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Effects of exogenous ecdysteroids on the female tick *Ornithodoros moubata*: Induction of supermolting and influence on oogenesis

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Abstract

Ingestion of 22,25-dideoxyecdysone (22,25-DDE) during the blood meal provoked supermolting in mated females *Ornithodoros moubata* (Acarina, Argasidae). Doses as low as 35 ng/ml blood induced molting in all ticks approximately 10 days after feeding which corresponds to the normal amount of time required for a nymphal molt. In addition, mated females oviposited after supermolting. Increasing dosage rates reduced the number of eggs produced. 5 µg/ml proved to be lethal.

Other ingested ecdysteroids like e.g. ecdysone, 20-hydroxyecdysone, makisterone A or ponasterone A could also induce supermolting, but never more than in 50 % of the females. However, the levels required were about 500 times greater than levels of 22,25-DDE. Lethal doses were 20 µg/ml for makisterone A, and 40 µg/ml for ecdysone and 20-hydroxyecdysone.

With all ecdysteroids tested, the supermolting females presented malformations of distal parts of the legs and of the mouth parts.

Topical application of the quantity of 22,25-DDE, ecdysone or 20-hydroxyecdysone corresponding to the lethal ingested dose was completely uneffective. Only application of 20 µg of makisterone A showed slight effects.

O. moubata seems very well protected from exogenous classical ecdysteroids since ingested doses as high as 10 µg/ml blood or topical doses of less than 20 µg per female have no effect. In contrast, a physiological dose of 10–20 ng 22,25-DDE ingested per female was effective. One can wonder why the females are so sensitive to this compound compared to the other ecdysteroids.

1 Introduction

The effects of injected or ingested exogenous ecdysteroids (ES) in immature insects are variable according to the species studied. There may be no apparent effect as in *Locusta* due presumably to an efficient detoxification mechanism (Feyereisen et al. 1976). In other insects, ES can accelerate apolysis and ecdysis (Wyatt 1972). But exogenous ES can also be toxic. They may severely disrupt larval growth and development (e.g. Robbins et al. 1968).

In adult insects, exogenous natural or synthetic ES like e.g. 22,25-dideoxyecdysone (22,25-DDE) can inhibit ovarian maturation and reproduction (e.g. KAPLANIS et al. 1972). The injection of 3.3 µg makisterone A or 25 µg 20-hydroxyecdysone inhibits vitellogenesis in *Oncopeltus fasciatus* and induces also the formation of a new adult cuticle (ALDRICH et al. 1981). This altogether with comparable observations of supermolting adult integument from *Cimex* or *Rhodnius* by parabiosis with a molting larva of *Rhodnius* (WIGGLESWORTH 1940), or from *Galleria* by implantation in immature stages (PIEHPO 1938, 1939), indicates that at least in some pterygote insects, the adult hypodermis can be hormonally stimulated to secrete a new cuticle.

As other arthropods, the immature ticks use ecdysteroids for molting control. Ecdysone and 20-hydroxyecdysone are present in nymphs of the argasid tick *Ornithodoros moubata* (Murray 1877 sensu WALTON 1962) (GERMOND et al. 1982) and of the ixodid tick *Amblyomma hebraeum* (DELBECQUE et al. 1978; DIEHL et al. 1982). Comparable to insects, high hormone titers are present at the beginning of new cuticle deposition. It is therefore not surprising that exogenous ES can also exert several effects in ticks. In ixodids, they can terminate larval diapause in *Rhipicephalus sanguineus* (SANNASI and SUBRAMONIAM 1972) or in *Dermacentor albipictus* (WRIGHT 1969). They inhibit oogenesis and cause high mortality in *Boophilus microplus* (MANSINGH and RAWLINS 1977). In argasids high concentrations of ingested 20-hydroxyecdysone, ponasterone A, or inokosterone induced supermolting in adults of *O. moubata* (species doubtful; KITAOKA 1972) or of *O. porcinus* (SOLOMON et al. 1982).

In this paper we report on the effects of several ES on supermolting and oogenesis in the argasid tick *O. moubata* (Murray 1877 sensu WALTON 1962). Special emphasis is placed on the synthetic 22,25-DDE which proved to be extremely effective in this species.

2 Material and methods

2.1 Animals

The argasid tick *Ornithodoros moubata* (Murray 1877 sensu WALTON 1962) colony was kept at 27 °C and 30–40 % relative humidity in the dark. Virgin females were obtained by isolation of the fed fifth instar nymphs. They were mated immediately after the blood meal.

2.2 Chemicals

Ecdysone, 20-OH-hydroxyecdysone and makisterone A were purchased from SIMES (Italy). Ponasterone A was a gift of Dr. Koolmann. 22,25-dideoxyecdysone was supplied by the Insect Physiology Laboratory, Beltsville Agricultural Research Center, USDA, Beltsville (USA).

2.3 Nutrition

Ecdysteroids were quantified gravimetrically or by spectrophotometric measurements of methanolic solutions ($\lambda = 242$ nm, $\varepsilon_M = 12\,000$). A few µl of the stock solution were mixed with fresh defibrinated pig blood. Females were fed artificially through a "Parafilm" membrane at 37 °C. After the blood meal, the females were kept in glass tubes which were half filled with a loose cotton plug. The females were weighed 24 hours after the blood meal to estimate the engorged weight after excretion of coxal fluid.

