Failure to Demonstrate Sterilans Effect of Juvenile Hormone Mimetics[1] in *Pieris brassicae* and *Galleria mellonella*

It has been demonstrated that juvenile hormone mimetics (JHM) have a sterilans effect in Hemiptera, e.g. *Dysdercus*[2] and *Pyrrhocoris*[3]. In both cases the treatment of the females with JHM results in the production of eggs in which the embryos do not fully develop or are not able to hatch from the eggs. The same sterilans effect is achieved if treated males copulate with untreated females, which indicates a transfer of the JHM from the male to the female. If such sterilans effects were generally produced in all insect groups, the JHM would represent very promizing candidates for insecticides with no, or negligible, toxicity in mammals. However, the following experiments with the great cabbage white *Pieris brassicae* and the greater wax moth *Galleria mellonella* show that the sterilans effect mentioned above is not a general characteristic of the JHM.

Egg maturation of *P. brassicae* is strictly imaginal[4] and can be prevented if the corpora allata are removed from freshly enclosed female butterflies[5]. The simplest method to prevent egg maturation is the decapitation of the females[6,7]. However, decapitated virgin females of *P. brassicae* will produce mature eggs if active corpora allata are implanted in their bodies[6] or if an JHM is applied topically[7,8]. In addition, it has been shown that males, treated topically on the thorax with an JHM, transfer the JHM to females during copulation[8].

The following JHMs have been tested: 1. an all-*cis*/*trans*-mixture of isomeres of ROELLER's compound[9]; 2. dichloro-farnesenic acid methyl-ester; 3. farnesenic acid ethyl-ester. All 3 compounds were first tested on their gonadotropic effect in *P. brassicae*; 200 µg applied topically per decapitated virgin female induced the maturation of respectively 139.8 ± 13.2, 137.6 ± 7.8, and 131.3 ± 19.9 eggs within 3 days. The results indicate similar juvenile hormone activities in *Pieris*. The 3 preparations have been selected for different reasons: preparation 1 because it contains the substance which may be the natural juvenile hormone of Lepidoptera[9], compound 2 because this and the closely related ethyl-ester have been used to demonstrate the sterilans effect in Pyrrhocoridae[2,3], and compound 3 as a reference, already well known from earlier work[7,8].

The first sterilans test was made with 4 groups of freshly mated females which, after copulation, were treated topically with 2 µl of either acetone (control) or a 10% acetone solution of one of the test compounds. Each female was then put in a polystyrene box of 1000 ml volume, containing an artificial flower with sugar water (10%) and a leaf of cabbage standing in a small vessel with water to keep it fresh. The cabbage leaves were replaced after 24 h and later on every second day until the butterflies died. The eggs deposited on each leaf being counted, the leaves were put in boxes containing some water and kept there until the larvae hatched (5 days after egg deposition) or longer. The abdomina of the dead females were dissected and the mature eggs in the ovaries counted. This value plus the number of eggs laid gave the number of eggs produced.

Table I shows that all groups survived the treatment by 12 to 16 days. Each group produced a mean number of 549 to 635 eggs, 494 to 604 being laid. The differences between the control group and the JHM treated groups are not significant at the 5% level. However, there were differences in the rate of oviposition as shown in the Figure. The data of Table II show that the JHM treated

Table I. Longevity, egg maturation, and oviposition of 4 groups of 9 mated females of *P. brassicae* treated topically with 2 µl of 10% acetone solution of 1. isomere mixture of ROELLER's compound, 2. dichlorofarnesenic acid methyl-ester, 3. farnesenic acid ethyl-ester

Compound	Longevity in days	Mean No. of eggs matured	Mean No. of eggs laid
1.	12.1	635.2 ± 62.9	570.2 ± 80.7
2.	12.4	633.0 ± 57.5	610.9 ± 67.7
3.	13.4	549.1 ± 49.7	494.3 ± 58.5
–	15.7	630.0 ± 74.3	604.1 ± 77.2

Control group = 2 µl of acetone.

[1] The author thanks Hoffmann-La Roche Ltd., Basle, for samples of the juvenile hormone mimetics.

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Table II. Mean numbers of eggs laid during indicated time intervals by those individuals of the groups indicated in Table I which survived treatment for at least 9 days

Time after treatment (h)	Control	1.	2.	3.
0– 24	48.0 ± 9.2	22.2 ± 5.4^{a}	26.3 ± 8.2	29.0 ± 8.2
24– 72	47.1 ± 16.8	113.3 ± 12.2^{b}	144.2 ± 19.2^{b}	102.3 ± 26.6
72–120	99.0 ± 19.8	207.9 ± 28.8^{b}	166.2 ± 19.2^{a}	119.8 ± 37.6
120–168	116.8 ± 15.0	118.1 ± 23.6	115.7 ± 16.3	102.9 ± 26.9
168–216	131.8 ± 14.2	78.1 ± 13.6^{a}	102.1 ± 23.1	96.5 ± 19.6

[a] and [b] Significantly different from control group at 5 and 1% level respectively.

groups reach the maximum rate of oviposition about 4 days earlier than the controls.

The eggs of all groups were normal at whatever time after treatment they had been collected. The hatching larvae fed normally, developed to normal second instar larvae and, if kept longer, to normal adults.

The results indicate that the treatment of freshly mated young females of *P. brassicae* with an JHM leads to accelerated egg maturation and oviposition, but does not cause harmful effects in the progeny.

