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Significance of critical developmental stage on starvation induced endocrine mediated precocious metamorphosis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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Abstract. Final (3rd) instar larvae of the coconut rhinoceros beetle *Oryctes rhinoceros* were found to undergo precocious metamorphosis when subjected to starvation; the beetles produced as a result were subnormal in size. However, the larva showed this kind of response to starvation only after attaining a certain critical stage of development; otherwise they died after surviving for a varying period, depending on their age. Topical application of the juvenile hormone analogue 'Kinoprene' (ZR 777) in sufficient doses at definite periods caused significant delay in the onset of precocious metamorphosis thus suggesting an endocrine involvement. Evidently, starvation might have caused a decrease in JH-titre which in its turn, triggered a chain of events leading to precocious metamorphosis.

Keywords. Coconut rhinoceros beetle; *Oryctes rhinoceros;* starvation; precocious metamorphosis; critical developmental stage.

1. Introduction

Oryctes rhinoceros (Coleoptera: Scarabaeidae) is a serious pest on coconut palm in the palm-growing belt throughout the world. During our studies involving starvation of the 3rd (final) instar field-collected larvae of this animal, we noted two types of response from the larvae to starvation: (i) some of them died without entering into pupation whereas others (ii) pupated after certain number of days of starvation. As the causes involved in this differential response are significant from the point of view of control of this pest, we conducted a detailed critical study of the various factors involved in this differential response of the larvae to starvation.

2. Materials and methods

For preliminary experiment (expt I) final (3rd) instar larva with head capsule width of 10–11 mm (Nirula 1955) were collected from field and introduced on (i) cowdung, (ii) fine sand, washed repeatedly with water to remove any nutrients and (iii) mixture of cowdung and sand in 1:1 ratio by volume, 10 larvae in each group. The medium was kept moist by periodical watering. Accumulating faecal pellets were removed and medium changed for fresh one if necessary. The larvae were introduced singly in separate plastic containers (10·5 cm high and 9 cm diameter) with lid which had holes drilled on them, filled one third the volume with the medium. The timing of various developmental events viz the onset of transition into prepupa, duration of prepupal and pupal stages were noted for all the larvae by daily observations after moving the superficial layers of the rearing media, causing least disturbances to the individuals. When cocoon was formed the observations were made after carefully breaking off a part of the cocoon.

For the following experiments, 3rd instar larvae of known age groups were obtained from a stock of '0' day old 3rd instar larvae, raised in the laboratory from field collected second instar larvae (head capsule width 5 mm) and maintained on cowdung.

To find out if the age of the larva had any effect on successful metamorphosis in a sand-alone medium, larvae of different age groups (15-days, 35-days and 40-days after moulting) were introduced on sand as above (expt II).

For the expt III, 48-day old larvae obtained at a time were divided into 3 groups and each group was then introduced into any one of the following media: (i) cowdung, (ii) riversand and (iii) dung:sand mixture (1:1 ratio by volume). Various developmental events upto adult emergence as well as the weight of the adults were recorded. This experiment was replicated so that 20 larvae were studied in each medium.

During the IVth expt, development of the 48-day old larvae in two media devoid of any food substance, a medium formed of the faecal pellets collected from the rearing containers and a medium of spongy rubber bits was observed after keeping a group of larvae in a medium of cowdung for control. All the starved larvae which pupated were allowed to emerge and the adults so emerged were studied.

A 1 µg/µl solution of 'Kinoprene' (ZR 777) (propyonyl 2e, 4e–3, 7, 11–trimethyl 2, 4–dodeca dienoate) in acetone was used for topical application. The solution/acetone was applied on the thoracic region of the larvae (without anaesthesia) at different stages of starvation, obtained by introducing 48-day old 3rd instar larvae in sand. The experiments were performed in the following manner.

Expt I: 50 µg hormone analogue applied on 48 day old larvae immediately before the initiation of starvation.

Expt II: 50 µg hormone analogue applied on the 8th day of starvation.

Expt III: 50 µg hormone analogue applied on the 8th and 12th days each of starvation (i.e. total 100 µg).

Expt IV: 50 µg hormone analogue applied on the 5th, 7th, 9th, 11th and 13th days each of starvation (i.e. total 250 µg).

