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Investigations with dichlorofarnesenic acid ethyl ester on the cotton stainer *Dysdercus cingulatus* Fabr.

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Dichlorofarnesenic acid ethyl ester, kindly supplied to us by Hoffmann-La Roche in Basle for experimental purposes, is a substance which shows a pronounced juvenile hormone activity. The substance is highly suitable for model experiments in that its breakdown in the insect body is made considerably more difficult by the two chlorine atoms so that it can be followed over a prolonged period in bioassay. At the dosage levels we used, the substance does not have a direct toxic effect on the insect so that the physiological activity can be observed practically without interruption by side-effects. We were particularly interested in the following possible effects :

1. Interference with metamorphosis.
2. Sterilization.
3. Stimulation of ovarian growth.
4. The possibility of transfer to untreated animals.

These effects have already been partly described in the papers of Scheurer and Luescher 1966, Luescher 1968, Norris and Pener 1965, Wigglesworth 1955 and earlier, with transplantation and ligature experiments, while the authors Dahm et al. 1967 and 1968, Masner et al. 1968, Röller et al. 1967, Romanuk, Slama and Sorm, 1967, Suchy et al. 1968, Wigglesworth 1968 and 1969 and Williams et al. 1967 and 1968 were mainly concerned with the activities of synthetic juvenile hormone or juvenile hormone derivatives.

In an initial series of experiments we treated *Dysdercus* larvae 5, 4 and 3 days before the imaginal moult. In insects treated 5 days before emergence, i.e. at the beginning of the final larval stage, with 10^{-5} and 10^{-6} g active ingredient per insect, disturbances in metamorphosis were so great that the insects died soon after moulting. The majority of insects treated 4 days before the final moult survived but were incapable of copulation so that they could not be followed up any further.

Treatment with the same dose 3 days before the final moult produced only slight deformations and the insects could be paired with untreated animals. With a dose of 10^{-5} g per animal the sterilizing effect was only obvious during 2–3 egg-laying periods after which it declined rapidly. With lower doses the effect could no longer be demonstrated.

These results indicate that when the substance is applied early, that is, at a time when adult differentiation is not very far advanced, pronounced interference with metamorphosis occurs.

We then investigated how the sterilization effect occurs if animals are treated at different intervals after the imaginal moult and if there is a basic difference in the effect if only females or only males are treated.

Males and females, which had been separated according to sex immediately after the final moult, were treated 6, 16, 32, 40, 60 and 86 hours after moulting ; they were then paired with untreated animals of the same age and the pairs observed individually.

When only the males were treated with 10^{-5} g active ingredient per animal we achieved a 95% effect, and when only females were treated with the same dose, a 99% effect. Sterilization was effective in that the embryos died during development within the egg. The effect was the same whichever partner was treated. We can conclude from this that the substance is transported from the males with the sperms, which themselves are apparently not damaged, since normal fertilization occurs. The time of treatment had no effect on the result.

Further, we observed that the life-span of treated animals was about 25% shorter than in untreated animals and that the total egg count was also reduced, by about 30% if the females were treated directly, and by 16% when the females mated with treated males.

Treated females began to lay on average 24 to 36 hours earlier and laid the individual egg groups more rapidly. The higher basal metabolism produced by this more rapid oviposition probably accounts for the shorter life-span.

In a further series of experiments we investigated whether the effect of dichlorofarnesenic acid ethyl ester also persists when the animals are allowed to copulate again with untreated animals after a single copulation with a treated partner. We were also interested as to whether the substance can be transferred from a female to a male and from this male to another female.

For this purpose we allowed a large number of untreated insect pairs to copulate, up to the first egg-laying, to be certain that the animals were capable of normal reproduction. After the first laying, the males in one series and the females in another series were replaced by treated animals of the same age. These pairs were left together for 36 hours to ensure that copulation took place. Finally the treated partners were removed and the original untreated partners returned.

We were able to show that in females which had copulated with a treated male, an average of 75–80% of eggs laid during the following three egg-laying periods were sterile but that this effect declined rapidly in further laying periods. When untreated males copulated with a treated female, they were only able to sterilize the original partner by about 16%. This means that there is only a slight back transfer of active ingredient from a treated female to an untreated male and from this male to an untreated female. If a treated female is paired with a new untreated male after each copulation the effect, which lasts throughout life when there is no change of partner, declines after only 4–6 egg-laying periods. Vice versa, a treated male which is given a new untreated female after each copulation can only sterilize the first female completely, the second is sterilised by 80% and in further females the effect declines rapidly to zero.

To summarise, we demonstrated conclusively that dichlorofarnesenic acid ethyl ester can produce all the important effects described in the literature when tested at the given dose levels on *Dysdercus cingulatus*.

Whether or not this is sufficient for practical application of this substance as a pesticide of the third generation cannot be determined at this stage in the investigations. In particular we have no evidence as yet from experiments on plants or outdoors.

In closing, we would sincerely like to thank the ETH Zurich, the Hoffmann-La Roche Company and Dr. R. Maag Ltd., Dielsdorf, for their most generous support.

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