmed (Yamasaki et al., 1980a). Paraquat induces an extensive area of oleoresin-soaked lightwood in the xylem of the pines (Roberts et al., 1973; Conley et al., 1977; Yamasaki et al., 1980b; Yamasaki and Sogo, 1982). The attraction of both sexes of *M. alternatus* arises from volatiles involved in the paraquat-induced lightwood (Yamasaki and Suzuki, 1982).

After landing on a dying (Katagiri et al., 1964) or paraquat-treated pine (Yamasaki and Sunago, 1983), a *M. alternatus* female moves to a site favorable

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for oviposition, cuts a characteristic pit in the outer bark, inserts her ovipositor into the inner bark through the pit, and deposits eggs. In the case of the paraquat-treated tree, the site of oviposition is restricted within the area over the lightwood (Yamasaki et al., 1980a).

The role of chemical stimulants in the oviposition of *M. alternatus* is not known. This paper presents evidence that nonvolatile oviposition stimulants for *M. alternatus* occur in the pine inner bark.

METHODS AND MATERIALS

Field Test Materials. For lightwood, 17-year-old *Pinus densiflora* Sieb. et Zucc. trees were frill-treated with paraquat (1,1′-dimethyl-4,4′-dipyridinium dichloride) early in May 1986 (Yamasaki and Sogo, 1982). The paraquat-induced lightwood was harvested in mid-July 1986. Five lightwood samples (7 × 35 × 2.5 cm) were immediately used in the field, and the remainder was used for collection of volatiles.

For methanol extract, air-dried inner bark (0.9 kg) of *P. densiflora* trees of the same age, harvested late in April 1986, was milled and extracted with boiling methanol (2.8 liters) for 3 hr. Filtrate was concentrated under reduced pressure.

For impregnating filter paper, a sheet (21 × 35 cm) of 0.2-mm-thick filter paper (dry weight 6.459 g) was impregnated with 10 ml of the concentrate (19.38 w/v%) using a pipet, dried in air, in vacuo at 80°C for 12 hr in the presence of desiccating agents, and weighed. After standing in air for two days, the sheet containing 30% methanol extract was weighed again.

Field Bioassay. The bioassay was conducted from 8:00 PM on July 15 to 8:00 AM on July 16, 1986, in a stand where severe mortality of pines was caused by nematodes.

Four of the fresh lightwood samples were wrapped with a sheet of the test filter paper with or without methanol extract or a sheet (21 × 35 cm) of freshly harvested inner bark of *P. densiflora*. A tinplate sheet (40 × 40 cm) to hold eggs was nailed to one of the two cross sections of the remaining lightwood sample without wrapper. After moistening the sheets of the test filter paper with a calculated volume of water, the baits with and without wrapper were hung on a wire 2 m above the ground at 5-m intervals along a ridge. The total number of *M. alternatus* attracted to each bait was counted at 8:30, 9:30, and 10:30 PM. The assayed wrappers were torn into pieces about 1 mm wide, and the number of eggs laid by *M. alternatus* was counted. To prevent hatching, eggs laid in parts awaiting examination were kept at 5°C.

Laboratory Test Materials. Lightwood volatiles (LW volatile) were collected by letting air from a cylinder pass through particles of the fresh lightwood and leading the gas into cooled traps (Yamasaki and Suzuki, 1982).

For test substrates, after being cut into 4 × 5cm pieces in order to remove volatiles, bark, inner bark, and about 6-mm-thick outer bark of 17-year-old *P. densiflora* trees, harvested late in April 1986, were first dried in air for two months, then in an air-circulating oven at 80°C for seven days, in vacuo at 80°C for seven days, and weighed. Pieces of 4 × 5-cm × 1.5-mm-thick veneer made of commercial lumber of *P. densiflora* also were dried and weighed. In addition to these substrates, 4 × 5-cm pieces of 1-mm-thick stainless steel were prepared.

For moistening these substrates, except the stainless steel, prior to laboratory bioassay, moisture content was achieved variously: (1) One series of 12 pieces was stood on an electronic balance, until the content reached 1.3%. (2) Another series to 5–6% by exposure to a cloud of steam. (3) A third to 18–56% by spraying with water, and, after 85–97% of a calculated water volume was adsorbed, the remainder was added using a syringe.