2.4 Scanning electron microscopy

Specimens were fixed in 70 % ethanol, gradually dehydrated with ethanol followed by acetone and then air-dried. They were gold sputtered and viewed in a PHILIPS stereoscan PSEM 500.

2.5 Statistical analysis of the results

Analysis of the results were performed with the informatic system Vax 11 VMS. Comparison of regression lines was conducted with a statistical method after HALD (1952).

3 Results

3.1 Induction of supermolting by ingestion of 22,25-dideoxyecdysone (22,25-DDE)

Mated females of *O. moubata* were fed with blood containing different amounts of 22,25-DDE (from 0.006 µg/ml to 5 µg/ml). The females were thereafter daily examined to see the effects of the treatment. Results are shown in table 1.

Table 1. Effects of different doses of ingested 22,25-dideoxyecdysone in mated females *Ornithodoros moubata*

Dose µg/ml blood	Number of treated females	Females dying within the first 10 days	Supermolting females	Ovipositing females
0.006	14	0	0	14
0.035	27	0	27	27[a]
0.07	12	4*	8	8[a]
0.1	13	3	10	10[a]
1	10	1*	9	9[a]
5	9	9*	–	–

* Dead animals presenting 2 cuticle upon dissection. – [a] In the case of supermolting females, oviposition occurred 10 days after exuviation.
The doses of 0.035 µg/ml and higher induced supermolting in all females.

The weakest concentration tested (0.006 µg/ml blood) had no visible effects on the females. Egg laying began within 10 to 18 days after the blood meal. This corresponds to the normal preoviposition time observed in our breeding conditions.

The dose 0.035 µg/ml induced supermolting of all females. As a maximum of 0.5 ml of blood is ingested by a female, 15 to 20 ng of ecdysteroid were effective.

Higher doses had the same effect, but mortality began to appear very rapidly, and 5 µg/ml blood was lethal. The animals died few days after the blood meal; however, dissections demonstrated the presence of two cuticles in most of the dead animals.

For every effective doses tested, the females ecdysed within 8 to 10 days after the blood meal. This corresponds to the time required for a nymphal molt. All "superfemales" showed some malformations of appendages, especially of the first pair of legs where claws and pulvilli were lacking (fig. 1, center and bottom). In most cases, these females were not able to take a new blood meal.

3.2 Effects of 22,25-DDE on oogenesis

Sampling hemolymph during the supermolting cycle of the mated females showed that hemolymph remained very clear indicating the absence of vitellogenin synthesis. Vitellogenin of this species is a dark hemoprotein which gives a brown colour to the hemolymph. Dissection of the females during this cycle also showed that the ovary remained in an early previtellogenic stage of oogenesis. Nevertheless, 10 days after molting each surviving female laid eggs. This time corresponds to the normal length of preoviposition period after a blood meal in the control females. Thus, vitellogenesis was inhibited during the supermolt cycle and started only shortly after ecdysis.

The number of eggs laid by the control females was directly proportional to the quantity of blood ingested (corr. coeff. 0.95) and also to the weight of fed females after 24 hours (corr. coeff. 0.94).

Fig. 2 shows the influence of 22,25-DDE ingested during the blood meal upon the number of eggs laid. In the case of the lowest dose (0.006 µg/ml blood) no statistical differences ($P < 0.05$) exist between the regression line of the experimental values, and the regression line of control females. Thus, this dose induces supermolting and has no effect on number of eggs laid. However, the regression lines of values obtained by ingestion of higher dosages are significantly different ($P < 0.05$) from the control. The curves have the same shape, but they are lower. In addition, it is possible to calculate the loss of egg production which increases with increasing doses; e.g. at the dose 0.1 µg/ml females produced 61 ± 7 (SD) eggs less per female than control ticks.

