Natural and synthetic materials with insect hormone activity

IV. Specific female sterility effects produced by a juvenile hormone analogue

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The juvenile hormone of insects is known to inhibit the process of insect metamorphosis. It is also known to stimulate ovarian growth in adult females of some species. It has been found recently that some substances with juvenile hormone activity also influence embryonic development. In the bug *Pyrrhocoris apterus* such substances, which prevent imaginal differentiation in metamorphosis, also affect the differentiation process of embryos at a certain stage of egg development (Sláma & Williams, 1966). This has been confirmed with other juvenile hormone analogues on embryonic development of silkworm eggs (Riddiford & Williams, 1967) and grasshoppers (Novák, 1967).

According to the above observations eggs treated with the substances show abnormal development of the embryos, which may pass successfully through the early stages of embryogenesis but are unable to complete differentiation. Usually the embryos do not develop beyond the stage of blastokinesis and die within the egg shells. The effect also appears when the substances are applied to or injected into females, which then deposit sterile eggs.

Among the juvenile hormone active materials so far known the most active one for *Pyrrhocoris* appears to be the compound recently discovered by Romaňuk, Sláma & Šorm (1967), which inhibits the larval–adult transformation in 0.001 μg quantities per specimen and unlike many other materials also stimulates ovarian development in adult females. This compound is a methyl ester of 3,7,11-trimethyl-7,11-dichloro-2-dodecenic acid which may be referred to as a ‘dihydrochloride’ of methyl farnesoate. Compared with the generally known juvenile hormone analogues this compound is about 10⁸ times more active than farnesol and about 10⁴ times more active than farnesylmethylether (Romaňuk et al. 1967).

Because of its extraordinarily high physiological effectiveness and relatively

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high specificity, this compound appears to be a good pesticide (Williams, 1967). Since the inhibition of embryonic development is one of the effective ways of exercising physiological insect control we have studied the sterilizing properties of the above-mentioned substance in more detail.

MATERIALS AND METHODS

The experiments were performed on adult females of *Pyrrhocoris apterus* L., fed on linden seeds and kept at 25 °C and 18 h photoperiodic illumination.

The analogue was applied externally to an uninjured cuticle of the abdominal tergites in a single 1 μl drop of acetone solution. The dose ranged from 0·01 to 100 μg per female. The experimental specimens were reared in special cages with nylon netting on the bottom through which the eggs dropped into a Petri dish. The eggs were collected daily and after counting they were incubated at 25 °C in separate Petri dishes. The hatching of larvae was checked at regular intervals and the eggs which showed inhibition of embryonic development were microscopically inspected. The number of white, unfertilized eggs, which do not develop at all and usually desiccate, was subtracted from the total number of eggs obtained. The final evaluation was made long after the normal larvae had hatched.

RESULTS

Normal active females of *Pyrrhocoris* lay eggs at regular intervals corresponding to reproductive cycles. About 60–80 oocytes develop simultaneously in one cycle and the ripe eggs are deposited without respect to insemination. The first oviposition takes place usually 5–7 days after adult emergence; the next cycles are shorter (Sláma, 1965). In our experiments we have evaluated the effect of the analogue on the eggs of the first batch (5–7 days) separately from the eggs deposited in the 2nd to 4th batches (8–15 days) and from the eggs deposited during the remainder of the oviposition period. The proportion of eggs which showed inhibition of embryonic development in these groups is presented in Fig. 1.

Almost all the eggs of the control group hatched into normal larvae, although a few, especially those laid in later cycles, failed to hatch for unknown reasons (perhaps because of desiccation). On the other hand, all the eggs laid by the females treated with 10 or 100 μg of the substance showed inhibition of embryonic development and none of them hatched even when laid more than a month after the substance was applied. This demonstrates that the substance causes a total and permanent sterility when 10 μg or more comes into contact with an adult female.

