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SHORT- AND LONG-TERM EFFECTS OF AZADIRACHTIN A ON DEVELOPMENT AND EGG PRODUCTION OF *RHODNIUS PROLIXUS*

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Azadirachtin A was given through a blood meal to 4th-instar larvae and to adult females of Rhodnius prolixus. *Development (ecdysis) and egg production were inhibited in a dose-dependent manner. Long-term experiments with subsequent four feedings on azadirachtin-free blood were performed with 4th-instar larvae and with adult females. Only in the low-dose azadirachtin larval groups (0.01 and 0.1 µg/ml of blood), development was partially restored; after a single 1.0 µg/ml treatment about 50% of the treated larvae were still alive 120 days later without any adult emergence. Similarly fed females had a dose-dependent lower survival and egg deposition rate. The results are discussed in relation to the mode of azadirachtin A action.*

Key words: azadirachtin – *Rhodnius prolixus* – development – egg production

Azadirachtin, a tetranortriterpenoid from the neem tree, *Azadirachta indica* A. Juss, is an insect growth inhibitor and therefore it plays an important role in the inhibition of insect development and reproduction (Rembold & Sieber, 1981; Garcia & Rembold, 1984; Garcia et al., 1984b; 1986; Rembold, 1984; 1987; 1989; Dorn et al., 1986). These effects are mainly due to interference with hemolymph ecdysteroid (Sieber & Rembold, 1983; Garcia et al., 1986, 1987; Rembold et al., 1987) and juvenile hormone (Rembold, 1984, 1987) titers.

In *Rhodnius prolixus* larvae, low doses of azadirachtin, given orally or by injection, cause moulting arresting and ecdysial stasis (Garcia & Rembold, 1984; Garcia et al., 1984b, 1986, 1987). These effects can be rescue by simultaneous administration of ecdysone (Garcia & Rembold, 1984). In adult females of *R. prolixus* the treatment with azadirachtin causes a reduction of vitellogenin in the hemolymph and of vitellin in the ovaries (Feder et al., 1988).

In insects, the overall picture about the effect of azadirachtin is far from complete. Usually, it is derived from short-term experiments. A profound understanding of azadirachtin mode of action on insects requires more information on long-term effects of this compound on the development and reproduction.

Therefore, the present study describes the long persistence of azadirachtin effects on these two biological parameters in *R. prolixus*. Accordingly, we also present data showing that permanent larvae, as induced by azadirachtin, are led to moulting by ecdysone ingestion after several weeks of the treatment.

MATERIALS AND METHODS

Insects – Fourth-instar larvae and adult females of *R. prolixus* were reared and maintained as described elsewhere (Garcia et al., 1975, 1984a). Virgin females were mated 1-2 days before the first feeding as adults. Groups of at least 25 insects, which had been starved for 15-20 days, were allowed to feed on citrated human blood through a membrane feeder (Garcia et al., 1984a).

Azadirachtin A and ecdysone treatments – Azadirachtin A (purified by one of us, HB) and ecdysone (Sigma Chemical Co.) were diluted in 1:4 ethanol-saline, and added to the meal as described previously (Garcia & Rembold, 1984) for oral treatment.

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Short- and long-term experiments – We first checked the data of the effect of azadirachtin A in short-term experiments, i. e., we observed the moulting and egg deposition until 30 days after the treatment. In this case we only considered the average of moulting and egg laid. For long-term experiments we also measured these biological parameters in the second-, third- and fourth-feeding sessions with blood without azadirachtin A. The blood meals therefore were given 30, 60 and 90 days after the treatment with azadirachtin A, respectively.

RESULTS

Azadirachtin A and development – The short-term effects of azadirachtin A on ecdysial arrest was studied in the following experiment. Four groups of at least 20 insects each (A-D) were fed on either blood alone (A) or on blood containing azadirachtin A (0.01 μg/ml, B; 0.1 μg/ml, C; 1.0 μg/ml of blood, D). Table I shows that in the short-term experiment the controls (group A), during the 30 days after feeding, moulted between 14-18 days. In the groups C and D of azadirachtin-treated larvae, during the same period of time none insects underwent ecdysis. In the lower dose of azadirachtin A (group B) this period was prolonged and only 15% of the insects moulted to fifth-instar. In all groups the larvae ingested a sufficient volume of blood to moult (Table I).