For fractions of water extract, the remainder (2 kg) of the fresh inner bark was reduced to particles and extracted with 3.9 liters of boiling water for 1.5 hr. The extract was fractionated as shown in Figure 1. Pieces of filter paper, 4 × 5 cm × 0.7 mm thick, containing the 30% fraction I were prepared in the same way. Pieces of laminated filter paper, 4 × 5 cm and 1.1 to 1.3 mm thick, containing 30% fractions II, III, IV, or V were prepared as shown in Figure 2.

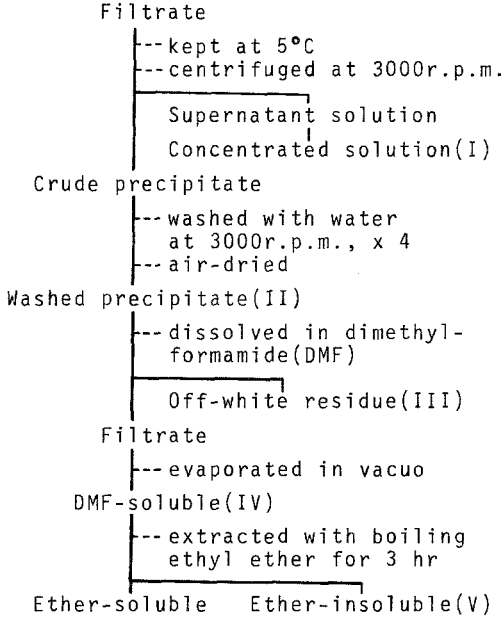

FIG. 1. Fractionation of water extract from fresh inner bark of *P. densiflora*.

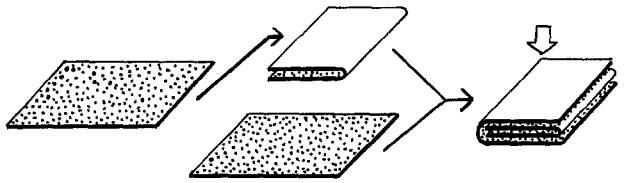

FIG. 2. Makeup of laminated filter paper containing powdery fraction II, III, IV, or V. Two 8.3 × 5-cm pieces (dry weight 0.708 g for two) of 0.2-mm-thick filter paper were moistened with water (0.9 ml), and dusted with the sample (0.212 g). One piece was folded in two, and placed on the other. The other was folded back. This laminated filter paper with the sample was pressed under a pressure of 50 kg/cm^2 for 1 min.

The pieces were dried in vacuo at 80°C for 12 hr, weighed, and moistened with a calculated volume of water immediately before the laboratory bioassay.

The Insect. P. densiflora trees surviving where many other trees were dying were frill-treated with paraquat early in July 1986. About 200 *M. alternatus* females attracted to these trees were caught in mid-July and held in individual glass bottles (Yamasaki and Suzuki, 1982).

Laboratory Bioassay. The apparatus consisted of a hardware cloth chamber (60 × 7 × 15 cm) without one side (60 × 7 cm), a tin-plated chamber (60 × 7 × 5 cm) without one side (60 × 7 cm), and a hardware cloth screen (63 × 10 cm), as shown in Figure 3. The hardware cloth chamber was partitioned into eight compartments using tinplate sheets wrapped with hardware cloth.

A series of eight test pieces moistened with a calculated volume of water as described above was fastened on the hardware cloth screen with hairpins (Figure 3). LW volatile (0.5 ml) was placed into each of three Petri dishes (2.5

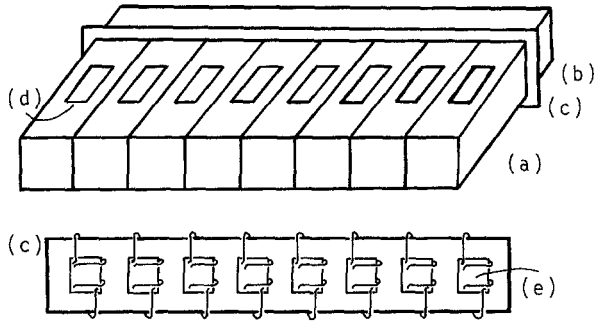

FIG. 3. Bioassay apparatus for *M. alternatus*: (a) compartment for each insect; (b) chamber in which LW volatile was placed; (c) hardware cloth screen; (d) insect entry hole (5 × 9 cm) with cover; (e) ovipositional substrates fastened on bed sheet (veneer or stainless steel) with hairpins or wiring.

