Western Pine Beetle: Field Response to Its Sex Pheromone and a Synergistic Host Terpene, Myrcene

Abstract. *In the field, both sexes of the western pine beetle,* Dendroctonus brevicomis, *are attracted by the female-produced bicyclic ketal* exo-*brevicomin; this response is enhanced by myrcene (a constituent of the beetle's host, ponderosa pine), which is not an attractant by itself. This synergism may be part of the phenomenon of the mass attack on its host.* Temnochila virescens chlorodia, *one of the principal insectan predators of this beetle, is attracted by* exo-*brevicomin alone.*

We report the identification of a host plant compound that synergizes the attractiveness of a bark beetle sex pheromone. *Exo*-brevicomin (*exo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane isolated and identified from female frass of *Dendroctonus brevicomis* (Coleoptera: Scolytidae) (*1*), is the first pheromone from a bark beetle found to be attractive alone under field conditions. The addition of myrcene (*2*), a natural component of *Pinus ponderosa* oleoresin, enhances the response of both sexes to *exo*-brevicomin in the laboratory and in the field. *Dendroctonus brevicomis* makes a mass attack and kills its host to reproduce. *Exo*-brevicomin and myrcene are part of the chemical basis of this phenomenon. Both sexes of *Temnochila virescens chlorodia* (Coleoptera: Ostomidae), one of the principal insectan predators of this bark beetle, are strongly attracted to *exo*-brevicomin alone.

Experiments were performed in 80- to 100-year-old ponderosa pine stands at both McCloud Flat, Siskiyou County, and near the south fork of Willow Creek, Madera County, California. Traps were affixed to the boles of trees whose breastheight diameter was 36 to 51 cm. All traps were hung 7.5 m above the ground. The test materials and controls were interchanged for each test. At Willow Creek, traps were strips of hardware cloth (30 by 61 cm; No. 4 mesh) coated with Stickem Special and hung on opposite sides of the bole. Trees were selected 21 to 90 m apart without regard to wind direction. At McCloud Flat the traps were formed into semicylinders that were joined to form a cylinder (61 cm high, 61 cm in diameter) around the bole. Selected trees were about 12 m apart in a line perpendicular to the prevailing winds.

The attractiveness of infested ponderosa pine bolts was compared to that of the test materials. The bolts were prepared by confining live, virgin female western pine beetles inside preformed tunnels (*3*). The infested bolts were stored in the dark at room temperature for 24 hours before use. The controls were traps that were not charged with infested bolts or test materials.

The compounds were obtained as follows. *Exo*-brevicomin was synthesized (*4*), and natural myrcene (Aldrich) was obtained 99.8 percent pure by sequential, preparative gas chromatography on Carbowax 20M and Apiezon L; purity was determined on a PDEAS (phenyldiethanolamine succinate) column.

A solution of *exo*-brevicomin (2 or 20 mg) in 0.7 g of Vaseline and 7 ml of pentane was added to a substrate consisting of 1 percent Carbowax 20M on 7 g of Chromosorb A, 20- to 30-mesh. A solution of 20 mg of myrcene in 1 g of Vaseline and 10 ml of pentane was added to 1 percent Carbowax 20M on 10 g of Chromosorb A, 20- to 30-mesh. An extract of 2 g of female frass in 1.5 ml of benzene, 0.7 g of Vaseline, and 7 ml of pentane was added to 1 percent Carbowax 20M on 7 g of Chromosorb A, 20- to 30-mesh. The *exo*-brevicomin and extract preparations were poured into an aluminum tube (30 cm by 7 mm inside diameter) and the myrcene preparation into a tube (45 cm by 7 mm inside diameter). The tubes were stored over solid carbon dioxide until used.