For each experiment, a group of larvae subjected to an identical treatment with acetone and another group of normal control (untreated) larvae were maintained. After each treatment, the larvae were returned to the respective medium. Development of all the larvae was followed and recorded.

3. Results and discussion

3.1 *Effect of various media on pupation of 3rd instar larva of unknown age*

Results of this experiment are shown in table 1. The larvae introduced on cowdung and dung-sand mixture showed a high variability in the timing of onset of metamorphosis (within $23\cdot9 \pm 16\cdot6$ and $24\cdot7 \pm 15\cdot0$ days, respectively). However, the larvae introduced on sand alone medium were found to show two kinds of response—50% of the larvae died within $21\cdot8 \pm 3\cdot5$ days without showing any signs of metamorphosis, while the others metamorphosed successfully within $12\cdot6 \pm 5\cdot9$ days. The duration of prepupal and pupal stages were similar for the 3 groups of larvae.

Table 1. The effect of different rearing media upon *O. rhinoceros* larvae of different age groups.

Expt. No.	Age of the larva (days)	Rearing medium	Mortality (%)	Time taken for initiation of metamorphosis after the beginning of experiment (days)	Duration of prepupal stage	Duration of pupal stage
		Cowdung	0	23·9 ± 16·6	9·6 ± 1·1	19·2 ± 0·9
I	Unknown	Sand	50	12·6 ± 5·9	9·2 ± 1·1	19·4 ± 1·0
		Dungsand mixture	0	24·7 ± 15·0	9·5 ± 1·4	19·3 ± 0·9
	15		100	—	—	—
II	35	Sand	100	—	—	—
	40		50	28·0 ± 2·0	11·5 ± 0·5	18·5 ± 0·5
		Cowdung	—	41·2 ± 7·3	9·8 ± 1·5	18·4 ± 0·7
		Sand	—	9·1 ± 1·5	10·4 ± 1·6	18·6 ± 1·2
III	48	Dungsand mixture	—	44·6 ± 6·4	10·0 ± 1·4	18·5 ± 0·7
		Faecal pellets	—	9·2 ± 0·8	10·0 ± 1·10	18·4 ± 0·5
IV	48	Spongy rubber	—	9·6 ± 1·0	10·0 ± 1·4	18·8 ± 0·8
		Cowdung	—	41·0 ± 4·5	9·4 ± 1·0	17·8 ± 1·3

3.2 *Effect of starvation on 3rd instar larvae of known age*

The high variability observed in the initiation of metamorphosis among the larvae introduced on the sand alone medium could be attributed to the fact that the field collected larvae were heterogeneous group of individuals of different age groups. Those that passed a critical age apparently showed a tendency to become prepupa within a few days while those which did not pass that critical age began metamorphosis only after attaining a stage of maximum growth possible for them on the medium on which they were introduced.

Some of the larvae introduced on sand alone medium died without undergoing metamorphosis while the others could metamorphose successfully, led to the assumption that there existed a certain critical stage of development for the larvae to become competent to metamorphose, those which had not reached this stage could not metamorphose and hence they died after surviving for a few days. Fifteen day old or even 35-day old larvae were found to be incompetent for metamorphosis when starved; when introduced on sand, they died after a survival for 13·3 ± 1·1 and 19·3 ± 3·6 days, respectively. Older the larvae, when they were subjected to starvation, the greater was the duration for which they survived; if the larvae were 40-day old when starvation started there was 50% mortality (table 1). Hence, it was evident that the final instar larvae may reach the critical stage of development just after the age of 40 days. However, 48-day old larvae were selected for all subsequent experiments, since they seem to be well beyond the critical stage, and all of them pupated when subjected to starvation.

3.3 *Effect of various media on pupation of 48-day old 3rd instar*

The 48-day old larvae reared on cowdung, initiated metamorphosis within $41{\cdot}2 \pm 7{\cdot}3$ days thereafter, those on river sand within $9{\cdot}1 \pm 1{\cdot}5$ days and those on dung sand mixture within $44{\cdot}6 \pm 6{\cdot}4$ days after beginning of the experiment. In other words, the larvae introduced on the sand alone medium were found to reveal a precocious onset of metamorphosis quite early. However, no significant change was observed in the duration of metamorphosis among these 3 groups of larvae (table 1). The prepupae, pupae and adult beetles produced as a result of precocious metamorphosis were much reduced in size. Whereas the controls weighed 6·2 g and those reared on dung sand mixture weighed 5·6 g, those reared on sand alone medium weighed only 3·7 g. The original larvae weighed 10·4–10·9 g.

