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Regulation of development and population growth of mealy bugs treated with Epofenonane, a JH active IGR*

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The reaction of homopterous insects in general and mealy bugs in particular to treatment with the insect growth regulator Epofenonane differs in several aspects from other insect species in accordance with their specific physiology. The dosage and time of application determine the type of response of the treated insects as follows: a) the larvae die in younger stages, mostly during ecdysis; b) the significantly smaller neotenic females remain sterile due to either restricted development and growth of ovaries or lack of mating; c) the males display various morphogenetic changes including supernumerary nymphal stages with remarkable regression of morphogenesis. The sum of all these effects observed in the laboratory results in full control of a mixed population in the field. The efficacy of Epofenonane, which is remarkably persistent outdoors, is comparable to the best commercial products used currently for the control of mealy bugs.

The scale insects belong to the worst pests of orchards and gardens. Their specific type of life makes them relatively invulnerable to most of the quickly degradable insecticides (BIVINS & DEAL, 1973; POE *et al.,* 1973), the efficacy of which has to be enhanced by the addition of mineral oils (BOBOYE & CARMAN, 1975). The use of compounds with longlasting effect, low mammalian toxicity and no adverse side effects would solve this problem. This, and the fact that the sedentary population of the scale insects show no or minimal migration, makes them an ideal target for juvenile hormone (JH) active insect growth regulators (IGRs). In fact, most of the experiments in this direction brought very promising results (BAGLEY & BAUERNFEIND, 1972; NASSAR *et al.,* 1972; STAAL *et al.,* 1973; SCHEURER & RUZETTE, 1974; BOBOYE & CARMAN, 1975; HAMLEN, 1975). These circumstances and the relative good persistence in the field of Epofenonane, a JH active IGR [6,7-epoxy-3-ethyl-1-(p-ethylphenoxy)-7-methylnonane, cis/trans mixture] (DORN *et al.,* 1976; HANGARTNER *et al.,* 1976) prompted us to use this IGR for the control of citrus mealy bugs. This report deals with the excellent results achieved in the field and offers a possible explanation of the mode of action on the basis of laboratory studies.

MATERIALS AND METHODS

The laboratory experiments were done on citrus mealy bug *(Planococcus citri* RISSO) on potato tubers (NELSON-REES, 1961) in ventilated plastic jars (250 ccm) at 25 °C, 60% rh and 16 h daily illumination. Epofenonane was emulsified in water and sprayed to the point of run-off. The insects were either

*These results were presented at the International Congress of Entomology, Washington, August 19–27, 1976.

examined as total mounts or fixed in Bouins or Carnoys fluid, sectioned in paraffin and stained with azan.

RESULTS AND DISCUSSION

Field trials

Epofenonane and Dimethoate were tested in a field trial against *P. citri* on replicated single trees of the grape fruit variety «Ruby Red» in the Rio Grande Valley of Texas. Citrus mealy bug populations remained at sub-economic levels until mid-summer when the second spray treatment was applied. Both chemicals provided equally effective control. Under the conditions of the test the 0.025% a. i. rate of Epofenonane was as effective as the 0.1% a. i. spray concentration. Epofenonane and Malathion were also tested in field trials against Comstock mealy bug on the alternate host plant white mulberry in Porterville, California. Epofenonane was applied twice in June and August at 0.1% a. i. spray concentrations. Reduction of the mealy bug populations of 99.5% on August 8 and 100% on October 2 attained with Epofenonane was comparable to the reduction provided with the standard Malathion treatment (MORENO, personal communication).

Laboratory experiments

Normal course of development

The life history of mealy bugs is highly specialized as is true of other scale insects. Most of the embryogenesis takes place within the female genital ducts (Fig. 1) and the first crawling larvae hatch shortly after the eggs are laid (Fig. 2). Sex differentiation in the larvae is first visible at the end of the second stage, when the genital rudiments start a vigorous growth and differentiation. The third stage larvae have fully differentiated ovaries and moult without any remarkable metamorphosis into the fourth stage which is, as a matter of fact, the neotenic female (Fig. 3). The male larvae, however, undergo a profound metamorphosis during the pronymphal (Fig. 4) and particularly the nymphal stage (Fig. 5) which follow the first three larval stages. So, the postembryonic development of females consists in 3, in males in 5 instars. The absence of extensive metamorphosis of females is reflected in their larva-like body in contrast to fully differentiated winged males (Fig. 6). The strain of the citrus mealy bug used in these experiments is obligatory bisexual, contrary to insects used by STAAL *et al.* (1973). The ovaries of the untreated virgin females grow considerably as reflected by the body weight (Table 1). The oocytes are resorbed, however, during vitellogenesis. Oviposition requires copulation, as has been reported by NELSON-REES (1961).