cm ID × 1.8 cm) inside the tinplate chamber. The apparatus was assembled with wire. Individual *M. alternatus* females were placed into each of the eight compartments. The bioassay was conducted from 8:00 PM to 8:00 AM. As a control, bioassay in the absence of LW volatile was conducted in another room (about 100 m away). The surface of the assayed materials was observed, and the number of eggs laid was counted. The percentage of ovipositional response was calculated as follows: Ovipositional response (%) = 100 × No. which responded/8 individuals.

RESULTS

Field Attraction and Oviposition of M. alternatus. Both sexes of *M. alternatus* were attracted to every bait in the field (Table 1). This table shows that at least one or two females were attracted to each of the baits (e.g., two females to the bait without wrapper). Four and 25 eggs laid by *M. alternatus* were present in or under the moistened filter paper with methanol extract and the fresh inner bark, respectively. It is impossible to compare the numbers of eggs (4 vs. 25) because the real number of the females attracted to each of the baits is not shown in this table. No eggs, however, were observed at the other baits; with that the two former observations are comparable.

Oviposition of M. alternatus *in Laboratory Affected by Free Moisture.* Figure 4 shows the effect of moisture content of pine inner bark at the onset of

TABLE 1. FIELD ATTRACTION AND OVIPOSITION OF *M. alternatus*

Bait	Sex	Total no. of *M. alternatus* attracted to each bait			Eggs laid (*N*)
		8:30 PM	9:30 PM	10:30 PM	
Lightwood (a)[a]	Female	1	2	2	0
	Male	1	4	2	
Lightwood (a) + moistened filter paper (b)	Female	0	1	1	0
	Male	1	2	2	
Lightwood (a) + air-dried filter paper containing 30% MeOH extract (c)	Female	1	0	1	0
	Male	2	3	0	
Lightwood (a) + moistened filter paper containing 30% MeOH extract (b)	Female	1	1	0	4
	Male	1	0	2	
Lightwood (a) + fresh inner bark (d)	Female	0	1	2	25
	Male	1	2	1	

[a]Moisture content: a, 20–35%; b, 50%; c, 9%; d, 67%.

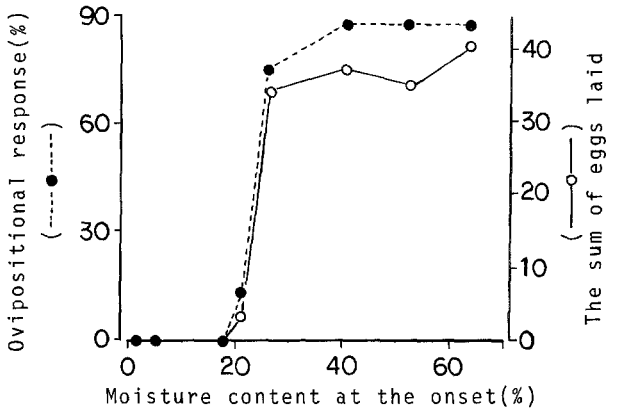

FIG. 4. Effect of moisture content of inner bark of *P. densiflora* on oviposition by *M. alternatus*. The laboratory test was conducted July 18–19, 1986, using LW volatile. Veneer with moisture content almost equivalent to that of the corresponding inner bark was used as a bed sheet. When fresh inner bark with 64% moisture was assayed, veneer with 56% moisture was used. Moisture contents of eight series of another four pieces which were placed outside their respective apparatus for 12 hr were substituted for those of inner bark after bioassay.

bioassay on oviposition by *M. alternatus*. Moisture contents of 1.3 and 5.0% at the onset increased to a level of 13.6–14.6% overnight. The contents of 18.0 and 21.0% decreased to levels of 16.4–17.0% and of 16.9–19.1%, respectively. The contents of 26.0% and more also decreased to a level of 21.7–26.4%. When inner bark with 18.0% or less moisture at the onset was assayed, neither egg laying nor the slight scoring of the inner bark prior to ovipositor insertion was observed, although a few scars produced by gnawing or feeding beetles were observed. Under inner bark with 21.0% moisture, three eggs were laid by one of eight beetles. Thirty-four eggs were laid in or under inner bark with 26.0% moisture by six of eight beetles, and in or under inner bark with 41.0% or more moisture 35–40 eggs were laid by seven of eight beetles. Besides the scars, openings resulting from ovipositor insertion, like pinholes of less than 1 mm ID in the respective scores also were observed in the assayed inner bark with 21.0% and more moisture.