3.3 Effects of other ingested ecdysteroids

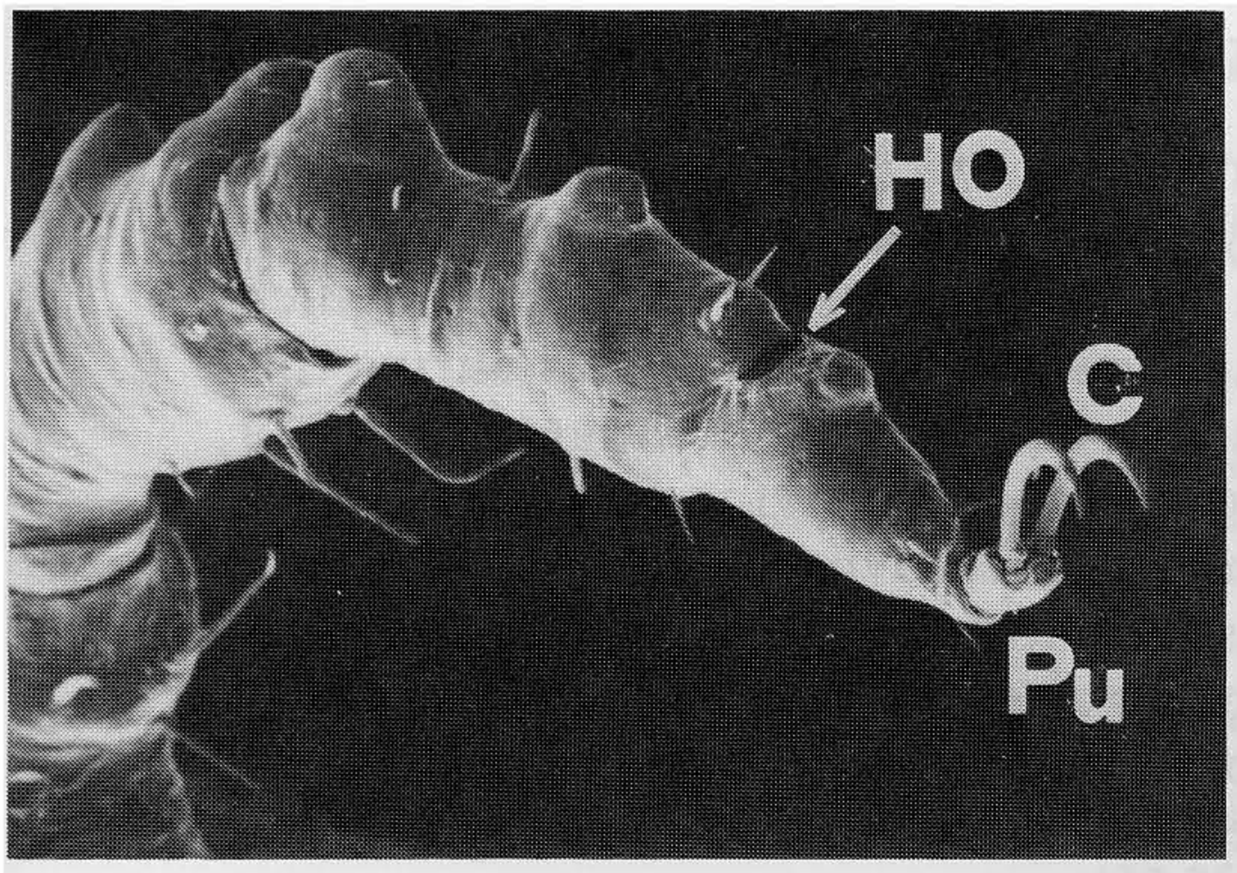
Effects of ingested ecdysone, 20-hydroxyecdysone, ponasterone A and makisterone A were investigated in mated females.

Preliminary experiments (data not shown here) indicated that 0.05, 0.1, 0.25, or 0.5 µg/ml blood of each of these ecdysteroids did not induce supermolting and had no effects on oogenesis. Thus, we investigated higher doses; Table 2 summarizes the results.

One, 2.5, and 5 µg/ml blood did also not provoke supermolting, but mortality appeared in few cases. Doses of 10 µg/ml blood of makisterone A induced supermolting in 2 of 16 females a few weeks after egg laying. Higher doses of makisterone A were lethal.

At doses of 20 µg/ml blood of ecdysone and 20-hydroxyecdysone, mortality was very high within the first 10 days after feeding, but supermolting was induced in 33 % ecdysone fed females and in 73 % 20-hydroxyecdysone fed females. In a few cases, the animals molted twice without another blood meal and died without ovipositing emprisoned within their second cuticle. However, one female laid eggs directly after the blood meal, and a few females oviposited a small number of eggs after supermolting once.

