Masking of the Aggregation Pheromone in Dendroctonus pseudotsugae Hopk.

Abstract. *The pheromone mask released by female* Dendroctonus pseudotsugae *Hopk. after the male stridulates stops aggregation of beetles in flight, but not arrestment of males, in an interaction of chemically and sonically induced behavior. This masking is viewed as a mechanism for survival through the regulation of attack and as a critical part of premating behavior.*

Dendroctonus pseudotsugae Hopk. (Coleoptera: Scolytidae) responds to Douglas-fir resin and several terpenes (*1*) and to the pheromone produced in the hindgut of a female boring into a suitable host (*2, 3*). This species shows constantly changing response behavior. After invasion of the host (occurring at a sex ratio of two females to one male, which lasts for several hours), mass attraction to the invaded host occurs in the ratio of two males to one female; then this attraction ceases very abruptly, although sparsely infested parts of the same trees may be colonized for some time (*2, 4, 5*). The cessation of attractiveness is too abrupt to result from a stoppage in the production of pheromone after mating, which can occur only after the male is inside the bark; instead, it was found to be the result of a masking of the pheromone (*6*). Field tests to clarify this mechanism are reported here. Such an interaction between chemically induced and sonically induced behavior was not observed earlier among bark beetles.

Fresh virgin beetles were used a day after their emergence from the bark of trees infested the previous year. After the sexes were separated, 30 females were introduced individually into a section of a Douglas-fir log, in holes made in the bark by a nail set (*2*). To prevent mating, these holes were covered with wire screen so that males could not enter them and yet the boring dust would be expelled by the female. In order to start behavioral testing at a certain time, it was necessary for all males to remain at the entry holes; therefore, the lower part of the screen was bent at right angles to the bark and formed a sort of platform where the males were placed. The males used were taken from an ice chest (about 5°C) and placed on the platform while they were still stupefied by the cold (prewarmed beetles were found to be excited and did not settle consistently). After preparation, the log section was placed in a screened cage in the forest. Testing for attraction began the first day after the females were introduced into the log sections, by which time they had bored about 1 cm from the entry hole and were producing considerable pheromone. Attracted beetles alighting on the screen were collected as they came.

In the tests with dead beetles, the males were killed by pressure between the thumb and finger just before being placed on the screen platform. The females were killed inside the gallery by a strong hit on the bark with a hammer just above the entry hole (with log sections from 25- to 30-year-old trees, the bark was only 0.5 cm thick).

In order to demonstrate the role played by stridulation, the elytral declivity of the male beetles was cut without damaging the muscles of the wing covers. The beetle was thrown slightly into the air over the table so that it dropped onto the table with its wings spread. By holding it between the fingers, one could excise the elytral declivity without forcibly spreading the wing.

In the terpene tests, a petri dish containing 1 percent solution of each terpene in 95 percent ethanol (*1*) was placed on the cage containing the log with masked pheromone. The results in Table 1 show that masking occurred when males were 1 to 2 cm from the holes containing pheromone-producing females and started to stridulate (Table 1, B). The attraction resumed as soon as the stridulating males were removed from the screen over the females (Table 1, C). The masking could easily be turned on and off at about 10-minute intervals simply by removing and returning the stridulating males to the attractive female-infested logs. Live females, residual frass, and even killed females previously producing pheromone in the bark attracted the beetles in flight (Table 1, A, D, and F) in the usual ratio of two males to one female. When males with excised elytral declivity were introduced, they could not stridulate and did not affect attraction (Table 1, H), but when they were allowed to enter the gallery, join the female, and mate, the mask was produced and subsequently no attraction

Table 1. Responses of flying Douglas-fir beetles to sections of logs with 30 virgin females and various treatments of 1-hour duration in McDonald Forest, Oregon, 7 May 1968, at 20° to 22°C.

Test	Treatment	Response	
		Type of behavior	No. and sex of beetles
A	No treatment (30 virgin females in log section)	Continuous pheromone attraction in the ratio 2 ♂ : 1 ♀	231 (158 ♂, 73 ♀)
B	30 live males added; they stridulated	Attraction stopped	0
C	Stridulating males removed	Attraction resumed	211 (146 ♂, 65 ♀)
D	Females killed by hammer	Residual attraction (from frass and the killed females) occurred	239 (171 ♂, 68 ♀)
E	30 males added to the killed females; they stridulated	Residual attraction continued despite stridulation	224 (151 ♂, 73 ♀)
F	Females removed from log and males introduced	Residual attraction continued despite stridulation	196 (136 ♂, 60 ♀)
G	30 killed males added	Attraction continued	243 (169 ♂, 74 ♀)
H	30 males with excised elytral declivity introduced	No stridulation occurred and attraction continued	235 (168 ♂, 67 ♀)
I	30 males as above, but screen removed to allow entry to females	No stridulation but attraction stopped	0
J_1	Oleoresin added after masking had stopped pheromone attraction	Attraction in the ratio 1 ♂ : 2 ♀	47 (15 ♂, 32 ♀)
J_2	α-Pinene, camphene, and limonene added after masking occurred	Attraction in the ratio 1 ♂ : 2 ♀	154 (42 ♂, 112 ♀) (results combined)

occurred (Table 1, I). Masking, moreover, did not affect the known primary attraction to host substances (Table 1, J_1 and J_2).

