

but in only one other insect, viz. *Galleria mellonella*, have several supernumerary larval instars been observed<sup>2</sup>. In the rest, e.g. *Pieris brassicae*<sup>3</sup>, *Pectinophora gossypiella*<sup>4</sup>, *Choristoneura fumiferana*<sup>5</sup>, *Manduca sexta*<sup>6</sup>, *Rhodnius prolixus*<sup>7</sup>, *Pyrrhocoris apterus*<sup>8</sup>, *Dysdercus cingulatus*<sup>9</sup>, *Bovicola limbat*<sup>10</sup>, etc., only 1 and rarely 2 abortive supernumerary moults take place. Thus *Galleria* belongs to the 1st category and the rest to the 2nd category.

It should be noted that the larvae of the 1st category occur in stored products or honey comb, habitats characterized by relatively constant ecological conditions, with food available more or less continuously, in which the prolongation of the larval life would not threaten survival. On the other hand, larvae of the 2nd category occur in habitats in which these conditions are subject to drastic changes and the whole cycle is adapted to such a changing environment so that undue prolongation of the larval life would normally mean death due to shortage of food or changes in the various environmental conditions. It would, therefore, appear that these 2 categories of insects are endocrinologically adapted to their respective environmental conditions. However, since life in the stored products or the honey comb has been secondarily evolved, it can be assumed that the condition observed in the 1st category represents a state of deviation from the normal. The chief endocrinological adaptations undergone by these insects are manifested by the ability of the larvae to undergo repeated moults by a single treatment with a juvenoid and the ability of the supernumerary larval instars to retain a fairly normal biology, including feeding, growth and moulting.

It is believed that in most insects, most of the juvenile hormone is metabolized<sup>11</sup> or excreted<sup>2</sup> after every moult so that the nature of the next moult is determined by the amount of the juvenile hormone secreted by the renewed activity of the corpora allata, and not by the JH carried over through a moult. Indeed the half-life of JH has been found to be only about 30 min<sup>11</sup>. In larvae of the 1st category,

it is obvious that a physiological mechanism has been evolved which ensures that the excess of the juvenoid supplied exogenously is not eliminated after the moult, at least not at the same rate as in other insects, and most of it is carried over to the next instar in an active state to help bring about another moult. At the same time, this excess does not interfere with the biology of the supernumerary instar. There is enough evidence to show that excess of JH interferes with the moulting process, which is one of the apparent causes of the non-viability of the supernumerary instars; but these larvae do not suffer from ecdysial failure despite having excess of a juvenoid.

It is clear that the hormonal mechanism controlling the larval-pupal moult in insects is a very complex phenomenon which has undergone adaptive changes and, therefore, is not identical in all cases; it deserves greater attention.

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## Field bioassay of male Douglas-fir beetle compound 3-methylcyclohex-3-en-1-one<sup>1</sup>

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**Summary.** Synthetic 3-methylcyclohex-3-en-1-one, or 3,3-MCH, decreased by 77% the flight aggregation of *Dendroctonus pseudotsugae* (Scolytidae) to sticky traps containing attractant (frontalin,  $\alpha$ -pinene, and resin). However, windthrown trees were protected by 3,3-MCH much less than is known to occur with the isomer 3,2-MCH.

The compound 3-methylcyclohex-2-en-1-one (3,2-MCH) released by *Dendroctonus pseudotsugae* Hopkins when male and female pair in the gallery<sup>2</sup> strongly inhibits aggregation of beetles to sticky traps<sup>3</sup> and felled trees<sup>4</sup>. We here report field bioassay of an isomer, 3-methylcyclohex-3-en-1-one (3,3-MCH), which was identified from volatile materials collected from live males, and is not known from females<sup>5</sup>.

**Materials and methods.** The effect of 3,3-MCH on aggregation of *D.pseudotsugae* was tested in MacDonald Forest near Corvallis, Oregon, from April 9 to May 16, 1978, on 3 windthrown Douglas-fir trees, each about 1 m diameter at breast height. The trees were 40–100 m apart, and partially shaded. On each log, 3,3-MCH was evaporated from 1/2-dram glass vials placed inside aluminum 35 mm film cans with perforated bottoms<sup>4</sup>. The cans were fastened at 3-m intervals for 18 m along both sides of the log. A similar 18-m section of each log was left as control, separated by 3 m from the treatment section. The treated section was towards

the butt on 2 trees and towards the crown of the other. The attacks, indicated by frass, were counted on 0.25-m<sup>2</sup> areas on both sides of the log, and the 2 counts combined as 1 0.5-m<sup>2</sup> sample every 0.5 m. 8 samples were counted for each treatment on each tree (n=24) and means were statistically compared by 2-way analysis of variance, taking into consideration variation among trees.

A possible inhibitory effect of 3,3-MCH on synthetic attractant composed of frontalin,  $\alpha$ -pinene, and resin was tested at 500 m elevation on Marys Peak, Siuslaw National Forest, near Corvallis. Each compound was evaporated from 1/2-dram glass vials in film cans placed inside sticky traps<sup>6</sup>. Traps with and without 3,3-MCH were placed as pairs spaced 30 m apart for each test. 3,3-MCH evaporated from the delivery system at 2 mg/day at 22°C. Temperature maxima on test days were 20–26°C. Beetles were collected from 2 or 3 replications of attractant traps with and without 3,3-MCH after all-day tests on April 10, May 7, 8, 18, and

20. Wilcoxon's nonparametric matched-pairs, signed-ranks test was used for statistical comparison of the 12 all-day sample pairs resulting; this test avoided the difficulty of variation due to differences in flight intensity on different days.