In the field, test material was delivered by aerating the tubes at a flow rate of 50 cm^3/min (*3*, *5*). Laboratory calibrations at this flow rate at 26°C over a 7-hour period gave a 65 percent rate of recovery of *exo*-brevicomin and a 55 percent rate of recovery of myrcene. No other products were detected. Thus we were certain that the compounds delivered were actually the original compounds rather than possible rearranged or degraded products. The elution rate from aeration tubes is relatively constant (*3*) as compared to the decreasing rates characteristic of evaporation alone. The small amounts of solvents are eluted in the first few minutes. Solvents have been shown to influence the response of bark beetles to their attractants (*3*).

In order of decreasing attractiveness, the sources were: female-infested bolts, myrcene with brevicomin, female frass extract, and *exo*-brevicomin (Table 1). Under the test conditions, neither the controls nor myrcene alone were attractive. The simultaneous release of myrcene and *exo*-brevicomin enhanced the field response of both sexes of *Dendroctonus brevicomis* ($\chi^2 = 64$; d.f., 3; $P < .001$). Response to as little as 2 mg of *exo*-brevicomin alone eluted over

Table 1. Mean number (range in parentheses) and sex ratio of *Dendroctonus brevicomis* trapped by treatments indicated. (A) Two tests, 9 October 1968; (B) four tests, 17–18 October 1968; (C) five tests, 21–24 October 1968. Results of the χ^2 test are expressed as the probabilities that deviation from the sex ratio of beetles caught by bolts (1:1) is due to chance alone.

Treatment	Tests			Trapped (males/females)	χ^2 test (P)
	A	B	C		
Exo-brevicomin (2 mg)	12 (9–15)	8 (2–26)	3 (0–4)	2.2	$< .001$
Myrcene (20 mg)		0	0		
Exo-brevicomin (2 mg) plus myrcene (20 mg)	28 (20–36)	16 (6–28)	14 (2–33)	2.2	$< .001$
Extract of 2 g of female frass		12 (2–29)	5 (1–10)	0.9	$< .5; > .3$
Control	0	1 (0–3)	0		
Bolt with 50 female attacks	96 (10–182)	27 (17–49)	16 (4–43)	1.0	

Table 2. Number and sex ratio of *Temnochila virescens chlorodia* trapped in response to female-infested bolts or to 20 mg of brevicomin in 4- to 6-hour test periods at McCloud Flat.

Test	No. trapped in September 1968				Sex ratio (males to females)
	24	24	25	26	
60 Female attacks in fresh bolt	0	3	11	17	1:0.75*
Brevicomin	33	53	94	73	1:1
Control	2	2	1	0	

* Probability (χ^2) that deviation from the sex ratio of beetles caught by brevicomin is due to chance alone is $.5 < P > .3$.

a 6-hour period demonstrates the sensitivity of the beetles to this material.

The female-infested bolt and frass extract attracted beetles in a 1:1 ratio of males to females (Table 1); but the ratio of beetles attracted by *exo*-brevicomin and by *exo*-brevicomin with myrcene was 2.2 : 1. We do not know whether the high proportion of males is caused by differences in concentration or by the absence of compounds. Besides myrcene, other components of the terpene hydrocarbon fraction from the extract of female frass synergize *exo*-brevicomin in laboratory tests (*1*, *2*). These components remain to be identified and tested in the field.

Temnochila virescens chlorodia, one of the principal predators of *Dendroctonus brevicomis* larvae and adults, has been observed to aggregate on trees that were under mass attack (*6*). The synchronous arrival of this predator and the western pine beetle on the host tree is a result, at least in part, of the predator's response to one of the pheromones produced by the bark beetle, namely, *exo*-brevicomin (Table 2). The female predator apparently must feed on adult bark beetles for reproductive maturation (*7*). This response to a component of the pheromone of the bark beetle may be the mechanism whereby high prey densities can be efficiently located. The predators then oviposit, and their larvae feed on the immature stages of the bark beetle.