The phenomenon of precocious pupation, induced by a sand-alone medium, could be regarded either as a response to the nature of the medium or as due to the effect of starvation induced by this medium, since it contained no organic matter that could be utilized as food. However, the larvae introduced on dung-sand mixture did not show precocious metamorphosis. Hence, it appeared that it was starvation and not the nature of the medium that induced precocious metamorphosis when the larvae were introduced in sand only. This was supported by the fact that two other media devoid of any food substance, a medium consisting of faecal pellets of the larvae collected from the rearing containers and a medium consisting of spongy rubber bits taken from worn-out foam cushions also could induce precocious metamorphosis when the larvae were introduced on them (table 1).

It may be noted that Nirula (1955) also pointed out the significance of food supply on development of *Oryctes rhinoceros* larva. However, he observed that the larval development was limited if food supply was exhausted before the end of the larval period. According to him, the two month old larvae remained alive for 14–20 days without any food, and continued larval life when food was available subsequently. He does not mention of starvation inducing pupation in the larvae. Catley (1969) also maintains that unfavourable climatic or nutritional conditions delay larval development which may be extended upto 14 months smaller than average size bettles being produced thereby. This may be correct when starvation is only partial, and when at least some nutrition is available to the animal. Present studies however clearly show the existence of a critical period in the life history of 3rd instar which is extremely important from the standpoint of survival during complete starvation. If the animal is subjected to complete starvation before the larva attains this age (40–48 days) the larva dies without pupating; after this stage, the larva will enter pupation about 9-days after starvation is induced, resulting in smaller adults ultimately. This is of paramount importance for the insect to tide over unfavourable condition imposed by starvation. It may thus be noted that the 3rd instar larvae enter pupation by about 57 days, whereas normally the control larvae take about 89 days, thereby cutting short more than 30 days of life history so that at least some of the animals can survive to reproduce, though the younger ones will succumb to adverse condition.

A precocious decline in JH-titre appeared to be the reason for the transformation of the starved larva into a precocious pupa. Application of a single dose of 50 μg of 'Kinoprene' (ZR 777) immediately before initiation of starvation did not produce any significant effect either upon the onset or duration of metamorphosis (table 2) or

Table 2. The effect of topical application of the JHA ZR 777 upon 48-day old third instar larva at different stages of starvation.

Expt. No.	Dosage and timing of application of JHA	Treatment	Time taken for the onset of metamorphosis (days)	Duration of prepupal stage (days)	Duration of pupal stage (days)
I	50 μg immediately before initiation of starvation	Hormone treated larva	10·7 ± 0·9	9·0 ± 0·8	18·7 ± 1·3
		Acetone treated control larva	11·0 ± 0·8	9·0 ± 0·8	18·0 ± 0·0
		Normal larva	10·3 ± 0·5	8·7 ± 0·9	18·7 ± 0·9
II	50 μg on the 8th day of starvation	Hormone treated larva	12·3 ± 1·3	8·3 ± 0·5	18·7 ± 1·7
		Acetone treated control larva	10·7 ± 0·9	9·0 ± 0·8	18·3 ± 0·5
		Normal larva	11·0 ± 0·8	8·7 ± 0·5	18·7 ± 0·9
III	50 μg each on the 8th and 12th days of starvation	Hormone treated larva	13·0 ± 3·3	10·0 ± 1·6	19·7 ± 1·3
		Acetone treated control larva	9·7 ± 2·1	9·7 ± 0·5	18·3 ± 0·5
		Normal larva	10·7 ± 1·3	9·0 ± 1·4	18·7 ± 0·9
IV	50 μg each on the 5th, 7th, 9th, 11th and 13th days of starvation	Hormone treated larva	17·3 ± 0·9	9·3 ± 0·9	18·7 ± 0·5
		Acetone treated control larva	10·7 ± 0·9	9·7 ± 1·3	20·0 ± 1·6
		Normal larva	9·7 ± 0·9	10·7 ± 0·9	19·0 ± 1·4

