Precocene II is no Anti-Juvenile Hormone in the Honey Bee, *Apis mellifera*

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The effect of precocene II on development of the honey bee, *Apis mellifera*, was studied *in vitro*. One- to two-day-old worker larvae (body weight 0.5 – 1.0 mg) were removed from the colonies, reared on royal jelly-yeast extract, and after 24 h were topically applied with different amounts (5 – 75 µg/larva) of precocene II. Toxicity was observed only with precocene doses of 50 µg/larva and more. The larval weight-gains declined with the increase of doses. The acetone-treated control had better survival and weight-gain as compared to the no-treatment control. The larval and pupal periods in the treated larvae remained unchanged as compared to the controls. The possibility of precocene acting as an antifeedant is discussed.

Ageratochromes were isolated from *Ageratum* plants in 1955 [1] and were subsequently synthesized [2]. These were renamed as "precocenes" as they induced precocious metamorphosis in immature hemipterans and prevented ovarian development in some adult insects [3, 4]. Since these effects were fully reversible by juvenile hormone (JH) application to precocene-treated *Oncopeltus fasciatus*, they have been called anti-juvenile hormones [3, 4]. Of the two related compounds, 6,7-dimethoxy-2,2-dimethylchromene (Precocene II) was more active than the corresponding 7-methoxy compound (Precocene I). Precocene II inhibited JH biosynthesis by cockroach corpora allata (CA) *in vitro* [5], and caused atrophy of CA in *Locusta migratoria* [6]; it was even called a "chemical allatectomizer" for *Apis mellifera* [7].

However, Slama [8] concluded that prothetelies in precocene treated seed-feeding hemipterans were due to its antifeeding action as prothetelic specimens always appeared among the larvae with most inhibited growth, close to lethality. Kelly and Fuchs [9] also proposed that precocene does not act as an antigonadotropic agent in adult *Aedes aegypti*, but induces a general toxic state in the treated animals. To test the possible action of precocene II as a "chemical allatectomizer" [7], we investigated its effect on development of the honey bee larva *in vitro*. Our results demonstrate that precocene is toxic at higher concentrations and causes decreased larval weight-gains, possibly through antifeeding action at lower concentration.

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Materials and Methods

One- to two-day-old worker larvae (body weight 0.5 – 1.0 mg) were taken from bee flight room colonies of *A. mellifera* for *in vitro* tests and were reared on royal jelly-yeast extract as described earlier [10]. Twenty-four hours after removal from the colony, the larvae (body weight approximately 8 mg) were topically applied with 1 µl acetone containing 5 – 75 µg of precocene II (a generous gift from Zoecon Corp.). Two controls were kept, one with acetone (1 µl) and the other without any treatment. Every experiment consisted of three replicates, each with at least 35 individuals.

Results and Discussion

Figure 1 gives the percentage of larval survival after precocene II treatment, recorded daily till the fifth larval instar. Lethality becomes progressively higher with increased dose. However, there is not much difference in the doses from 5 to 25 µg/larva (86.6 – 82.8% survival to L 5); toxicity is only high at 50 and 75 µg precocene. Maximum survival to pupal and adult stages, of all the tests, is observed with dose of 5 µg/larva (64.7 and 58.0%; Table I). As also observed in earlier experiments [11], acetone treatment results in higher increase of larval weight as compared to no-treatment (741 vs. 706%).

There is a progressive decline in larval weight-gains as the dose is increased, except in the 10 µg treatment. Pupal mortality was not high in controls as well as in treatments; no morphological abnormalities were seen in the dead pupae. We did not observe

Table I. Effect of precocene II on the development of honey bee larvae

Treatment (per larva)	Number of larvae *	Percentage survival to			Larval weight-gain *** %
		L5 **	Pupa	Adult	
None	105	92.3	57.1	51.4	706
Acetone	105	90.4	60.0	55.2	741
5 µg Precocene II	105	86.6	64.7	58.0	638
10 µg Precocene II	105	85.7	57.1	56.1	688
25 µg Precocene II	105	82.8	52.3	45.7	629
50 µg Precocene II	107	59.8	19.6	16.8	597
75 µg Precocene II	105	54.2	20.0	20.0	563

* Approximate weight 8 mg.
** Fifth larval instar.
*** Increase in 48 h after treatment.

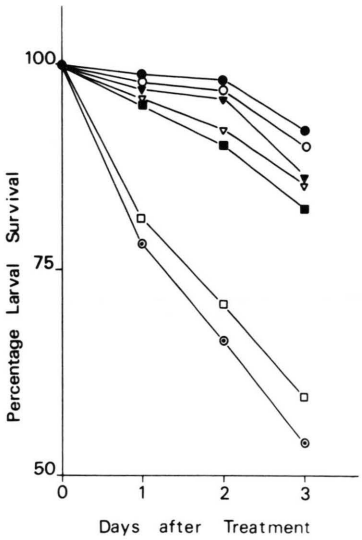

Fig. 1. Percentage of honey bee larval survival after precocene II treatment, daily recorded till fifth larval instar. No treatment (●); 1 µl acetone/larva (○); precocene II treatment/larva, 5 µg (▼), 10 µg (▽), 25 µg (■), 50 µg (□), 75 µg (⊙).

any change in the duration of larval and pupal periods in precocene treated larvae as compared to the controls.

With only few exceptions, all the compounds which showed antifeeding effects on larvae also delayed or suppressed the maturation of the oocytes in the ovaries of the adult females [8]. Certain antifeedants can produce developmental changes which suggest disturbance of the endocrine integrity. For example, the antifeedant azadirachtin caused growth disruption in insects, possibly by interfering with normal hormone balance [12]. The earlier reports [3, 4, 6] of precocious adult formation in precocene treated hemimetabolous insects could possibly be due to its antifeeding action. However, this hormonal disturbance was not observed in the honey bee, a holometabolous insect. The observation that precocene had no effect on the development of *Graphosoma italicum*, which is resistant to a large number of antifeeding compounds due to its adaptation for feeding on aromatic seeds of Umbelliferous plants [8], also supports that antifeeding effect is the primary mode of action of precocene. In the present study, the decreased weight-gains of the treated honey bee larvae are also indicative of antifeeding action of precocene.

Precocenes were called anti-juvenile hormones because subsequent JH treatment reversed the precocene effect. A specific anti-juvenile hormone should suppress or prevent the action of exogenous JH [8], or the simultaneous application of both to an insect should result in its normal development. However, according to Bowers [13] mixed application of precocene and JH will only show JH effects. Precocene, like in the present study, caused only a general toxic effect in *A. aegypti* [9], a holometabolous insect. However, this toxic effect was obseved in honey bee only at a very high dose (50 µg/larva, approximate larval weight 8 mg). Based on our results it can be interpreted that precocene acts as an antifeedant at lower doses and possibly through this disturbs the endocrine processes. At higher doses precocene is a general toxicant.

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