Oviposition of M. alternatus *in Various Substrates.* Materials tested in the laboratory are shown in Table 2. Bed sheet alone and filter paper with or without the bed sheet did not induce oviposition by *M. alternatus*. Scars different from those on inner bark were left on the filter paper, but were not observed on the bed sheet alone. Outer bark containing 5–6% moisture elicited no oviposition.

TABLE 2. LABORATORY OVIPOSITION OF *M. alternatus* IN VARIOUS SUBSTRATES WITH AND WITHOUT VOLATILES (JULY 22–25, 1986)

Substrate	LW volatile present[a]		LW volatile absent[a]	
	Ovipositional response (%)	Eggs laid	Ovipositional response (%)	Eggs laid
Veneer	0	0	0	0
Stainless steel	0	0	0	0
Filter paper	0	0	0	0
Filter paper + veneer	0	0	0	0
Filter paper + stainless steel	0	0	0	0
Outer bark[b] + veneer[b]	0	0	0	0
Outer bark + veneer	0	0	12.5	2
Outer bank + stainless steel	12.5	2	0	0
Inner bark	87.5	42	75	42
Inner bark + veneer	87.5	49	87.5	39
Inner bark + stainless steel	87.5	38	87.5	40
Bark + veneer	87.5	53	100	38
Bark + stainless steel			75	42

[a] Volatiles extracted from paraquat-induced lightwood.
[b] Moisture content at the onset was 5–6%; all others were 49–56%, except stainless steel.

On the other hand, when outer bark with 49–56% moisture was assayed, one of eight beetles laid two eggs; otherwise, no eggs were observed. Pits, slots, or galleries of various sizes in every series of the outer bark were 9–13 in number. In contrast to these results, even in the absence of LW volatile, 42, 39, and 40 eggs were laid in or under moistened inner bark by six, seven, and seven of eight beetles, respectively. In or under moistened bark 53, 38, and 42 eggs were laid by seven, eight, and six of eight beetles, respectively. On average, 21 pits were left on a series of eight bark pieces.

Oviposition of M. alternatus *in Laboratory Affected by Fractions of Water Extract.* Five fractions of water extract were assayed (Table 3). Beside scars, small openings were observed in every series, except dried filter paper containing fraction IV, which elicited no oviposition. One of eight beetles laid two or three eggs under moistened filter paper with fraction I. One beetle laid four eggs under moistened filter paper with fraction III in the presence of LW volatile. On the other hand, moistened filter paper with fractions II, IV (Figure 5), and V elicited ovipositional responses of 75% of the 17 or 20 eggs laid, 87.5% of 21 or 18; 87.5% of 20; and 75% of 24, respectively.

TABLE 3. EFFECT OF FRACTIONS OF WATER EXTRACT ON OVIPOSITIONAL RESPONSE OF *M. alternatus* (JULY 26–28, 1986)

Material with veneer[a]	LW volatile present		LW volatile absent	
	Ovipositional response (%)	Eggs laid	Ovipositional response (%)	Eggs laid
Filter paper containing 30% fraction I	12.5	3	12.5	2
Laminated filter paper containing 30% fraction II	75	17	75	20
Laminated filter paper containing 30% fraction III	12.5	4	0	0
Laminated filter paper (a) containing 30% fraction IV	0	0	0	0
Laminated filter paper containing 30% fraction IV	87.5	21	87.5	18
Laminated filter paper containing 30% fraction V	87.5	20	75	24
Inner bark	87.5	42	87.5	38

[a] Moisture contents of materials and veneer at the onset, respectively, were 55 and 50–56%, except the case of (a); in this case filter paper material and veneer, respectively, containing 6% and 5% moisture were used.

