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PRECOCENES: POSSIBLE MEANS TO CONTROL THE BEE MITE, VARROA JACOBSONI (OUDEMANS)

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Abstract. Allatocidines, precocene I, precocene II and several synthetic derivatives with the similar effects were tested using the bee mite, *Varroa jacobsoni*. Presented results indicate that precocenes and their synthetic derivatives tested produce considerable effect on adult females of the bee mite. The most effective methods of administration were direct or indirect topical application and fumigation. Precocenes exhibited repellent and toxic effects on the bee mite, but precocene I was also toxic for honey bees. The synthetic allatocidines showed about 10 times higher activity than the natural ones and very low toxicity for honey-bee. A possible mode of action of these compounds on the bee mite is discussed.

The first report about the appearance of the bee mite, *Varroa jacobsoni* (Oudemans) in Europe was from Haragsim and Samšiňák (1972). Nowadays the mite badly endangers the colonies of European honey bee, because the mite is able to reproduce not only on the drone larvae as in the case of its original host, Indian bee *Apis cerana*, but also on the larvae of workers.

Most of insect endo- and ectoparasites and parasitoids feed on the haemolymph of their hosts. It is evident that the parasites respond to changes in hormonal millieu of their hosts (Beckage 1983).

Hänel (1983) observed that after application of juvenile hormone (JH III) to the bee larvae that afterward served as hosts for adult females of *Varroa jacobsoni*, the number of parasite's progeny significantly increased. It seems that the reproduction of the mite is somehow dependent on the level of juvenile hormone in the host larvae. So we assume that a decrease in the juvenile hormone level in the honey bee depresses the multiplication of the parasite. Therefore precocens seemed us to be a suitable tool to control the bee mite.

MATERIALS AND METHODS

The experiments were performed on the bee farm infested by the bee mite, *Varroa jacobsoni* (Oudemans). The farm is located in the middle of mountain forest in Northeast Slovakia, district Levoča. The bee hives were opened, the frames with honey combs and bees were taken out, the bees with attached mites were transferred into experimental cages 100 × 100 × 60 mm in diameter, made of wood. One wall of the cage was provided by glass, the opposite by the wire net. About 50 or 100 bees were placed in each cage.

Precocenes were kindly provided by prof. W. S. Bowers. The synthetic compounds were prepared according to the original Czechoslovak patent No. 252629 registered under the number 10339 since 1983.

Precocenes were applied per os in a sugar solution (60 % sucrose in water) in the doses of 0.5 μg/ml or 2.5 μg/ml and also by the method of indirect topical application: the acetone solution of precocene was poured on a petri dish, the solvent was allowed to evaporate and the dish with precocene (0.5 μg/cm²) was put in the cage with parasitized bees for 6 or 8 days. During this time the cages were incubated in the super, separated from the bee sworm by a layer of silk and felt.

More efficient methods of application of allatocidines were spraying and fumigation. The solutions for spray were prepared from distilled water and emulgators (e.g. Triton X-100 about 0.2%). Fumigation was performed with thick filter paper (like Whatman No. 3) impregnated with amonium

nitrate. The tested compounds were dissolved in acetone and the solution in appropriate dose was applied on the paper strip. After evaporation of the solvent the paper strip with some allatocidine was placed with the experimental cage in the super and the paper strip was lighted and let to smoulder slowly in the closed super. The bees with attached mites were exposed to fumes for 30 min. The experimental cages were controlled daily, the dead bees and mites were removed from the cages and counted. The living bees were examined for the presence of living mites at the end of experiment. Each experiment was repeated no less than three times. The figures and tables represent the average values from all repetitions.
All the micrographs have been made by the scanning electron microscope Tesla BS 300, anode potential 150 kV.

RESULTS

Topically applied allatocidines produced strong repellent effects. The exposed *Varroa* females abandoned their hosts and tried to escape from the treated area. After some time they died.

The methods of topical application (both direct by spraying or indirect using contaminated petri dishes) showed significant effects: precocene I caused 83 % mortality in *Varroa* females, but also 59 % mortality in the exposed bees. Precocene II caused about 80 % mortality in the mites, only 5 % in the bees in comparison with acetone controls (see Figs. 1, 2). Similar results were obtained when precocenes were applied by fumigation (see Table 1).

Fig.1.