The possible nature of the mass attack in the western pine beetle is suggested by this study. Myrcene is a prominent constituent of the xylem oleoresin of two host species, *Pinus ponderosa* (*8*) and *P. coulteri* (*9*), of the western pine beetle in California. *Exo*-brevicomin attracts low numbers of beetles initially, but the mass attack probably depends upon the release of myrcene and other unknown compounds from the host as a result of boring activity by the female.

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Structural Determinant of a Ribosomal Protein: K Locus

Abstract. *One of the ribosomal proteins (30S-8B) of* Escherichia coli *B has a greater electrophoretic mobility than the homologous protein (30S-8K) in* E. coli *K. The amino acid compositions, tryptic peptides, and cyanogen bromide peptides of these two homologous proteins unambiguously indicate that the genetic locus which determines the electrophoretic mobility of protein 30S-8 is the structural gene for this protein.*

There are two genetic loci which affect the structure of ribosomal proteins. One is the locus which determines the streptomycin phenotype of the ribosomes; this is expressed in the structure of at least one of the proteins of the 30*S* ribosomal subunit (*1*). The second locus is that of "K character," which is expressed in the electrophoretic mobility of one of the 30*S* proteins (*2*). Comparison of the 30*S* proteins from K strains of *Escherichia coli* with those from other strains of *E. coli* reveals a marked difference in the electrophoretic mobility of a protein which we designate as 30*S*-8 (*3*).

The identification of a protein which is altered in some manner with a genetic locus does not necessarily establish that the locus in question is the structural gene for that protein. It is possible that the phenotypic alteration is a consequence of the action of some enzyme which modifies the structure of the protein but does not determine the primary structure of the protein (for example, by acylation). We now show that the K locus determines the primary structure of the K protein.

Protein 30*S*-8 from B strains (30*S*-8B) has a greater electrophoretic mobility on polyacrylamide gels than protein 30*S*-8 from K strains (30*S*-8K) (*2*). This suggests that 30*S*-8B is either smaller or more positively charged than 30*S*-8K. When the molecular weights of the two proteins are compared by chromatography on Sephadex or by equilibrium centrifugation, they both appear to be of approximately the same size: between 22,000 and 25,000 daltons. Therefore, 30*S*-8B must have a greater net positive charge than 30*S*-8K, and we would anticipate that this would be reflected in the amino acid compositions of the two proteins.

The data in Table 1 confirm this expectation. There are significant differences in the mole percentage of eleven amino acids for 30*S*-8B and 30*S*-8K. The numbers of amino acids per molecule of protein 30*S*-8 can be calculated from the data on the mole percentage composition and from the molecular weight of the protein. By physical measurements, the molecular weight of 30*S*-8B is 22,000 daltons (*4*), and the methionine content of this protein is almost precisely 4 moles per 22,000 daltons; therefore, all of the data on amino acid composition have been presented at moles per 22,000 daltons. Significantly, there are three additional arginines and one more lysine in 30*S*-8B, which accounts for the greater electrophoretic mobility of 30*S*-8B relative to 30*S*-8K.

Table 1. Comparison of amino acid compositions of ribosomal proteins 30*S*-8B and 30*S*-8K. The compositions were measured as described (*4*) and are expressed as moles per 22,000 daltons.

Amino acid	Composition (moles/22,000)		Difference
	30*S*-8K	30*S*-8B	
Lysine	15.8	17.2	+ 1.4
Histidine	3.2	2.8	
Arginine	19.8	23.0	+ 3.2
Aspartic	16.2	15.4	− 0.8
Threonine	7.0	7.2	
Serine	16.0	13.0	− 3.0
Glutamic	22.4	23.8	+ 1.4
Proline	7.6	8.2	+ 0.6
Glycine	15.2	12.6	− 2.6
Alanine	24.2	23.0	− 1.2
Valine	17.0	19.2	+ 2.2
Methionine	4.0	4.0	
Isoleucine	5.4	6.0	+ 0.6
Leucine	17.0	15.2	− 1.8
Tyrosine	3.8	3.8	
Phenylalanine	5.4	5.2	