in the morphological characteristics of the prepupae, the pupae or adult beetles, when compared to the acetone-treated and normal control larvae. This ineffectiveness of the single dose of 50 μg of ZR 777 might be because exogenous hormone could not persist in the body until the endogenous JH-titre decreased due to starvation. Part of the analogue might have also been lost during movement of the larvae through the medium. When 50 μg of ZR 777 was applied to the larvae on 8th day of starvation also, no significant effect on metamorphosis was revealed (table 2). Apparently because by the time the JHA was applied, the larval pupal transition already switched on. Hence it appeared that a significant effect on metamorphosis could be expected if the JHA was applied in sufficient dose at that critical stage, after the JH has declined and before the metamorphic changes have switched on. When 50 μg was applied each on the 8th and 12th days of starvation, a single individual showed a significant delay in the onset of metamorphosis; it revealed the signs of transition into prepupa only on the 17th day of starvation while the acetone-treated and normal control larvae showed this transition within 9·7 ± 2·1 days and 10·7 ± 1·3 days, respectively. However, the remaining hormone treated larvae did not show any effect of the hormone. But when the JHA was applied repeatedly for 5 alternate days from the 5th day of starvation, 50 μg at a time, all the larvae showed significant delay in the onset of metamorphosis (table 2). Presumably, repeated application of ZR 777 at short intervals, from a fairly earlier stage of starvation, ensured the presence of this JHA at the critical stage of JH-decline in all the larvae in spite of any individual variation that might have existed in the timing of this decline. Also, repeated application might have produced a prolonged and possibly a cumulative effect. However, all the larvae began to metamorphose within a few days (3–5 days) after receiving the last dose. This indicated that the effect of 50 μg of ZR 777 could last only for a short duration.

Although the initiation of metamorphosis was delayed by the JHA, it could not affect the duration of metamorphosis; also, it did not produce any apparent abnormalities in development. These observations are in agreement with the view that in many species of Coleoptera, the usual effect of JHAs on the last larval instar is characterized by a delay of pupation; due to special hormonal condition controlling larval-pupal transformation in these insects, development in the last larval instars appears to be suspended for as long as the JH remains in the body. Such hormonal condition prevent the juvenoids from causing disturbance of metamorphosis in these species (Slama *et al* 1974).

It was thus revealed that in *O. rhinoceros*, metamorphosis could not ensue as long as the JH-titre remained high. Precocious pupation observed in the starved larvae might have hence been due to a decline in JH-titre. In otherwords, starvation appeared to induce a decline in JH-titre in these larvae.

Starvation was reported to cause a rise in concentration of JH in the blood of 5th instar *Manduca sexta* larvae (Bhaskaran and Jones 1980; Nijhout 1975) producing supernumerary larvae. However, in *Oncopeltus fasciatus* starvation caused a reduction in JH-titre in the haemolymph (Rankin and Riddiford 1977). A similar mechanism might have been in operation in the starved larvae of *O. rhinoceros*. Though starvation-induced precocious metamorphosis is extremely rare in insects, there is a striking similarity of the present findings on *O. rhinoceros* with those of D'Angalo *et al* (1941) on tadpole. They found that the stage of the tadpole at the time of food withdrawal was critical for metamorphosis. Starvation after a certain critical stage accelerated metamorphosis in tadpole, but before that stage, it caused a delay in metamorphosis.

The existence of a certain critical stage for onset of metamorphosis may be explained as due to the fact that most of the organs can achieve differentiation in response to hormones only after attaining a certain stage of development (Wigglesworth 1954). A threshold size for metamorphosis has been reported in the tobacco hornworm (Nijhout 1975) in which however, starvation of the mid 4th instar larvae produced undersized 5th instar larvae which then underwent one or more extra larval moults. Also, 5th instar larvae (2 days after the moult to 5th instar) starved at body weight of less than 4 g, underwent delayed moulting into larval-pupal intermediates (Nijhout and Williams 1974).

That starvation can evoke precocious metamorphosis may be of adaptive significance to the animal in that it enables the larvae to escape from conditions of severe food shortage by transforming itself into the adult, with an entirely different food preference.

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