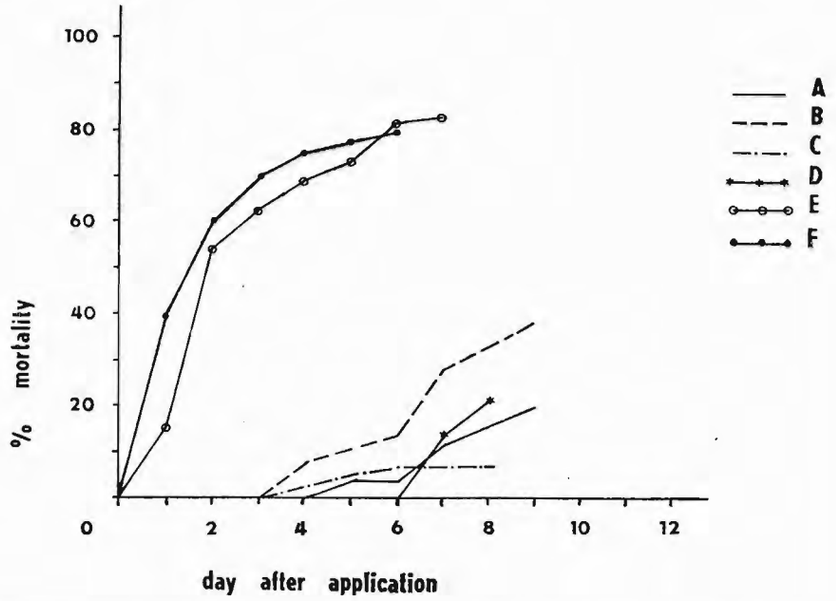

Fig. 1. Mortality of *Varroa jacobsoni* females after application of: A — sugar solution; B — dtto with acetone (control); C — dtto with precocene I; D — dtto with precocene II; E — indirect topical application of precocene I; F — indirect topical application of precocene II.

Table 1. Activity of allatocidines (estimated as % of mortality) in *Apis mellifera* and *Varroa jacobsoni*: a — *Apis mellifera*, b — *Varroa jacobsoni*

Compound No.	Method of application and doses									
	Peroral	Dose	Topical	Dose	Spray	Dose	Fumigation	Dose
1.	a — 2 b — 8	2 µg/ml sucrose sol.	59 83	0.5 µg per cm²	— —	— —	30 86	50 µl on strip/cage
2.	a — 18 b — 21	2 µg/ml sucrose sol.	5 80	0.5 µg per cm²	7 81	70 µg in acetone per cage	6 80	dtto
3.	a — b —	—	—	—	2 100	40 µl/cage in emulsion	0 62	10 µl on strip/cage
4.	a — b —	—	—	—	—	—	2 100	dtto

The perorally applied precocenes produced very low effects on both bees and mites. In the case of bees, only 2 % of exposed workers died after peroral application of precocene I and about 18 % in the case of precocene II.

As concerned *Varroa* females, precocene I caused about 8 % mortality and precocene II about 21 % in comparison with the controls. Thus the method of peroral application was abandoned and synthetic allatocidines were applied topically or by fumigation.

Fig. 2.

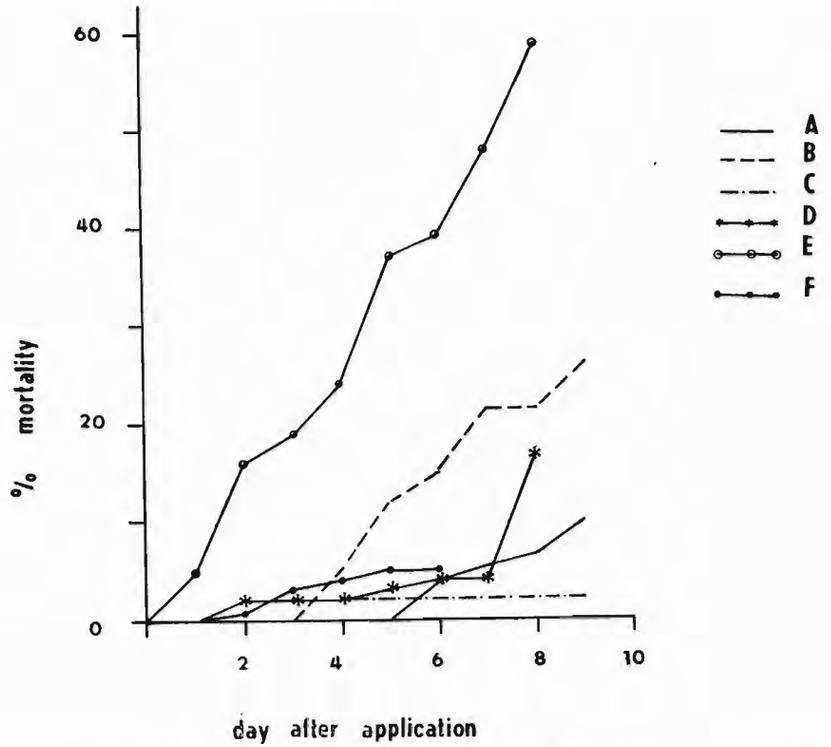

Fig. 2. Mortality of bees after application of: A — sugar solution; B — dtto with acetone; C — dtto with precocene I; D — dtto with precocene II; E — indirect topical application of precocene I; F — indirect topical application of precocene II.