All females supermolting after the ingestion of ecdysone, 20-hydroxyecdysone and makisterone A presented the same malformations as previously described for 22,25-DDE. In addition, the eversible Géné's egg waxing organ remained evaginated.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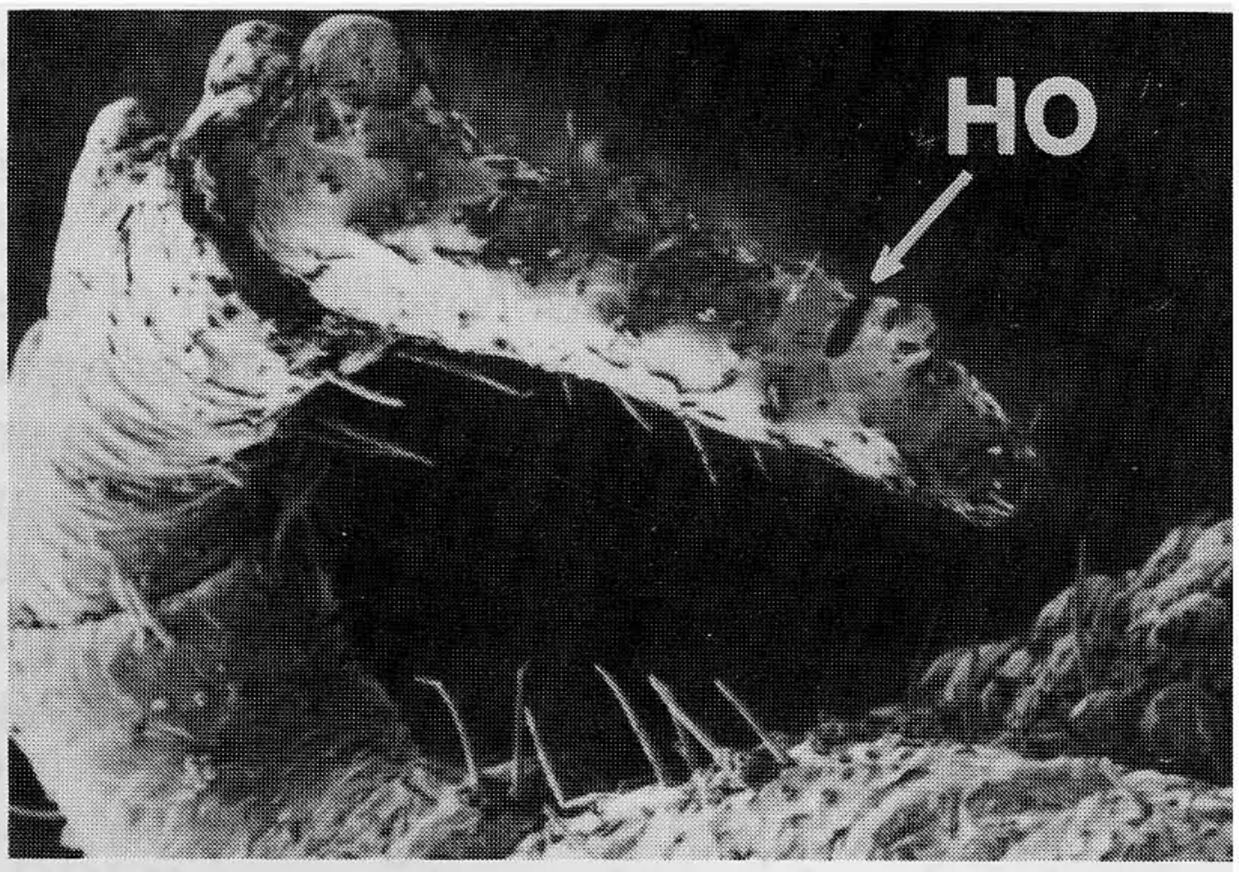
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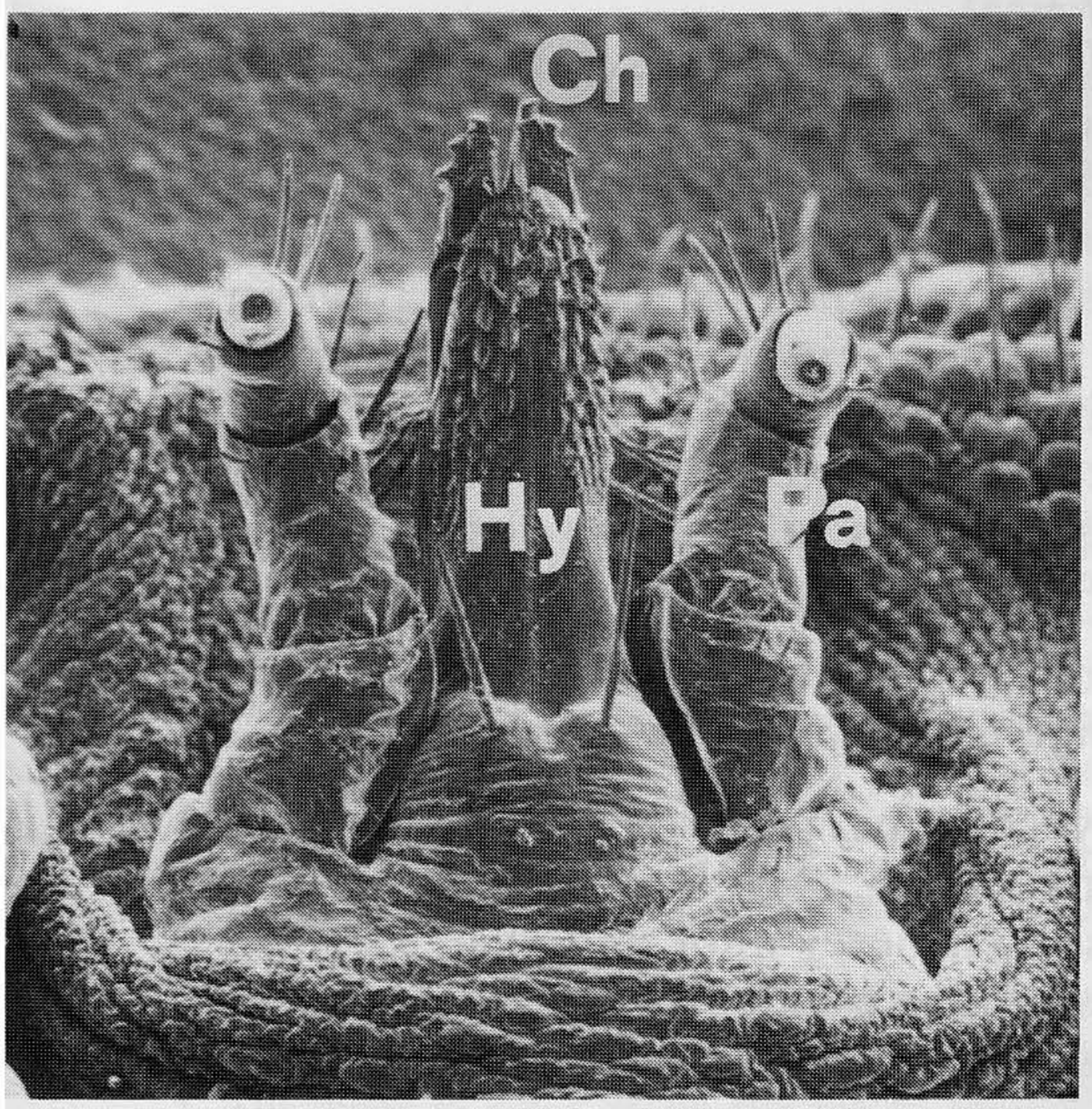
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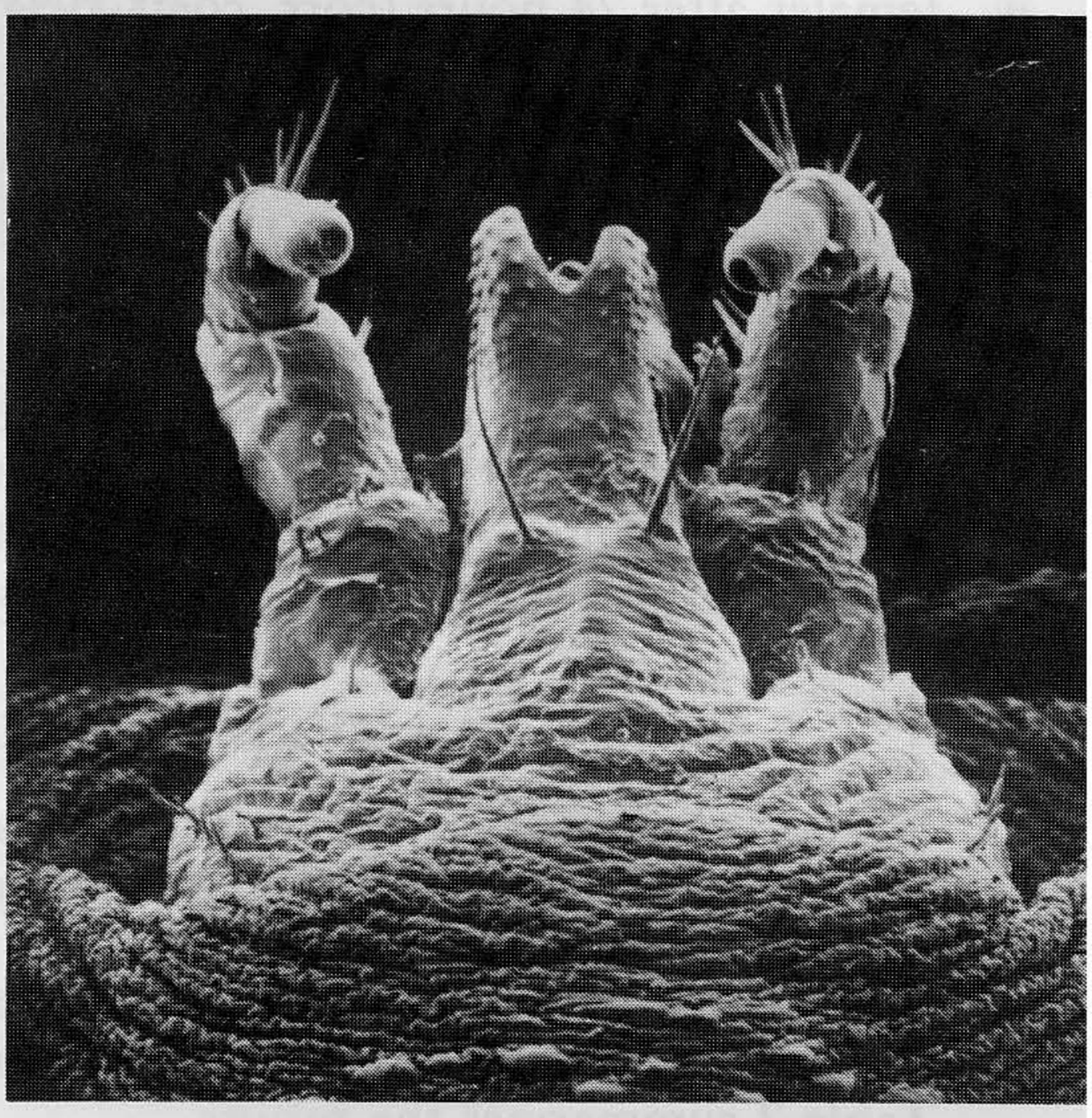