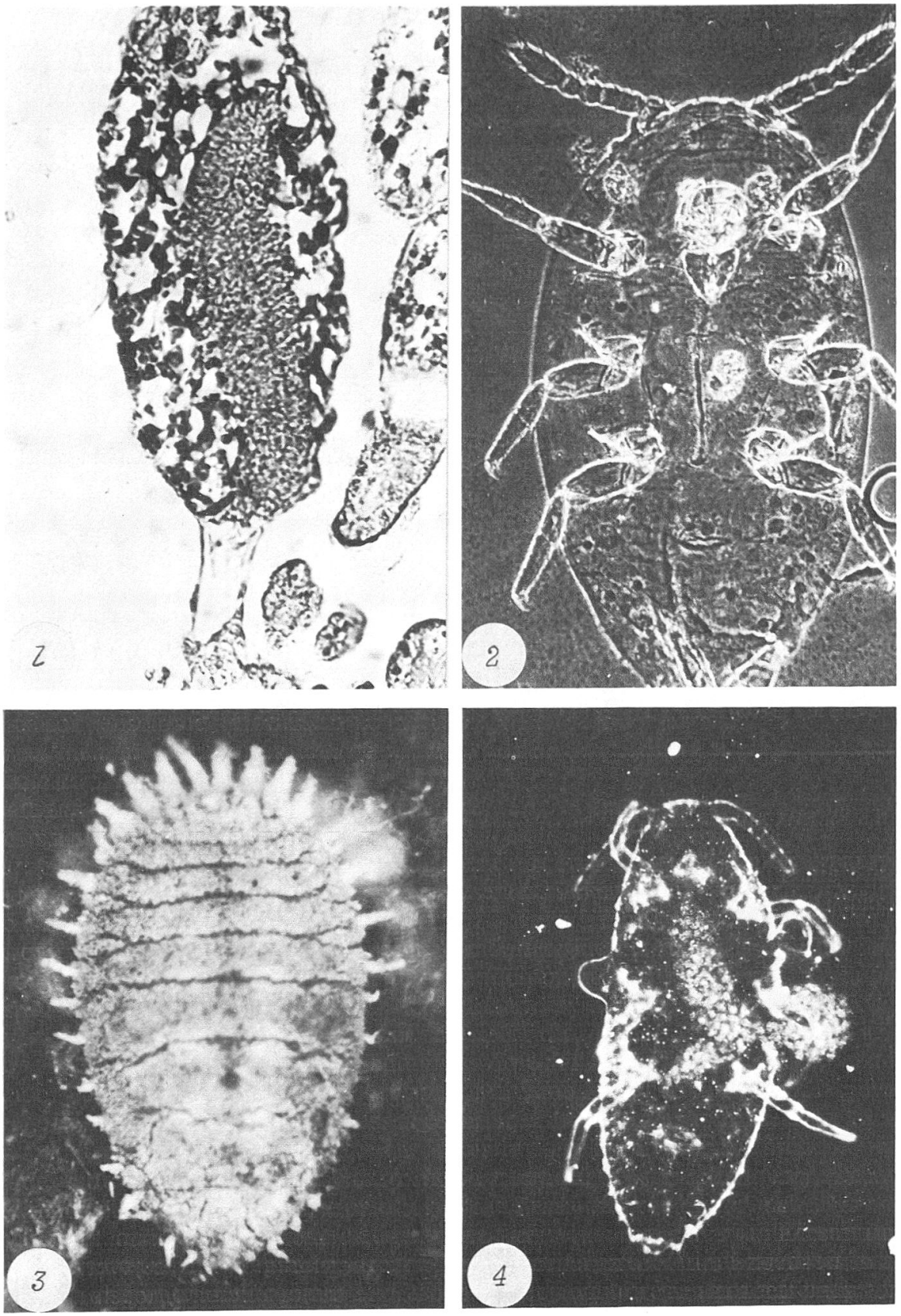
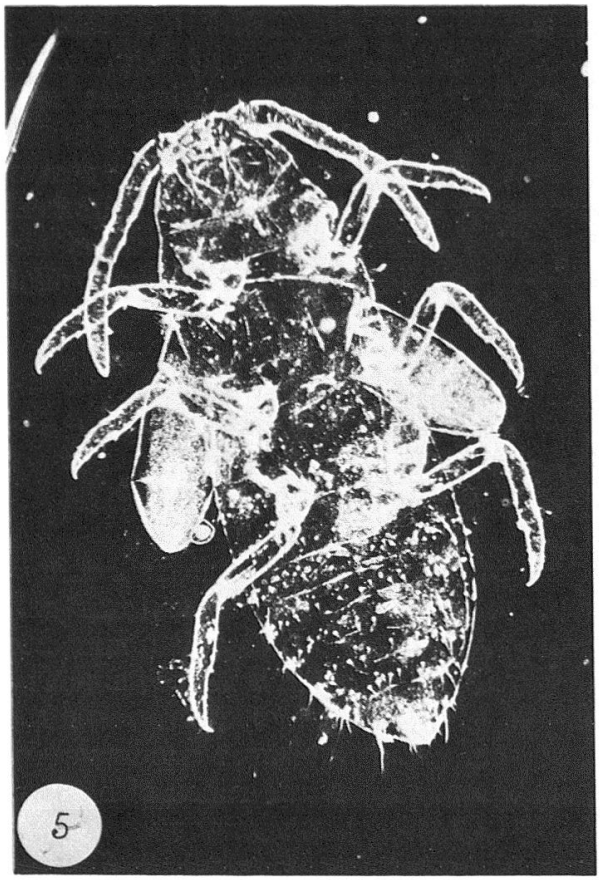
