

no significant differences in the amounts of residual proteolytic activity on their surfaces as indicated by the Azocoll assay. It appears that the aggregation lag of enzyme-dissociated cells (i.e. return of adhesiveness) is not due to a necessary inactivation of adsorbed enzyme; EDTA-treated cells aggregate immediately even though they exhibit the same negligible surface proteolytic activity as the trypsin-treated cells. This interpretation conforms with earlier reports¹ and with our own unpublished observations that the addition of soybean trypsin inhibitor to trypsin-dissociated cells does not abolish the aggregation lag. Enzymatic disaggregation procedures are known to produce cellular injury^{6,7} and it seems likely, therefore, that the aggregation lag represents a 'recovery' period during which

cells disrupted by enzymatic action regenerate or repair certain surface components.

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Olfactory-induced muscle potentials in *Dendroctonus frontalis*: Effects of trans-verbenol and verbenone¹

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Summary. Potentials from antennal muscle movement induced by stimulation with the pheromone frontalin were recorded simultaneously with the electroantennogram (EAG) from *Dendroctonus frontalis*. The beetle-produced pheromones verbenone and trans-verbenol were found to decrease the muscle potential activity elicited by frontalin.

In recent years numerous insect pheromones have been referred to as attractants because they elicit positive orientation behavior from insects in laboratory bioassays and field tests⁴⁻⁶. More recently, additional chemicals have been identified which, although unattractive when presented alone, modify the response of insects to attractants. These chemicals have been classified primarily into 2 groups: those which increase and those which decrease the response of insects to the attractants. However, until recently neither the behavioral⁷ nor neurophysiological⁸ roles of any of these compounds were known.

Frontalin (1,5-dimethyl-6,8-dioxabicyclo [3.2.1] octane) was identified as an attractant for *D. frontalis* and found to be moderately attractive in field tests⁵. However, the host tree terpene α -pinene and the pheromone trans-verbenol⁹ were found to increase trap catches substantially when combined with frontalin^{10,11}. When the pheromone verbenone⁹ was added to attractant mixtures the response of beetles was significantly reduced¹⁰.

Payne¹² recorded muscle potentials from electroantennogram (EAG)¹³ preparations of *D. frontalis* stimulated by the bicyclic ketals, frontalin and exo-brevicomin (exo-7-ethyl-5-methyl-6,8-dioxabicyclo [3.2.1] octane⁴) and the host tree terpenes α -pinene and 3-carene. Muscle potential activity was considerably greater in response to pheromone stimulation than to stimulation by the terpenes. These muscle potentials were thought to originate from the antennal muscles which are presumably involved in antennal raising and orientation.

The purpose of this investigation was to determine the effects of verbenone and trans-verbenol on these muscle potentials.

Materials and methods. Adult beetles newly-emerged from brood material collected in East Texas were used. EAG's were recorded using Ag-AgCl capillary electrodes. The recording electrode was implanted in the antennal club. The indifferent electrode was placed either in the antennal scape or head capsule¹⁴. Muscle potentials were recorded in a similar manner with the preamplifier in the AC recording mode. In some cases a high pass filter was also used. Recordings were also made from single sensilla basiconica within the sensory bands^{15,16}, by implanting tungsten elec-

trodes in the sensillum base. Muscle potentials were induced by stimulating the antenna with frontalin or frontalin + α -pinene, and the effects of verbenone and trans-verbenol on these potentials were studied. 9 male beetles and 7 female beetles were used.

Stimulus compounds were delivered as 5- μ l samples placed on filter paper and inserted into glass cartridges. The muscle potential stimulus (e.g., frontalin) was placed in 1 cartridge; trans-verbenol or verbenone in the other. Presentation of stimuli was facilitated by 2,3-way solenoid operated air valves¹⁴.

Results. Figure 1 shows the relationship between the EAG and the muscle potentials during frontalin and α -pinene stimulation. Stimulation with verbenone and trans-verbenol immediately (i.e., with msec) following muscle potential induction resulted in reduction of the potentials in both male and female beetles (figure 2).

The delay between the beginning of sensory activity and the onset of muscle potential activity was determined by recording the EAG and the muscle potentials simultaneously but on separate channels (figure 3). The response on 1 channel was recorded in the DC mode with a low-pass

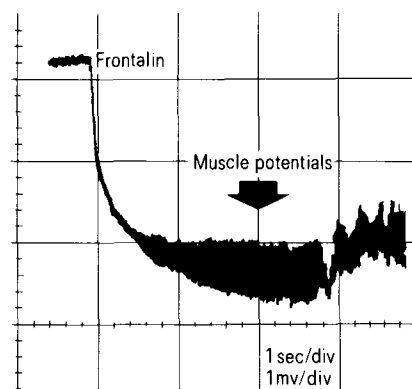


Fig. 1. EAG recorded from *D. frontalis* male in response to frontalin. Muscle potentials are recorded only after some degree of EAG depolarization.