The females which received 1 μg of the substance laid completely inviable eggs in the first four reproductive cycles. Later there was some recovery. Approximately 40% of the eggs remained affected whereas the rest hatched.
normally. When the dose was decreased to 0.1 μg the eggs were significantly affected though there was no longer a total inhibition. Most inhibition then occurs among the eggs of the first cycle and the proportion of malformed embryos decreases considerably in later cycles. The females treated with 0.01 μg of the substance deposited about 40% affected eggs in the first cycle. The effect almost disappeared towards the end of the reproductive period, indicating that the minimal effective dose per female is near 0.01 μg. Additional experiments in which 0.001 μg was applied to the females revealed no effects on egg development.

These results show that the methyl-farnesoate ‘dihydrochloride’ is a very potent sterilizing agent with a long-lasting effect which causes developmental deficiencies followed by death of practically all eggs laid by a female which has once been brought into contact with as little as 1–10 μg of the substance. The effect gradually disappears when smaller amounts are used.

![Graph showing inhibition of embryonic development by methyl-farnesoate ‘dihydrochloride’ after external application to freshly emerged adult females of *Pyrrhocoris*.](image)

In the original experiments we applied the substance at different time intervals during the first reproductive cycle, i.e. at the 0, 1st, 2nd, 3rd, 4th and 5th day after adult emergence. In all cases we obtained the same effect as shown in Fig. 1. This indicates that the substance acts in all stages of oogenesis including the stage after which the chorion is formed. The substance readily penetrates the integument and is carried through the haemolymph into the oocytes. This agrees with the recent findings of Slama (unpublished) that a considerable amount of the substance may be found in the haemolymph 10 min. after external application.

The compound used does not appear generally toxic to adults even when used in rather large concentrations (0.5 mg./spec.). Besides its effect on embryonic development and inhibition of metamorphosis it exhibits high juvenile hormone
gonadotropic action. It stimulates ovarian development of allatectomized females and so it increases female fecundity. Treated females usually have a shorter life-span, which is most probably a result of intensive reproductive activity. The total number of eggs produced by the treated female is the same, or may be even larger than that of the controls.

The female of *Pyrrhocoris* may produce about 400–500 eggs during the whole reproductive period. As shown in Fig. 1, almost all these eggs may be affected by a single application of 1 µg of the substance. This suggests that only about 0·00025 µg of the substance would be necessary to inhibit embryonic development of one egg through the female’s body.

Another aspect of the sterility effects of the analogue used is concerned with the development of larvae which hatch from eggs deposited by females treated with intermediate doses. As shown in Fig. 1, the females treated with 0·01 or 0·1 µg of the substance produced a mixture of inviable and hatchable eggs. A certain proportion of the hatched larvae may die just after eclosion. The surviving larvae, however, usually die in course of the 2nd or 3rd instar for no apparent reason and only very few of them are able to reach the adult stage. This seems surprising since it has been found that the substance has no effect at all on the development of 1st to 4th instar larvae. We assume, therefore, that the critical change leading to this inhibition of larval development must have taken place already during embryonic development. If we take into account also this effect on larval development, the dose of 1 µg of the substance per female may be considered in practice to cause total inviability.

**DISCUSSION**

The results show that the effects of the ‘dihydrochloride’ of methyl-farnesoate on embryonic development of *Pyrrhocoris* are in a full agreement with the original observations of Sláma & Williams (1966), who have used another material with juvenile hormone activity, the so-called ‘paper factor’. This factor has been shown to consist of two compounds: juvabione (Bowers, Fales, Thompson & Uebel, 1966) and dehydrojuvabione (Černy et al. 1967). The difference is merely in activity, the ‘dihydrochloride’ being about 10³ times more active both in the juvenile hormone tests performed on larvae and in its effect on the embryos.

The results are also in accord with the findings of Riddiford & Williams (1967), who used a lipid extract from abdomens of adult male cecropia moths and a synthetic material prepared by Law, Yuan & Williams (1966) in tests on silkworm eggs. In our experiments the minimal active dose per egg appears to be more than 1000 times lower than is the case in silkworms. In addition we have obtained permanent sterility of the females just by a simple external application of 1 µg quantities of the substance, which suggests that it does not get metabolized or excreted as easily as it may be the case in cecropia.
Morphological examination of the inhibited embryos revealed that embryonic development was blocked at the same stage as previously reported by Sláma & Williams (1966) and Riddiford & Williams (1967). We have obtained more uniform results when the substance has acted through the female’s body than when applied to freshly laid eggs. In agreement with the preliminary results of Riddiford & Williams (1967) we have also observed mortality in the larvae obtained from eggs which survived lower doses of the substance. It is possible that this effect on *Pyrrhocoris* larvae is connected with an induction of dominant lethal mutations. In our laboratory we have at present two kinds of recessive mutants of *Pyrrhocoris* (white and yellow pigment) which originated from eggs from a treated female. Indeed it is known that some of the chemosterilants produce dominant lethal mutations if applied in low doses (see Auerbach, 1958; Purdom, 1960; La Chance & Crystal, 1963, 1965; La Chance & Rieman, 1964; Bořkovec, 1966).