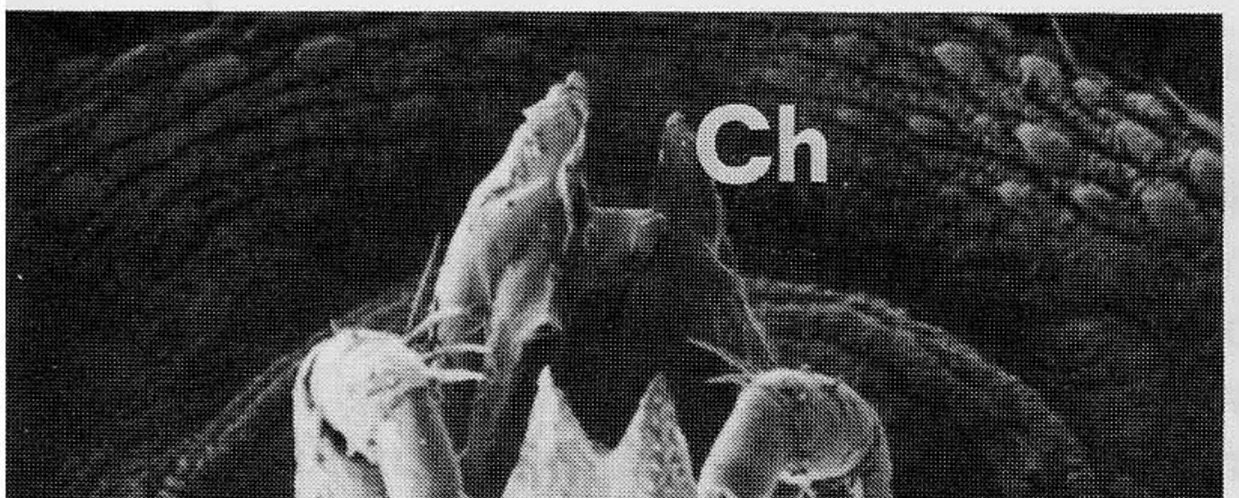
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3.4 Effects of topically applied ecdysteroids