In order to study the effects of azadirachtin A on the long-term experiment, we checked the insects of the groups B, C and D, after the second-, third- and fourth-feeding sessions with fresh blood without azadirachtin A, i. e., the insects after the treatment with azadirachtin A in the first blood meal, received three blood feedings without azadirachtin A. The results showed that the inhibition of moulting caused by ingestion of azadirachtin A could not be reserved in the group D, which received the higher dose of azadirachtin A, after three subsequent blood meals without it (Table II). The number of moulting in this group was zero until 120 days after the treatment. This observation was specially evident comparing these results with those observed in the group B, which received 0.01 μg azadirachtin A/ml of blood in the first blood meal. This group had several insects which underwent ecdysis after each of the three feeding without azadirachtin A. The group C, which received 0.1 μg/ml of blood in the first blood meal, present only a small number of moulting during 120 days of observations (Table II). A low number of treated insects which underwent ecdysis moulted to adult stage after a blood meal and produced eggs normally (not shown).

Permanent larvae and ecdysone treatment – To show that the insects treated with 1.0 μg azadirachtin A/ml of blood (group D) in the first meal did not present any health or intoxication complications, the group of 12 survivors (Table II, feeding session 4) was divided into two groups for another blood meal. One group was offered 5.0 μg ecdysone/ml of blood and other served as control. Four of the ecdysone fed larvae moulted into the next fifth-instar whereas none of the controls underwent any moult.

Azadirachtin A and egg production – Four groups of at least 25 adult females (E-H) received different doses of azadirachtin A in the first feeding-session as adults. The doses (μg/ml of blood) were: 0.1 μg (F), 1.0 μg (G), 5.0 μg (H); a control group (E) received blood with the solvent only. Only insects that ingested at least 2.5 times their body weight were used. Egg deposition after the first blood meal is shown in Table III. Azadirachtin A drastically decreased egg production in *Rhodnius* at the 1.0 μg/ml and 5.0 μg/ml of blood (groups G and H). For the period of 30 days after feeding, the control females attained an averade of 27 eggs, whileas in the azadirachtin A treated groups at the higher doses only 8.5 eggs or even less were produced (Table III). The group treated with 0.1/μg azadirachtin A/ml of blood (group F) did not differ significantly from the control (group E).

The results obtained from long-term experiment are illustrated in Table III. The azadirachtin A effect on egg production was still presented at the group H, which received the higher dose of the compound in the first blood meal, by the third fresh blood meal without azadirachtin A. The group G (treated with 1.0 μg/ml in the first meal) presented only partial reversion in the egg deposition after 120 days of the treatment (Table III). The group F had deposited eggs in the same rate if compared with the controls.

The eggs laid by the experimental groups F and G were not affected in their viability; however, eggs from the group H presented only 45% of eclodibility.

TABLE I

Short-term experiments on the effects of azadirachtin A, on the intermoulting period and ecdysial arrest of 4th-instar larvae of *Rhodnius prolixus*. Groups of at least 20 insects each

Groups	Azadirachtin A μg/ml	Blood ingested mg	Intermoulting period range in days	% moult[a]
A	–	93.7 ± 8.3[b]	14 – 18	100
B	0.01	94.2 ± 6.3	25 – 30	15
C	0.1	91.4 ± 5.6	–	0
D	1.0	94.1 ± 7.7	–	0

[a] The experiment terminated 30 days after feeding.
[b] Mean ± SE.