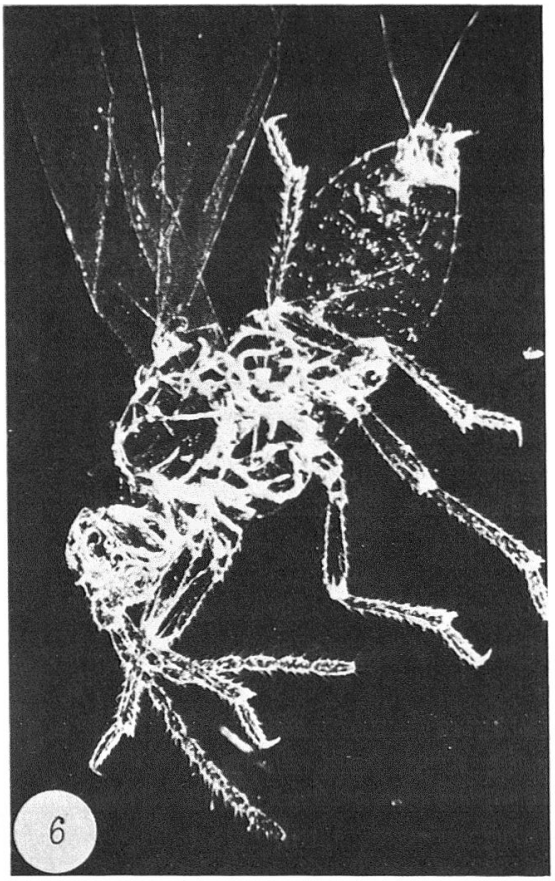
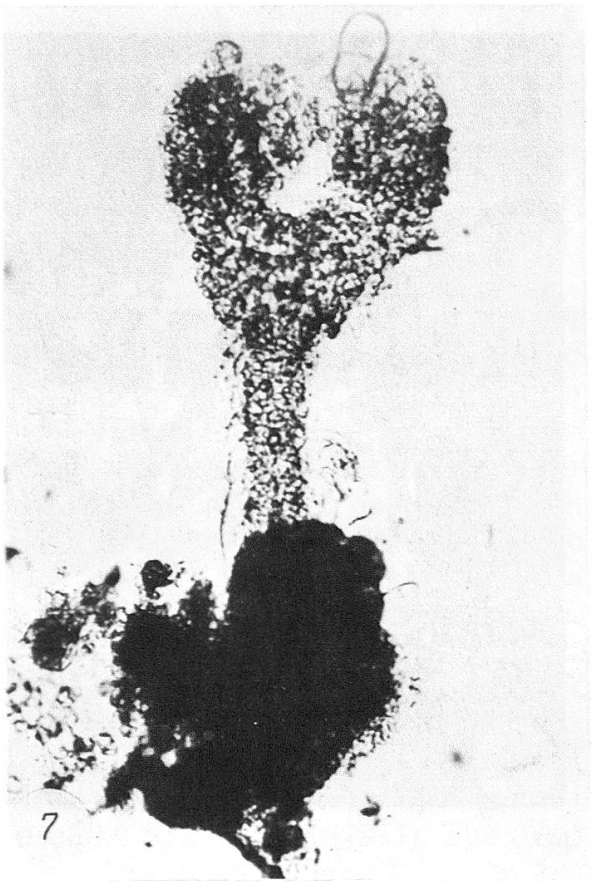