Methanolic solutions of the four ES ecdysone, 20-hydroxyecdysone, makisterone A and 22,25-DDE were topically applied onto the dorsal cuticle of mated females immediately after the blood meal.

Two groups of 15 females were each treated with 40 µg of ecdysone or 20-hydroxyecdysone. This dose which was lethal when ingested did not provoke

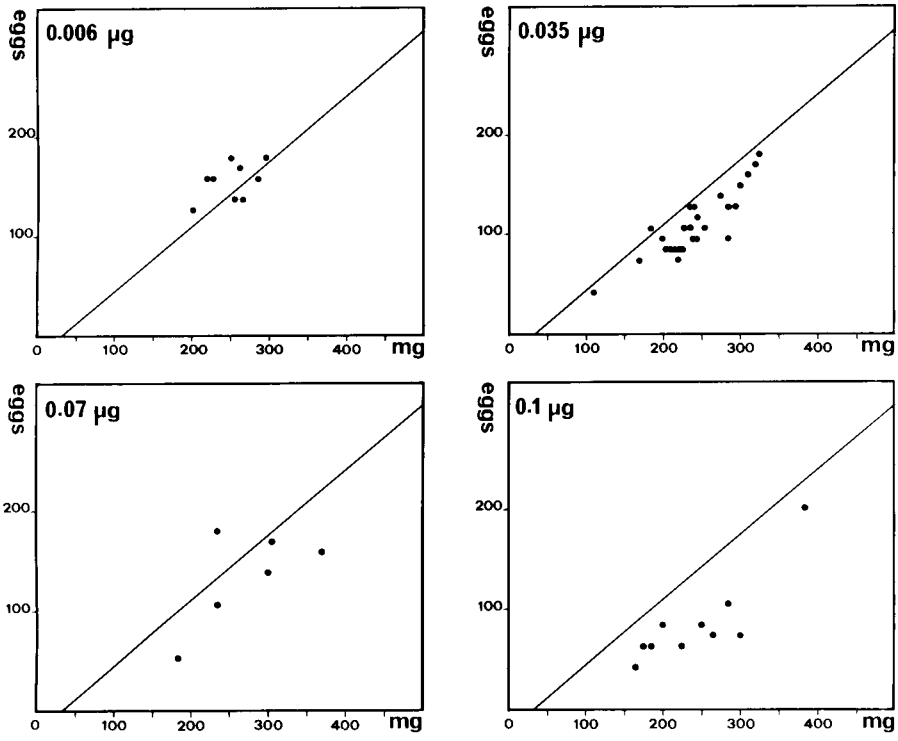

Fig. 2. Effects of different doses of 22,25-dideoxyecdysone ingested by mated females *Ornithodoros moubata* on egg-yield. Normally, mated females oviposit a number of eggs proportional to their engorged weight 24 hours after the blood meal. The corresponding regression line appears on each of the 4 diagrams (equation: Number of eggs = 0.663 × weight of engorged females – 21.422; corr. coeff. 0.94). Dots represent the experimental values for the 4 doses tested. Only the 3 higher doses induced supermolting with a corresponding decrease of number of eggs per mg engorged weight. Differences between the regression lines are statistically different at the level of $P < 0.05$

Fig. 1. Malformations of distal parts of appendages observed in supermolted females *Ornithodoros moubata* after ingestion of 22,25-dideoxyecdysone. – *Top left:* Distal part of a leg in a normal female. The tarsus has numerous sensillae and shows the sensory Haller's organ (HO). The extremity of the Pulvillus (Pu) presents 2 claws (C). – *Top right:* In the supermolted females, the claws and the pulvillus are lacking. – *Center left:* Normal mouth parts of a female *O. moubata:* Ch = chelicerae, Hy = hypostome, Pa = palpus. – *Center right and bottom:* Various degrees of malformation and atrophy of hypostome and chelicerae in supermolted females. Only the palpus remains unaffected. These females were unable to take a new blood meal

Table 2. Effects of the ingested ecdysteroids ecdysone, 20-hydroxyecdysone, ponasterone A and makisterone A in mated females *Ornithodoros moubata.* Supermolting is induced only in a few cases with high dosages of ecdysteroids (10 µg/ml or 20 µg/ml)