It is clear that masking is triggered by stridulation of the male in proximity to the attractive female. Presumably the sound of the stridulation is received by a phonoreceptor of the female, and so the release of the masking substance is controlled by the central nervous system. This phenomenon indicates the interplay of possible messengers, as both auditory and olfactory stimuli are present.

Although the mask stopped the attraction of flying beetles, the arrested males whose stridulation triggered the mask were observed to remain at the screened entry holes for at least 4 days after the mask occurred. It is possible that the continuously fresh frass, produced as the female constructs the gallery, is sufficiently attractive to hold the arrested males there. However, it seems more likely that male arrestment with stridulation, or "calling," represents a critical stage in the secondary process of attraction of the Douglas-fir beetle. In other words, attraction of the beetles in flight can be stopped by masking, but not the premating behavior of the arrested males whose stridulation triggered the masking. Release of the masking substance may be considered part of the female mating behavior also; it occurs not only when stridulating males approach but also during the prolonged nudging movements that characteristically precede mating in this species and, of course, after mating. Perhaps this phenomenon, when investigated further, will shed some light on the complex interrelation of population aggregation and sex behavior in those bark beetles whose survival depends not only on finding a mate but also on mass attack of the host tree (*7*).

After the female Douglas-fir beetles reemerge from the bark and reattack new hosts (summer attack), they again produce the aggregating pheromone (*2*). Whether the mask also occurs during this second flight and aggregation was not investigated.

The survival value of the mask is clear. It tends to distribute evenly the available males, while both preventing overcrowding with resultant brood mortality and allowing the mass attack necessary to overcome host resistance (*6*). This phenomenon gives a new dimension to the already intricate mechanism of host and insect forms of attraction in this destructive bark beetle. It complicates our present effort to establish a control system by manipulating the aggregant pheromone, but it may ultimately offer a new possibility of control if saturation quantities of the masking substance can be used to prevent aggregation of the flying population to especially valuable or vulnerable stands of Douglas fir.

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References and Notes

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Membranous Structures Associated with Translation and Transcription of Poliovirus RNA

Abstract. *Poliovirus RNA and proteins are synthesized in association with distinct membranous structures that were separated by means of isopycnic centrifugation of cytoplasmic extracts in discontinuous sucrose-density gradients. Viral RNA is replicated in a structure that contains rapidly labeled replicative intermediate RNA and viral RNA polymerase associated with the smooth membrane fraction. In sucrose gradients this viral RNA replication complex is distributed at densities in the range of 1.12 to 1.18 grams per cubic centimeter. Viral proteins are synthesized on polyribosomes bound to membranes and sediment with polyribosomes at densities of less than 1.25 grams per cubic centimeter.*

Host cell membranes are involved in the replication of picornaviruses, a group of small animal viruses free of lipids, which contain RNA as their genetic material. As shown by cell fractionation, the synthesis of both viral protein and viral RNA is associated with membranous structures in the cytoplasm of infected cells (*1–3*). Furthermore, proliferation of cellular membranes after virus infection has been detected by electron microscopy (*4, 5*) and by incorporation of radioactive choline (*6*).

Cytoplasmic membranes can be separated by a method of isopycnic centrifugation described by Bosman *et al.* (*7*). Using a modification of this technique, we have isolated the membranous structures associated with translation and transcription of poliovirus RNA. Our results indicate that each process is associated with a distinct membranous structure.

The S3 HeLa cells in suspension were infected with poliovirus, treated with actinomycin D, and incubated at 37°C as described (*8*). After harvest and washing, the cells were swollen in hypotonic buffer, RSB [0.01*M* tris(hydroxymethyl)aminomethane, *p*H 7.4, 0.01*M* KCl, and 0.0015*M* $MgCl_2$], for 20 minutes and then broken open with 15 strokes in a tight-fitting Dounce homogenizer. Nuclei and cell debris were removed by centrifugation at 900*g* for 10 minutes, and the supernatant cytoplasmic extract was made 30 percent (by weight) in sucrose by addition of 60 percent sucrose in RSB (by weight). A discontinuous gradient was formed by layering sucrose-RSB solutions in the following order: 3 ml of 60 percent sucrose, 7 ml of 45 percent sucrose, 7 ml of 40 percent sucrose, 10

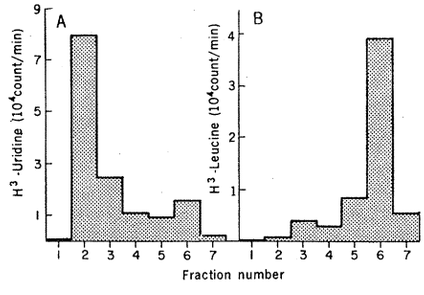

Fig. 1. Distribution of pulse-labeled viral RNA and proteins. (A) Viral RNA. S3 HeLa cells (4.1×10^8) were infected with poliovirus type 2 and treated with actinomycin D (5 μg/ml) as described (*8*). At 3.25 hours after infection the culture received a 2.5-minute pulse of uridine-5-^{3}H (20 μc/ml). Cytoplasmic extract was prepared and analyzed by means of isopycnic centrifugation through a discontinuous sucrose gradient. A portion of 0.2 ml from the pelleted material of each fraction was analyzed for trichloroacetic acid–precipitable radioactivity (*8*). (B) Viral protein. The same procedure as described in (A) was used except the culture received a 3-minute pulse of leucine-^{3}H (20 μc/ml).