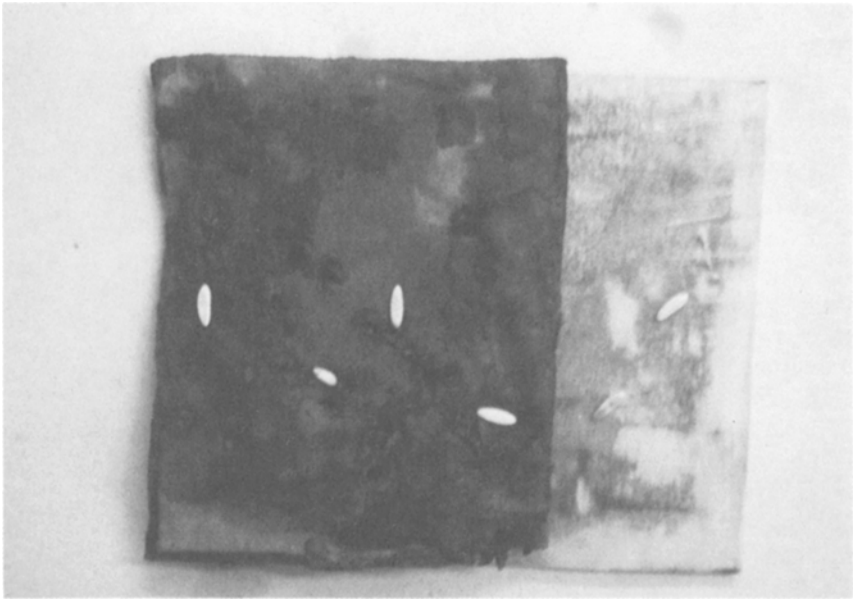

FIG. 5. Six eggs laid under moistened filter paper with fraction IV by a *M. alternatus* female. The assayed substrate is turned inside out and placed on veneer. This beetle laid two other eggs in the substrate. Openings resulting from ovipositor insertion also are shown.

DISCUSSION

It is evident from Tables 1–3 that oviposition of *M. alternatus* is not evoked by volatiles, but by nonvolatiles occurring in the inner bark of *P. densiflora*. The oviposition stimulants are extractable in methanol, dimethyl formamide, or water, but are insoluble in ethyl ether. The ovipositional response elicited by fraction II, IV, or V was equivalent to 86–100% of that elicited by inner bark as a control, whereas the number of eggs laid was only 40–60% of that laid in the inner bark (Table 3).

The bed sheet was found to be unnecessary for oviposition (Table 2), but it is convenient to prevent the loss of eggs laid. Oviposition of *M. alternatus* appears to be independent of cambium or xylem of host pine.

It has been known that in the sphingid *Manduca sexta* L. (Sparks, 1973) and noctuid *Anadevidia peponis* F. (Ichinose and Sasaki, 1975) moisture itself evokes oviposition. In *M. alternatus*, however, free moisture must be present in order for the chemical stimulants from inner bark to elicit oviposition (Figure 4, Tables 1 and 3). Critical moisture content necessary for oviposition appears to be between 18% and 21% under these experimental conditions. Contact chemoreception through intervention of free moisture has been confirmed in some butterflies such as *Byasa alcinous* K., *Leudhorfia japonica* L., *Papilio xuthus* L., *P. machaon hippocrates* C. et F., *P. macilentus* J. (Nishida, 1977), *P. protenor demetrius* C. (Ichinose and Honda, 1978), and *P. bianor dehaanii* C. et F. (Abe et al., 1981). In *M. alternatus*, however, chemoreceptors have not been identified.

The behavior of cutting a pit in the outer bark prior to ovipositor insertion was independent of moisture content. Moisture content of the outer bark of a living pine varies with atmospheric conditions, unlike that of inner bark. It was alluded that volatiles from bark of dying pines evoke this behavior (Yamane et al., 1975). In this study, however, the behavior of cutting a pit in outer bark was observed even in the absence of LW volatile. On inner bark with free moisture, the behavior of slightly scoring it prior to ovipositor insertion was observed. On filter paper with fractions I to V of water extract, no pit-making behavior was observed. Nonvolatiles in outer bark may induce this behavior. In the absence of LW volatile, fraction III elicited no oviposition (Table 3), but 10 openings were produced by four of eight beetles. Ovipositor insertion appears to be induced by a wide variety of factors, in addition to oviposition stimulants, involved in pine inner bark.

The present results suggest the feasibility of controlling *M. alternatus* by using oviposition stimulants in combination with attractants produced by paraquat-induced lightwood without insecticide application.

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