	Doses µg/ml blood	Number of females	Number of females dying during first 10 days	Super-molting females	Ovi-positing females	Females ovipositing after supermolting	Number of females dying after 10 days
ecdysone	1	10	0	0	10	–	0
	2.5	15	1	0	14	–	0
	5	20	2	0	18	–	3
	10	10	0	0	10	–	0
	20	15	10[a]	4	1	2	0
	40	9	9	–	–	–	–
20-hydroxy-ecdysone	1	10	0	0	10	–	0
	2.5	15	0	0	15	–	1
	5	20	2	0	18	–	0
	10	10	2	0	8	–	2
	20	15	5[a]	10[b]	0	2	0
	40	7	7	–	–	–	–
ponaste-rone A	1	10	0	0	10	–	0
	2.5	15	3	0	12	–	0
	5	17	0	0	17	–	0
	10	20	2	0	18	–	0
makiste-rone A	1	9	0	0	9	–	0
	5	7	0	0	7	–	0
	10	16	2	2[c]	5	0	0
	20	10	10	–	–	–	–
	40	7	7	–	–	–	–

[a] One dead female presented 2 new cuticles upon dissection. – [b] Two females ecdysed, then remained imprisoned in a new secreted cuticle. – [c] Ecdysed a few weeks after egg laying.

any affects. The females laid a normal amount of eggs within a previposition period of 10 to 30 days.

Three other groups of 15 females were treated with either 500 ng, 2 µg, or 5 µg 22,25-DDE. No effects were obtained.

The only ES which showed slight effects was makisterone A. The dose of 20 µg per female provoked supermolting in only 1 of the 11 treated females and death in 2 others. The remaining animals laid a normal quantity of eggs.

4 Discussion

4.1 Effects of ecdysteroids on supermolting

Our results clearly show that the adult integument of the female tick *O. moubata* is able to respond to exogenous ES. The molting cycle is realized within 8 to 10 days which correspond approximatively to the length of the molting cycle of the fifth instar nymphs. However, malformations of the distal part of legs and of mouth parts occur, especially after 22,25-DDE treatment. This fact has not been previously noticed by other authors which also obtained supermolting of argasid ticks (KITAOKA 1972; MANGO et al. 1976). Perhaps a precocious presence of high levels of ES in the hemolymph accelerates the molting processes and causes disturbance in the development of the distal parts

of the appendages. This may be compared to the "hyperecdysionism" phenomenon reported by WILLIAMS (1968) with incomplete differentiation of adult organs in pupae of *Samia cynthia* after treatment with large doses of ES.

Our experiment points out the great difference of sensitivity of the female *O. moubata* to the different ES tested. The synthetic 22,25-DDE provokes supermolting with doses about 500 times lower than ecdysone, 20-hydroxyecdysone, or makisterone A. In view that the mean quantity of blood ingested by a female is about an half ml, only 15 to 20 ng of ingested 22,25-DDE provoked supermolting in every female. In fact, this minute quantity represents a physiological dose seeing that about 10 ng of 20-hydroxyecdysone are present in the fifth instar nymphs at the moment of the ES peak which probably initiates the cuticle synthesis (GERMOND et al. 1982). We must note that it is the first time that a such minute quantity of ingested ES is reported to be efficient on supermolting of ticks.

The small quantity of 22,25-DDE induces only one supernumerary molting cycle, while the larger quantity of the other ES may provoke several consecutive molting cycles without any additional blood meals. *O. moubata* possesses a blocked midgut which does not communicate with the hindgut. Even during molting, the midgut content is not evacuated (ENIGK and GRITTNER 1952). Thus, part of the large quantity of natural ES ingested probably remains in the intestine and diffuses slowly over a long time period into the haemocoel to provoke several molting cycles. However, in the absence of a blood meal the animals died, presumably from starvation, most often after 2 supermolts, and sometimes after 3.

In addition, the lethal quantities of ingested ES are higher for natural ES (10 to 20 µg per female) than for the synthetic 22,25-DDE (about 2.5 µg per female). Thus the female tick *O. moubata* appears to be very resistant to exogenous natural ES.

The sensitivity seems to vary also according to the mode of application of exogenous ES. *O. moubata* is very sensitive to low quantities of ingested 22,25-DDE while it is unaffected by topical application of quantities corresponding to lethal ingested doses.

Differences of sensitivity to various ES have been previously reported in other argasid ticks. Female *O. porcinus porcinus* can supermolt after it has ingested blood containing 2 to 4 µg 20-hydroxyecdysone or ponasterone A/ml (MANGO et al. 1976). However, 16 mg 2-deoxyecdysone/ml are necessary to induce the same effect without mortality (SOLOMON et al. 1982).