filter; the response on a 2nd channel was recorded in the AC mode with a high-pass filter. The initial olfactory response (i.e., initial EAG depolarization) preceded muscle potential recordings by ca. 75 msec for both frontalin and verbenone stimulation. This delay was thought to be a product of afferent conduction, neural integration and

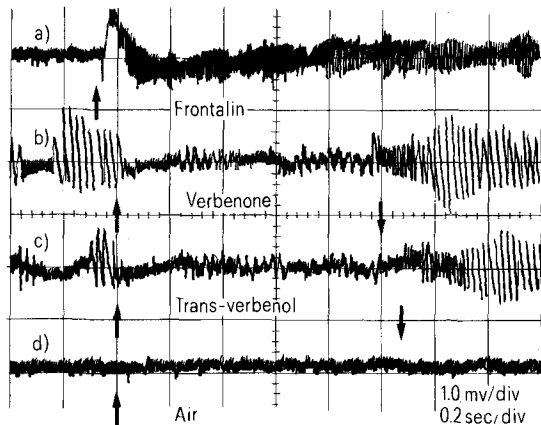


Fig. 2. Frontalin-elicited muscle potentials, *a* recorded with amplifier in AC mode. Verbenone and trans-verbenol reduce frontalin-induced potentials in *b* and *c*. Lower trace *d* is stimulation by air. Arrows indicate stimulus duration. Frontalin stimulus is off during stimulation by other compounds.

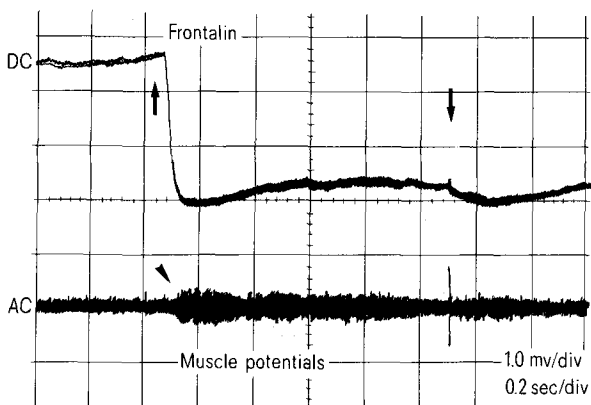


Fig. 3. Upper trace is frontalin EAG recorded in DC mode with a low-pass filter. Lower trace was recorded simultaneously in AC mode with high-pass filter. A delay of ca. 75 msec is observed from the onset of the olfactory response to the recording of muscle potential activity.

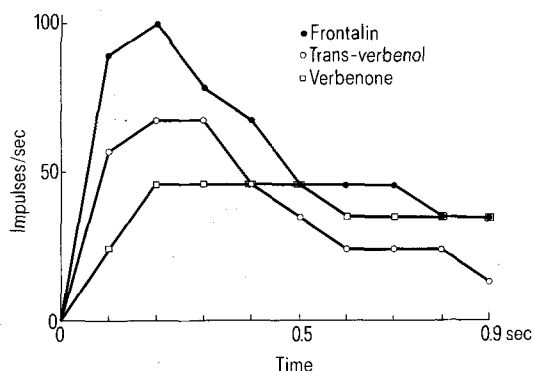


Fig. 4. Response of a single sensillum basiconicum cell to frontalin, transverbenol and verbenone. Ordinate is impulses/sec over each 0.1 sec. Abscissa is time. Stimulus duration 1 sec.

effluent transmission of olfactory-perceived information to the muscles.

Using the EAG and a technique involving the differential adaptation of antennal acceptors¹⁷, both verbenone and trans-verbenol were found to interact with some, but not all of the acceptors for frontalin¹⁴. Data from single unit recordings further verified interpretation of the EAGs. In addition, recordings from single olfactory cells associated with sensilla basiconica stimulated by frontalin, verbenone or trans-verbenol elicited phasic-tonic responses (figure 4). Although, frontalin, verbenone and trans-verbenol could interact with acceptors on the same cell, the number of impulses elicited by the latter 2 compounds was considerably fewer than by frontalin. The effects of verbenone and trans-verbenol stimulation on frontalin-induced muscle potentials are possibly indicative of the relative interaction of the former 2 compounds with the frontalin acceptors (figure 3; see also Dickens and Payne¹⁴).

Payne¹² noted that muscle potentials elicited by the host terpenes, α -pinene and 3-carene, were smaller than those elicited by the bicyclic ketals, frontalin and exo-brevicomin. The differences in the size of the muscle potentials Payne¹² observed were probably also due to differences in the interaction of the compounds with the antennal acceptors.

The decreased muscle potential activity elicited by verbenone, trans-verbenol and the host terpenes might indicate similar interactions of these compounds with the frontalin acceptors. Since the muscle potentials were considered to be related to antennal raising and orientation movements¹², these compounds might be considered arrestants of muscle potential activity. In this regard, the increased attractiveness (trap catch) of frontalin with the addition of a host terpene⁵ and/or trans-verbenol¹⁰ might be due to arrestment of the beetles on baited traps. The decreased response of beetles to attractant mixtures plus verbenone might be due to a general decreased flight activity in the vicinity of the traps.

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