A second experiment was made to test whether or not in the first experiment the females had been treated too late to produce a sterilans effect, and whether or not perhaps repeated treating with JHM would produce this effect. Of the 4 groups of freshly enclosed virgin females selected, 2 were treated topically with 2 µl/female of 10% dichloro-farnesenic acid methyl-ester (pretreated groups) on the same day. All females were mated on the third imaginal day and put individually in boxes as described above. One day later the females of one of the untreated and one of the pretreated groups were treated with the JHM as mentioned above. Thus from the 4 groups 3 had been treated with the JHM: 1. on the first, 2. on the fourth, and 3. on the first and fourth imaginal day.

The results were essentially the same as in the first experiment. The 3 groups of treated females laid the mean number of respectively 499, 521, and 497 eggs, all eggs and larvae being perfectly viable. The females treated on the first imaginal day (groups 1 and 3) began to oviposit at 8 to 10 h after copulation. One half of the females treated 1 day after copulation (group 2) began to oviposit a few hours after treatment, the other half about 24 h later, i.e. on the same day as the controls.

These results confirm those of the first experiment, and also show that neither the treatment of freshly hatched virgin females nor a double treatment before and after mating has any influence on the development of the embryos and the viability of the larvae.

In a third experiment pupae were treated topically with the JHM as above. 1–2 days later the butterflies eclosed. 4 females hatching 1 day after the treatment were mated on their third day with males from treated pupae. They produced a normal number of perfectly viable eggs. 2 females which had eclosed 2 days after the treatment of the pupae were mated on the second imaginal day with normal males and then treated once more with the JHM. These females laid only 142 and 284 eggs respectively, which, however, were perfectly viable.

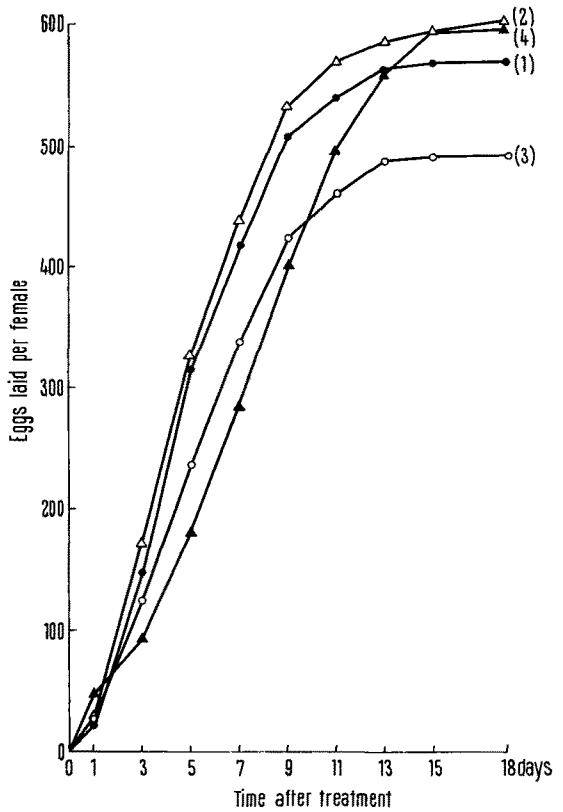
In a fourth experiment virgin females were mated with males which had been treated topically with farnesenic acid ethyl-ester 24 h earlier. The females produced normal offspring.

In a fifth experiment freshly laid eggs of *P. brassicae* were sprayed with acetone solutions containing 1–10% farnesenic acid ethyl-ester or were treated with the pure JHM. All the eggs developed normally and gave healthy larvae.

Thus all the results indicate that the tested juvenile hormone mimetics cannot produce a sterilans effect in *P. brassicae* if applied topically.

Similar treatments of pupae of the greater wax moth *Galleria mellonella*, which matures the eggs already in the pupal stage, gave no sterilans effect either, although treatments were made at different times of the pupal stage, beginning at the day of pupation till shortly before eclosion of the moths. In this case, however, the moths have not been treated, nor has it been proved beyond doubt that topically applied JHM can penetrate the intact pupal cuticle, though the latter is suggested by the occurrence of malformed adults in some pupae treated shortly after pupation[10].

The results of these investigations indicate that sterilans effects of juvenile hormone mimetics are not produced in all insects and suggest that such effects may be restricted to certain groups or species of insects.



Computed numbers of eggs (ordinate) laid by groups of mated females treated with isomere mixture (1.), dichloro-farnesenic acid methyl-ester (2.), farnesenic acid ethyl-ester (3.), and pure acetone (4.) in the course of their survival time (abscissa).

Zusammenfassung. Drei «topical» applizierte Substanzen mit Juvenilhormonwirkung beschleunigten bei weiblichen *Pieris brassicae* Eireifung und Oviposition, ergaben aber keinen Sterilisationseffekt, wie er bei gewissen Wanzen festgestellt wurde. «Topical» mit Dichlorfarnesensäuremethylester behandelte Puppen von *P. brassicae* und *Galleria mellonella* ergaben normal fortpflanzungsfähige Adulttiere.

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[10] Since this paper was written, further experiments with the codling moth *Laspeyresia pomonella* have been performed. In species too neither of the substances 1 and 2 mentioned in Table I had a sterilans effect.