TABLE II

Long-term effects of azadirachtin A on morphogenesis of 4th-instar *Rhodnius prolixus* larvae

Feeding session (30 d)	Days after treatment	4th-instar larvae (n) at end of session				Moults/deads in respective sessions			
0	0	25	29	27	23	0	0/0	0/0	0/0
1	30	23	27	25	21	22/1	5/1	0/5	0/3
2	60	0	21	20	18	0	5/1	2/1	0/4
3	90	0	15	17	14	0	3/2	1/1	0/2
4	120	0	10	15	12	0	6/0	3/0	0/2
aza A (μg/ml)		0	0.01	0.1	1.0	0	0.01	0.1	1.0

DISCUSSION

The results presented in this paper clearly indicate that azadirachtin A is an effective growth inhibitor for the bloodsucking bug *R. prolixus*. The inhibition of either development or egg laying is dose dependent and only partially reversible in the lower doses used by three subsequent fresh blood meals without azadirachtin A (Tables II and III). In short-term experiments the compound in all three concentrations drastically inhibited the ecdysis. Egg laying was also affected by the two higher doses of the compound (Table III). These results are in total agreement with previous reports from our laboratory (Garcia & Rembold, 1984; Garcia et al., 1984b; Feder et al., 1988) which showed that azadirachtin interferes in both biological events by extensive changes in the endocrine control of the insect's growth and egg formation.

Also, in this paper for the first time, the long persistence of the effect of azadirachtin A on development and reproduction is well proven. Azadirachtin A, for example, in the higher concentration used, decreases or even abolishes the moulting and egg deposition during at least 120 days after its application (Tables II and III). Two hypotheses for an explanation of this long-term effect of azadirachtin A can be discussed. First, azadirachtin A induces permanent changes in the endocrine regulation of these biological parameters. In this respect, it is now well know that *Rhodnius*, such as *Locusta migratoria* (Rembold et al., 1988), rapidly excretes dihydroazadirachtin A, but a constant quantity of this compound was recovered from the head and visceral structures some weeks after its application (Garcia et al., 1989). It is therefore possible that some minute concentration of azadirachtin A remains bound in the endocrine tissues switching off neuro-endocrine stimulation and/or the endocrine organs persisting there for a long time. The absence of active prothoracic glands or ovaries which produce ecdysteroids in larvae and adult females of *R. prolixus*, respectively, should also explain these results. In support for this hypothesis, it was shown that prothoracic

TABLE III

Number of eggs deposited by adult females of *Rhodnius prolixus* in short- and long-term experiments. The insects received azadirachtin A in different doses in the first bloodmeal (feeding session 1). The egg deposition was observed each 30 days after each feeding

30 days session	µg azadirachtin A ml blood	Days after treatment	Females at end of session	eggs/female/deads
0	0	0	26	0
	0.1		27	0
	1.0		27	0
	5.0		29	0
1	0	30	26	27.0/0
	0.1		26	23.5/1
	1.0		25	8.5/2
	5.0		26	5.5/3
2	0	60	26	23.5/0
	0.1		22	25.2/4
	1.0		23	10.5/2
	5.0		22	5.5/4
3	0	90	23	18.9/3
	0.1		18	19.2/4
	1.0		20	13.5/3
	5.0		18	6.5/4
4	0	120	19	19.7/4
	0.1		15	18.1/3
	1.0		17	12.8/3
	5.0		14	7.5/5

glands and ovaries produced only small amounts of ecdysteroids *in vitro* followed azadirachtin A treatment of *R. prolixus* (Garcia et al., 1987). A decrease in ecdysteroid synthesis was observed even when normal prothoracic glands and ovaries were incubated *in vitro* with azadirachtin A (Garcia et al., 1987; Feder et al., 1988). It is also known that juvenile hormone controls vitellogenesis in *R. prolixus* (Davey, 1980). Since azadirachtin depresses the production of juvenile hormone in *L. migratoria* (Rembold, 1984), we could imagine that in *Rhodnius* the same occurred. However, juvenile hormone 0-III could not be detected in *R. prolixus* (Feder et al., 1988) even when using an extremely sensitive microanalytical technique as described by Rembold & Lackner (1985).

Secondly, an intoxication of the animals by azadirachtin A could be discussed on the basis of our results. Our own data, however, showed only low effect of the compound on mortality (Tables II and III). Furthermore, ecdysone application in permanent larvae, as induced by the higher dose of azadirachtin A treatment, led the insects to moult. This finding demonstrates that the insects had only a hormonal deficiency even 120 days after the treatment. Consequently, any toxic effects of the compound can be disregarded for explaining the long-term effect in *R. prolixus*.

Studies to elucidate the persistent effect of azadirachtin A are being developed in our laboratories.

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