According to Sláma & Williams (1966) substances with juvenile hormone activity may be used not only as potent pesticides against insect larvae but also as selective ovicides. As suggested by Bořkovec (1966), an ideal sterilant would have high selectivity, absence of side effects, low toxicity and environmental safety. At the present time even the best sterilant will not fulfil all these requirements. However, the substance we have used seems to fulfil most of the requirements for an ideal sterilant. It acts specifically on certain insects whereas it is completely or almost completely inactive on others such as many Lepidoptera, some Coleoptera, Diptera, and even some other species of Heteroptera. This shows that the substance is highly specific. The ancillary increase in fecundity in fact favours its sterilizing action. The toxicity is very low or absent not only for insects but also for other animals including mammals. Another advantage seems to be in its contact action and very low effective dose, which is much lower than for other chemosterilants. There might be a certain danger arising from possible mutagenic effects but the substance proved to have no general mutagenic effect on insects where it has no physiological activity, as revealed by *Drosophila* tests. It is unlikely to have any effects on higher animals by analogy with the juvenile hormone of insects (cf. Williams, 1967).

This compound and others to be described form a qualitatively new group of insect sterilants which are non-toxic, highly specific and very effective compounds with juvenile hormone activity. Their effect is further reinforced by the probable dominant lethal mutation induction in survivors of low doses. The use of these compounds for integrated pest control represents the combination of two methods: chemosterilization and the introduction of deleterious genes into a population (see F.A.O. Report, 1965).
SUMMARY

1. The synthetic compound methyl farnesoate ‘dihydrochloride’, which is a potent juvenile hormone analogue for some insect species, causes inhibition of embryonic development when applied externally to adult females of Pyrrhocoris.

2. As little as 1 µg of the substance per specimen is enough to induce permanent sterility of the females. Doses down to 0.01 µg/spec. cause partial sterility followed by recovery towards the end of the reproductive period. After contact application the substance penetrates rapidly through the integument and is taken up by the oocytes at any stage of oogenesis. The minimum effective quantity per egg in females showing permanent sterility is approximately 0.00025 µg.

3. The substance is highly effective, has high specificity, very low or no toxicity, unimportant side effects, and long-lasting action. For these reasons it is thought to be a very good and specific sterilizing agent.

RÉSUMÉ

Substances naturelles et synthétiques ayant l'activité d'une hormone d'Insecte. 4. Effets spécifiques de stérilité chez les femelles, produits par un analogue de l'hormone juvénile

1. Quand on l'applique extérieurement à des femelles adultes de Pyrrhocoris, le ‘bichlorhydrate’ de méthylfarnésoate, composé synthétique, analogue puissant de l'hormone juvénile pour quelques espèces d'Insectes, provoque une inhibition du développement embryonnaire.

2. 1 µg de substance par individu suffit pour induire la stérilité permanente des femelles. Des doses allant jusqu'à 0,01 µg/individu provoquent une stérilité partielle suivie d'une récupération, vers la fin de la période de reproduction. Après une application par contact, la substance pénètre rapidement à travers le tégument et est absorbée par les oocytes à n'importe quel stade de l'oogénèse. La quantité minimale efficace par œuf, chez les femelles présentant une stérilité permanente, est d'environ 0,00025 µg.

3. La substance est hautement efficace, possède une spécificité élevée, une toxicité très faible ou nulle, des effets parallèles sans importance et une action de longue durée. Pour ces raisons, on suppose qu'elle constitue un très bon agent stérilisant spécifique.

REFERENCES


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