Fig. 1-4: Egg with embryo in the oviduct, Bouin, azan (1); larva of the first instar, lactophenol (2); neotenic female of the fourth instar (3); and male pronymph, lactophenol (4).







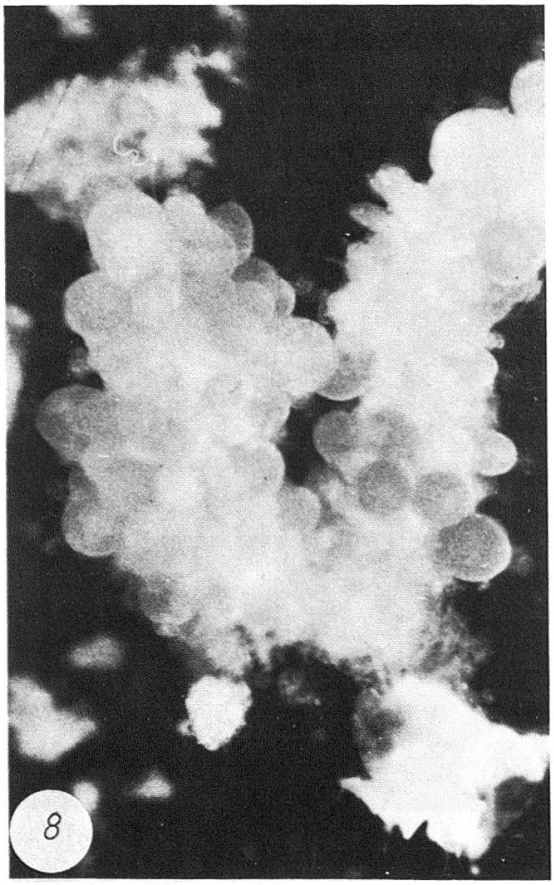


Fig. 5–8: Male nymph, lactophenol (5); mature male, lactophenol (6); ovarial rudiments of a female reared on potato treated with 0.1% Epofenonane since the first larval instar, enlargement 100x (7); and fully differentiated functioning ovaries of untreated female, enlargement 25x (8).