3,3-MCH was synthesized by ChemSampCo, Columbus, Ohio. GC/MS indicated 95% purity and 0.6% of the active 3,2-MCH isomer present. The 3,3-MCH was held at  $-15^{\circ}\text{C}$  until needed, and replaced in the field weekly to prevent conversion into enough of the more stable 3,2-MCH to interfere with the tests. After 1 year, the purity of 3,3-MCH held at  $-15^{\circ}\text{C}$  was unchanged according to GC/MS. Frontalin 98.5% was also from ChemSampCo, and  $\alpha$ -pinene 95% from K & K Laboratories, Plainview, N.Y.

**Results and discussion.** Response of flying *D. pseudotsugae* to attractant in sticky traps was about 77% less than response to the control traps, a highly significant difference. However, the sections of windthrown trees with 3,3-MCH placed along them did not have significantly fewer attacks than the untreated portions of the trees (table). These results are in marked contrast to previous tests of 3,2-MCH using the same delivery system. Addition of 3,2-MCH to sticky traps baited with frontalin and  $\alpha$ -pinene near Hood River, Oregon, decreased the beetle catch by 96%<sup>3</sup>. Also, 3,2-MCH reduced by 96%<sup>4</sup> the number of attacks on felled trees in 3 locations in Oregon and Idaho.

Response of flying *Dendroctonus pseudotsugae* to field test of 3,3-MCH during spring flight: A, attacks per 0.5 m<sup>2</sup> bark sample on windthrown trees; B, beetles collected on sticky traps to attractant with 3,3-MCH and without (control)

Test	Control		With 3,3-MCH		n	Significance
	$\bar{x} \pm 95\% \text{ CI}$	$\delta/\bar{x}$	$\bar{x} \pm 95\% \text{ CI}$	$\delta/\bar{x}$		
A Trees	$6.9 \pm 1.65$	—	$4.1 \pm 1.05$	—	24	NS $p < 0.05$
B Traps	$9.1 \pm 3.03$	4.4	$2.1 \pm 2.08$	3.8	12	** $p < 0.01$

Preparation and testing of 3,3-MCH is difficult because it begins to convert into its more stable 3,2-isomer at temperatures favorable for beetle flight. Our field bioassay of 3,3-MCH was begun in 1976, but the results suggesting that it had an inhibitive effect on flight aggregation were invalidated when a later GC/MS of our stock compound revealed a 5–8% conversion to the active 3,2-MCH isomer<sup>7</sup>. Since our field tests have shown 3,3-MCH to have only a slight effect on flight aggregation, at about the same level as several MCH analogues tested<sup>8</sup>, its biological function remains obscure. Since it is less stable than its isomer, it is possible that 3,3-MCH is a precursor to 3,2-MCH, or it may have more subtle primer or releaser effects during male premating behavior in the gallery, as does 3,2-MCH<sup>7</sup>.

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## Microorganisms seen by scanning and transmission electron microscopy in Legionnaires' disease from human lung<sup>1</sup>

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**Summary.** In addition to several anomalous structures, other general forms of definitely rod-shaped microorganisms have been found by scanning and transmission electron microscopy in the lung tissue taken at autopsy from a patient who succumbed to confirmed Legionnaires' disease with extensive necrotizing lobar pneumonia. The microorganisms were greatly varied in size and shape. They were micrographed in the act of fission. These forms have been found to some extent throughout the tissue. No nickel was demonstrated, either in the lung tissue or in the microorganisms.

An epidemic of acute febrile respiratory disease broke out among persons attending the American Legion convention in Philadelphia in 1976, and earned for it the name 'Legionnaires' disease'. Although an organism has since been isolated and cultured from the lungs of patients with the disease<sup>2,3</sup>, only 2 studies of it directly in tissue sections have been reported by transmission electron microscopy (TEM)<sup>4,5</sup> and none by scanning electron microscopy (SEM). In the present report both SEM and TEM are being used to study the causative organism more thoroughly, and preliminary results are described.

The lung tissue was taken at autopsy from a patient who died in June 1977 with extensive necrotizing lobar pneumo-

nia. The diagnosis of Legionnaires' disease was made by specific fluorescent antibody and silver impregnation staining<sup>3</sup> and was confirmed by the Communicable Disease Center in Atlanta, Georgia, USA.

**Materials and methods.** The specimens of formalin-fixed autopsy material were washed in veronal acetate buffer. A part was separated for TEM and the remainder processed for SEM. For SEM the pieces were critical-point dried in CO<sub>2</sub> and sputter-coated with gold-palladium after dehydration in graded ethanols, including 3 changes of absolute alcohol. Micrographs were taken at 20 kV in an ETEC Autoscan. For TEM other pieces were post-fixed in phosphate buffered or veronal acetate buffered osmic acid,