The synthetic allatocidines (Fig. 3 C, D) possess higher activity against *Varroa* females than the natural ones: Allatocidine No. 3 induced 100 % mortality in the exposed *Varroa* mites and only 2 % in the exposed bees, even the dose applied by spraying was about two times lower than that of precocene II.

The fumigation was not so effective, it required to apply higher doses than 10 µl per fumigant strip.

Allatocidine No. 4 had greater effect on *Varroa* females than allatocidime No. 3. After fumigation with the same doses it caused 100 % mortality in mites and only 2 % in the treated bees (see Table 1).

The obtained data allow us to state that allatocidines possess promising activity against the bee mite, *Varroa jacobsoni*. The most effective methods are topical application and fumigation.

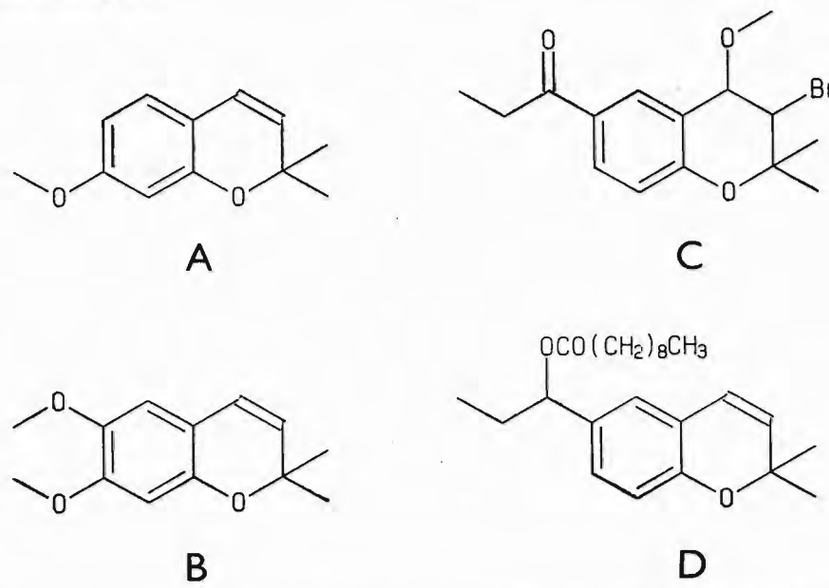

Fig. 3. Precocenes and their derivatives: A — 2,2-dimethyl-7-methoxy-2 H-benzopyrane (precocene I); B — 2,2-dimethyl-6,7-dimethoxy-2 H-benzopyrane (precocene II); C — 2,2-dimethyl-3-bromo-3,4-dihydro-4-methoxy-6-propionyl-2 H-benzopyrane; D — 2,2-dimethyl-6-(1-decanoyloxypropyl)-2 H-benzopyrane.

DISCUSSION

Precocenes are known as substances with anti-allatotropic activity on hemimetabolic, rarely holometabolic insect species (Bowers 1982, Němec and Němcová 1984).

The toxicity and allatocidal effects of precocene II in honey bee were studied by Rembold et al. (1979), and Fluri (1983). The former authors concluded that precocene II had no allatocidal effects on the honey bee, while Fluri (1983) estimated the toxic doses of precocene II for each honey bee cast. He found that the toxic doses precocene II for worker bee is 50 µg per specimen, but above 200 µg for the queen or drone. According to our data (see Table 1) *Varroa* females are more sensitive to the allatocidines than the honey bee.

Precocenes were also tested on several species of Acarida. Leahy and Booth (1980), Hayes and Oliver (1981), Dees et al. (1982), and Khalil et al. (1983) on a different species of ticks (Ixodidae) implied that the contact or fumigant application precocene II decreased oogenesis and egg eclosion, but not spermiogenesis. The application of precocene II also delayed the intermoulting period in the young ixodid nymphs and increased their "ecdysial mortality", but it had no influence on their morphogenesis. The topical application of precocene II impaired sucking and oviposition in the adult tick females, but their sexual attractiveness was quite normal.

The effect of allatocidines on *Varroa* females was rapid. The attached mites left their hosts within several hours after application and then they died probably due to the toxicity of the compounds or they perished as a consequence of starvation.

The abandonment may be a consequence of the changes in nutrients content in the haemolymph of bee host (Sadov 1978) or it may be caused by deterring and repelling effects of applied compounds as have been observed with precocenes by Sláma (1978) and Rembold et al. (1979).