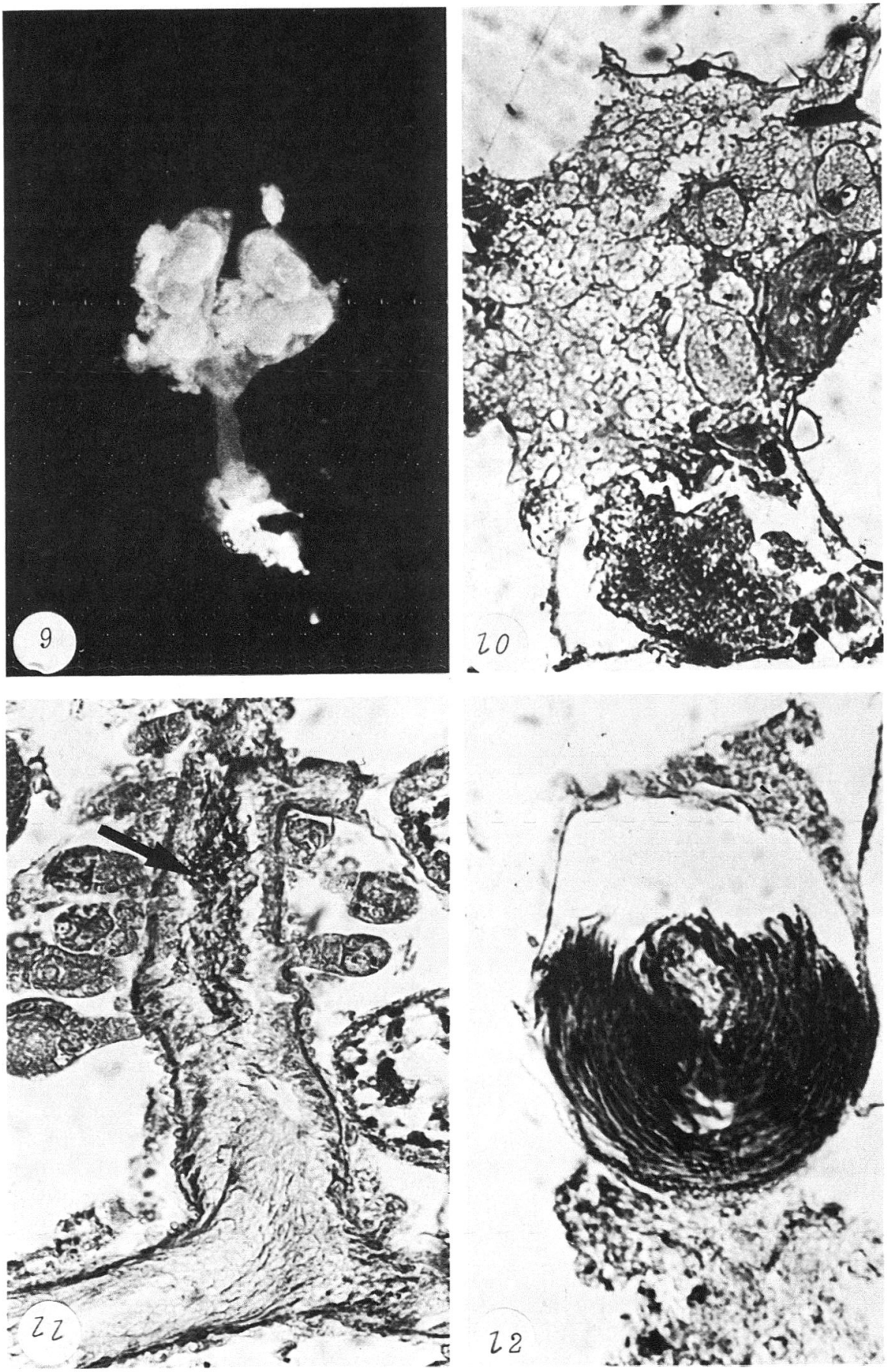


Fig. 9-12: Same as fig. 7, dosage: 0.01%, enlargement 25x (9); same as in fig. 7, atrophied ovarien tissue, Bouin, azan (10); sperm in the oviduct (arrow) of a female reared on potatoes treated with 0.1% Epofenonane since the third larval instar, transferred as adult on an untreated tuber and provided with untreated males, enlargement 250x (11); and sperm in the seminal receptacle of the female treated as in fig. 11, enlargement 250x (12).

Effect of Epofenonane on the larval ecdysis and metamorphosis of males

Epofenonane, like other JH active IGRs, seems to limit the activity of ecdysone (MASNER *et al.,* 1975) which disturbs both ecdysis and metamorphosis. Actually a frequent mortality during the first ecdysis of the crawling larvae living on the treated potatoes was observed as reported by BOBOYE & CARMAN (1975) and MORENO *et al.* (1976) in *Aonidiella aurantii* MASKELL. The moults of later larval stages are rarely disturbed. The ecdysis of the male pronymphs and particularly the nymphs into adults is, however, drastically affected. The sensitivity of the metamorphosing males towards Epofenonane is understandable by virtue of the latters JH activity. The inhibition of metamorphosis is here, however, accompanied by an exceptional feature of regression of the number of pores on the anal plate of the supernymph compared to the nymphal structure. This is to our knowledge the only case of reversal of metamorphosis in the intact insect (VOGEL *et al.,* in prep.).

