

pyrene concentrations, by use of equation 1, assuming, for the various parameters, values similar to those found by Vanderkooi and Callis¹⁴ (figure, solid line).

If the pyrene concentration is raised above 1 μM , the experimental points exhibit a negative deviation which cannot be accounted for by simple diffusion theory and has been attributed¹⁴ to an alteration in membrane structure caused by insertion of several pyrene molecules.

In the presence of insulin, it can be easily seen that the experimental data can be simulated by decreasing the value of the translational coefficient D from 2.8×10^{-8} to $1.4 \times 10^{-8} \text{ cm}^2 \cdot \text{sec}^{-1}$ (figure, dashed line) and/or by decreasing the value of the partition coefficient β (figure, dotted line). The latter of these 2 possibilities, i.e. that insulin decreases the solubility of the dye in the membranes, seems rather unlikely, since it would involve a gross and generalized effect of the hormone on the structure of the membrane lipid bilayer. The former possibility, instead, gives further support to the hypothesis⁶⁻⁸ that insulin causes a decrease of membrane fluidity. The effect on lateral diffusion would even appear to be more marked than that on the overall lipid microviscosity observed by Luly and Shinitzky⁸, possibly because the variations in the rate of pyrene excimer formation give specific information on the mobility in the plane of the membrane.

Due to the physiologically low hormone concentrations used, the effect observed upon addition of insulin appears

not to be attributable to a nonspecific cross-linking of membrane receptors by insulin dimers; nor does concanavalin A duplicate the insulin effect.

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Endocrinological adaptations in insects

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Summary. Insect larvae respond differently to juvenoid treatment. Larvae occurring in relatively constant ecological conditions with abundant food, e.g. *Trogoderma granarium*, *Tribolium castaneum*, *Corcyra cephalonica* and *Ephesia cautella*, can undergo several extra larval moults, show growth with each moult, may moult 2–3 times with 1 treatment and do not suffer ecdysial failure. Larvae adapted to changing ecological conditions, e.g. *Spodoptera litura*, *Earias fabia*, *Papilio demoleus*, *Euxoa spinifera* and *Attacus ricini*, rarely undergo supernumerary moults, and the supernumerary instars suffer from ecdysial failure. It is suggested that the former have evolved an endocrinological adaptation.

We have studied the effect of feeding and topical application of some juvenoids on the larvae of two Coleopteran species viz. *Trogoderma granarium* and *Tribolium castaneum* and 7 Lepidopteran species viz. *Corcyra cephalonica*, *Ephesia cautella*, *Spodoptera* (Prodenia) *litura*, *Earias fabia*, *Papilio demoleus*, *Euxoa spinifera* and *Attacus ricini*, and we find that they can be divided into 2 categories on the basis of their response to the juvenoid treatment.

Larvae of 1 category, including *T. granarium*, *T. castaneum*, *C. cephalonica* and *E. cautella*, easily undergo repeated supernumerary larval moults when the last normal larval instar and successive supernumerary instars are topically treated with adequate quantities of the juvenoids, or when they are reared in food media containing adequate quantities of the juvenoids in the food throughout. Treated either way with appropriate quantities of the juvenoids, 100% larvae undergo supernumerary moults. *T. granarium* may thus undergo upto about 23 (in female) and 19 (in male) supernumerary larval moults, *T. castaneum* 13 supernumerary moults and *C. cephalonica* and *E. cautella* 22 supernumerary moults. Further, upto a certain number of supernumerary moults (about 15 in female and 12 in male in *T. granarium*, 8 in *T. castaneum*, 16 in *C. cephalonica* and in *E. cautella*), the larvae continue to grow in size, and, when removed from the hormone-mixed media or when topical

treatment with the juvenoid is not given any more, they undergo a few supernumerary larval moults and then pupate and complete the metamorphosis to produce 'giant' pupae and adults. If treated longer with the hormone, though the super larvae may still continue to moult, they tend to become reduced in size and are unable to pupate and finally die. Moreover, in these forms, it is interesting to note that a single treatment with a juvenoid given to the normal last larval instar or a supernumerary larval instar may cause 2 or 3 extra moults to take place. Supernumerary larval instars in all these cases seldom show signs of ecdysial failure.

Larvae of the other category, including those of *S. litura*, *E. fabia*, *P. demoleus*, *E. spinifera* and *A. ricini*, undergo supernumerary larval moults with difficulty. With heavy and properly timed doses of the hormone given topically or via the gut, a small percentage of the larvae only may produce 1 (*S. litura*, *E. fabia*, *E. spinifera*, *A. ricini*) or rarely 2 (*P. demoleus*) supernumerary larval instars. The supernumerary larval instars are, as a rule, non-viable, do not pupate and usually suffer from ecdysial failure. If, rarely, a supernumerary larval instar does metamorphose, an abnormal adultoid is produced.

Supernumerary larval instars have been reported to be produced by juvenoid treatment in several other insects,

but in only one other insect, viz. *Galleria mellonella*, have several supernumerary larval instars been observed². In the rest, e.g. *Pieris brassicae*³, *Pectinophora gossypiella*⁴, *Choristoneura fumiferana*⁵, *Manduca sexta*⁶, *Rhodnius prolixus*⁷, *Pyrrhocoris apterus*⁸, *Dysdercus cingulatus*⁹, *Bovicola limbat*¹⁰, 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 metabolized¹¹ or excreted² 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 min¹¹. 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-one¹

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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, α -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 gallery² strongly inhibits aggregation of beetles to sticky traps³ and felled trees⁴. 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 females⁵.

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 bottoms⁴. 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-m² areas on both sides of the log, and the 2 counts combined as 1 0.5-m² 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, α -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 traps⁶. 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