Great differences in the sensitivities of several argasid species to supermolt induction by exogenous ES do also exist. Some are very sensitive, as e.g. female *Argas arboreus* in which topical application of only 1 µg 20-hydroxyecdysone, inokosterone, or cyasterone provoked supermolting (AHMED and BASSAL 1982). *A. persicus* and *O. tholozani* were much less sensitive to ingested ES than *O. p. porcinus* (MANGO, 1979). On the other hand, *A. japonicus* failed to supermolt when ES were applied by dipping, continuous substrate contact, or incorporation in the blood meal (KITAOKA 1972). KITAOKA (1972) reported that in *O. moubata* 8 µg inokosterone or 20-hydroxyecdysone per ml blood were sufficient to provoke 100 % supermolting in the females, and 1 µg ponasterone A per ml, 50 %. In contrast, our results showed that a minimum of 10 µg/ml (makisterone A) or 20 µg (ecdysone or 20-hydroxyecdysone) was required to induce an adult molt. However, in view of the wide range of minimum concentrations of ES inducing supermolting in the different species,

we cannot exclude the fact that we may have a different strain of *O. moubata*, or even a different species. Indeed, the taxonomic status of the *Ornithodoros* group was only recently elucidated in detail (WALTON 1979).

4.2 Effects of ingested ecdysteroids on oogenesis

During the molting cycle induced by ingested ES, the ovaries of mated engorged females remain in a precocious stage of oogenesis (previtellogenesis). In the case of 22,25-DDE, incorporation of the vitellus begins only after ecdysis. The female oviposits 10 days after the molt, which is within a normal preoviposition time of nontreated females, indicating that the 2 processes of molting and oocyte maturation cannot coexist. 22,25-DDE only delays vitellogenesis until ecdysis, but does not prevent it. In the case of small doses, we can imagine that no trace of 22,25-DDE remains in the animal after the molting cycle because total inactivation or degradation of the product has probably taken place. Then, a normal oogenesis can occur. However, with increasing dosages of 22,25-DDE, there is a decrease in the number of eggs laid. In these cases, inactivation or elimination of the product or of its metabolites may not be completed, and the persistance of minute quantities during the vitellogenic cycle could decrease the egg yield. This effect of 22,25-DDE on oogenesis has been reported in insects (ROBBINS et al. 1968; EARLE et al. 1970; KAPLANIS et al. 1972).

The highest doses of the natural ES tested could also delay the beginning of vitellogenesis. However, in few cases, when several molting cycles are induced, the vitellogenesis is completely prevented. In *O. p. porcinus*, SOLOMON et al. (1982) reported also disturbances of vitellogenesis by ES. However, in this species the yolk deposition could begin and was arrested during the supermolt cycle, then the vitellus was resorbed. In *O. moubata* (species uncertain) KITAOKA (1972) showed an inverse relation between the percentage of supermolting and ovipositing females in function of the dose ingested.

This inhibitory effect of high dosages of ES on vitellogenesis has been also reported in insects. In female *Acheta domestica* injected with 10 µg 20-hydroxyecdysone (CHUDAKOVA et al. 1982) or in female *Oncopeltus fasciatus* injected with 3.3 µg makisterone A (ALDRICH et al. 1981), oogenesis is arrested.

In conclusion, it appears that small doses of ingested synthetic 22,25-DDE have profound effects in the argasid female *O. moubata* (Murray 1877 sensu Walton 1962). This compound provoked disturbance of vitellogenesis; in addition, it induced supermolting with atrophy of mouth parts which prevents the females from taking a new blood meal and from maturing an additional set of eggs. The lethal dose is also very low (5 ppm in the blood). This great efficiency may permit us to envisage a possible future use of this compound or related synthetic ES in the perspective of tick control by a systemic method.

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Zusammenfassung

Wirkungen exogener Ecdysteroide auf die Weibchen der Zecke Ornithodoros moubata: Auslösung einer Superhäutung und Einfluß auf die Oogenese

Die Aufnahme von 22,25-DDE mit der Blutmahlzeit bewirkte eine Superhäutung bei den begatteten Weibchen von *O. moubata*. Dosen von weniger als 35 ng/ml Blut führten zu der Häutung bei allen Zecken etwa 10 Tage nach der Nahrungsaufnahme, was der normalen Zeit einer Nymphenhäutung entspricht. Nach der Superhäutung legten die Weibchen Eier ab. Zunehmende Dosen des Wirkstoffs reduzierten die Zahl der Eier. Eine Dosis von 5 µg erwies sich als letal.

Andere mit der Nahrung aufgenommene Ecdysteroide: Ecdyson, 20-Hydroxyecdyson, Makisteron A oder Ponasteron A lösten ebenfalls eine Superhäutung aus, jedoch höchstens bei 50 % der Weibchen. Jedoch waren die hierfür benötigten Dosen 500× größer als jene von 22,25-DDE. Die letalen Dosen betrugen bei Makisteron A 20 µg/ml sowie bei Ecdyson und 20-Hydroxyecdyson 40 µg/ml.

Bei allen getesteten Ecdysteroiden zeigten die supergehäuteten Weibchen Deformationen des distalen Teils der Beine sowie der Mundteile.

Die topikale Applikation jener Mengen der zuletzt genannten 3 Wirkstoffe, die der letalen Dosis über die Nahrung entsprach, erwies sich als völlig unwirksam. Lediglich Makisteron A in einer Dosis von 20 µg pro Weibchen zeigte leichte Wirkung. Im Gegensatz dazu war eine physiologische Dosis über die Nahrung von 10–20 ng 22,25-DDE pro Weibchen wirksam. Es ist erstaunlich, daß die Weibchen dieser Verbindung gegenüber so empfindlich sind, verglichen mit den anderen Ecdysteroiden.

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