The specific reaction of mealy bugs towards the JH active IGR is especially remarkable in experiments using lower concentrations (0.001%). This dose is still high enough to prevent the metamorphosis of males, and the treated population consists exclusively of smaller but otherwise seemingly normal females (Table 1) which cannot reproduce parthenogenetically. An eventual migration of mobile males from outside could, however, change the situation, if the females originating from the treated larvae were actually normally differentiated, as they look like. A closer inspection showed that this was not the case.

Sterilisation of females with Epofenonane

The females, as mentioned above, do not undergo any spectacular outer metamorphosis. Their ovaries, nevertheless, differentiate. Permanent contact with JH active IGR limits the differentiation of the ovaries similarly as described for bugs (MASNER, 1969). The number of activated oocytes depends on the dose and stage of development at the time of the beginning of treatment (Table 1). Most efficient is the treatment at the first stage with 0.1% when the ovaries remain at the stage of the young second instar larva (Fig. 7) compared to the fully functioning ovaries (Fig. 8). The lower doses allow differentiation of a few oocytes which may eventually get some yolk and are later resorbed (Fig. 9). The tissues, both germ cells and mesodermal, are completely atrophied and regeneration is not possible (Fig. 10). This is demonstrated in an experiment where such treated females are transferred to untreated potatoes and provided with fertile males. No growth of oocytes was observed, and the ovaries remained atrophied as we have seen before (Table 1).

Females treated at the beginning of the last third instar are somewhat larger (Table 1). Their ovaries contain few growing oocytes, but no eggs are deposited as long as they remain on treated potatoes, even when males are added. The males are not attracted by the females either due to the lack of pheromone production similarly as found by MORENO *et al.,* (1976) in *A. aurantii* or due to changes of mating behaviour as reported by FOCKLER & BORDEN (1973)

Table 1: Sterilisation of females of the citrus mealybugs exposed to potato tubers treated with Epofenonane since the indicated larval stage. The resulting females were eventually transferred from treated to untreated tubers and untreated males were added, where indicated.

set-up stage of larvae	dosage (%)	arrangement of the resulting females	inspected females			eggs	larvae
			number	body weight (mg)	ovaries		
1	-	control ♀♀	8	5.00 ± 2.40	big, empty	-	-
	-	♀♀+♂♂	35	3.70 ± 1.77	full	many	≈ 500
	0.1	perm. contact ♀♀	23	0.60 ± 0.12	small, empty	-	-
	0.01	♀♀	25	1.08 ± 0.19	few eggs	-	-
	0.001	♀♀	18	2.40 ± 1.17	half full	-	-
	0.1	♀♀+♂♂	14	0.56 ± 0.23	small, empty	-	-
	0.1	treat.→untreat.♀♀	4	0.60 ± 0.18	small, empty	-	-
	0.1	♀♀+♂♂	20	0.62 ± 0.17	small, empty	-	-
2	0.1	perm. contact ♀♀	8	0.88 ± 0.27	small, empty	-	-
3	0.1	perm. contact ♀♀	6	1.03 ± 0.40	few eggs	-	-
	0.1	♀♀+♂♂	12	1.03 ± 0.20	few eggs	-	-
	0.1	treat.→untreat.♀♀+♂♂	4	0.95 ± 0.20	small, empty	70	64

for the striped ambrosia beetle and by OBERLANDER *et al.,* (1975) for *Plodia interpunctella.* The females transferred to untreated potatoes are mating with the added males as documented by the sperm found in oviducts (Fig. 11) and receptacles (Fig. 12), and a few eggs with viable embryos are oviposited (Table 1). The fertility of those females is, however, limited just on the few eggs differentiated before the onset of treatment. The morphogenesis of the ovaries is apparently precluded by the Epofenonane treatment during the last larval stage which causes permanent sterility.

CONCLUSION

The excellent results of the field trials are largely due to the relatively high outdoor stability of Epofenonane and, above all, to a very sensitive reaction of the mixed population of mealy bugs which is affected at every stage of development. The inhibition of ecdysis in larvae is complemented by inhibition of metamorphosis of males and limited morphogenesis of the ovaries of females. The result is a very small population of surviving females, the fertility of which is limited on few oocytes differentiated during the postembryonic development before the treatment. This determines also the optimal time for the treatment in the field - the time when the population is still in the larval stage. Thus a good control even of a heterogenous population in the field is practicable.

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