The synthetic allatocidines would not be activated by oxidation of C-3, 4 double bonds like natural precocenes providing highly reactive epoxides, because they have in some cases no such double bond (e.g. compound No. 3). In spite of it, they have a very high biological activity. However, it would be explained by the fact that halogenide derivatives are also potent alkylating agents. The mode action of these compounds is not known, but it is worth discovering.

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ПРЕКОЦЕНЫ — ВОЗМОЖНЫЕ СРЕДСТВА ДЛЯ БОРЬБЫ С КЛЕЩАМИ ПЧЕЛ *VARROA JACOBSONI* (OUDEMANS)

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Резюме. Изучали влияние про-аллатоцидинов, прекоцена I, прекоцена II, и некоторых синтетических веществ обладающих похожее действие, на клеща *Varroa jacobsoni*. Изученные аллатоцидины обладают сильным биологическим действем на взрослые самки этого клеща при применении удобного метода. Самыми эффективными методами оказались местное применение и окуривание. Было доказано токсическое и репеллентное действие прекоценов на клещей, прекоцен I оказался также токсическим для пчел. Синтетические про-аллатоцидины были десять раз больше действительны, чем натуральные, но их токсичность для пчел была очень низка.

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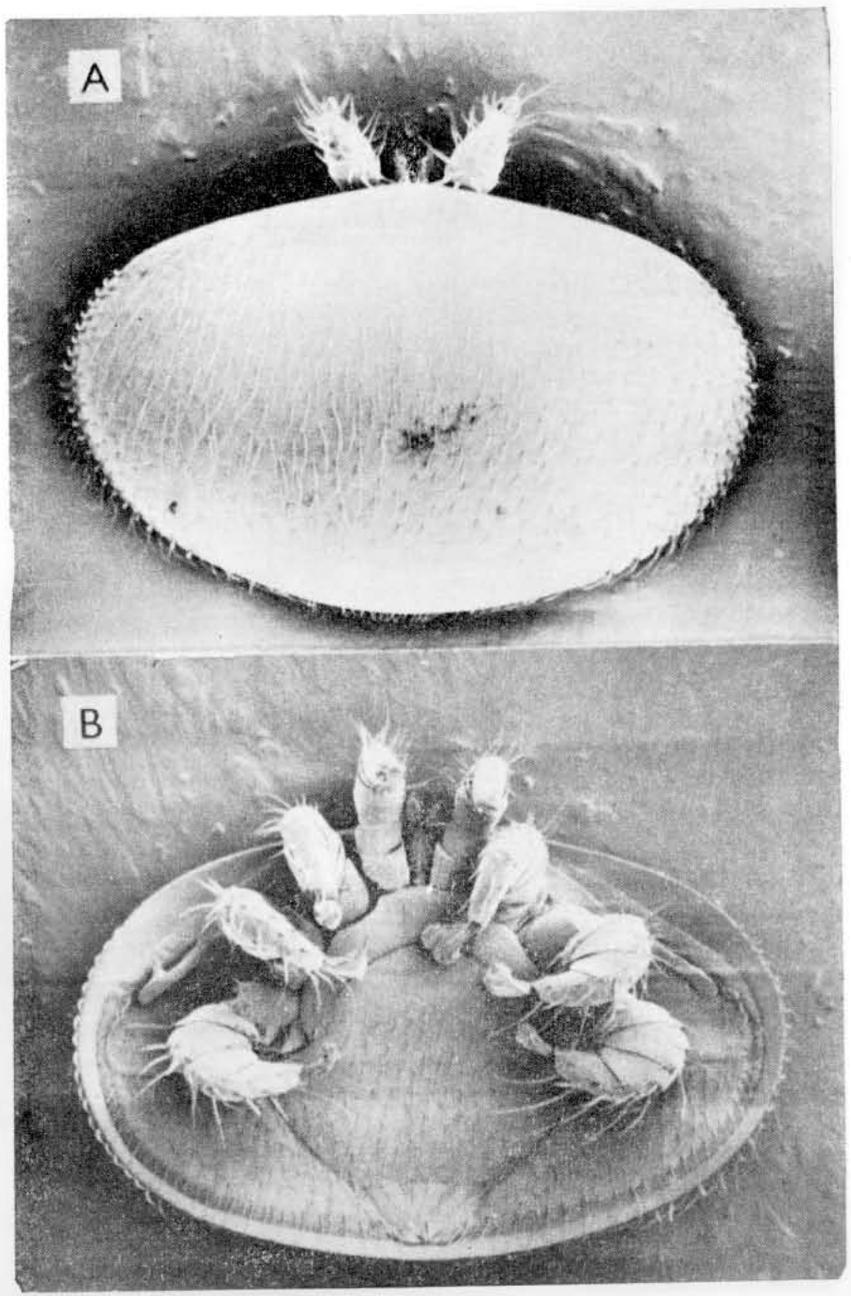
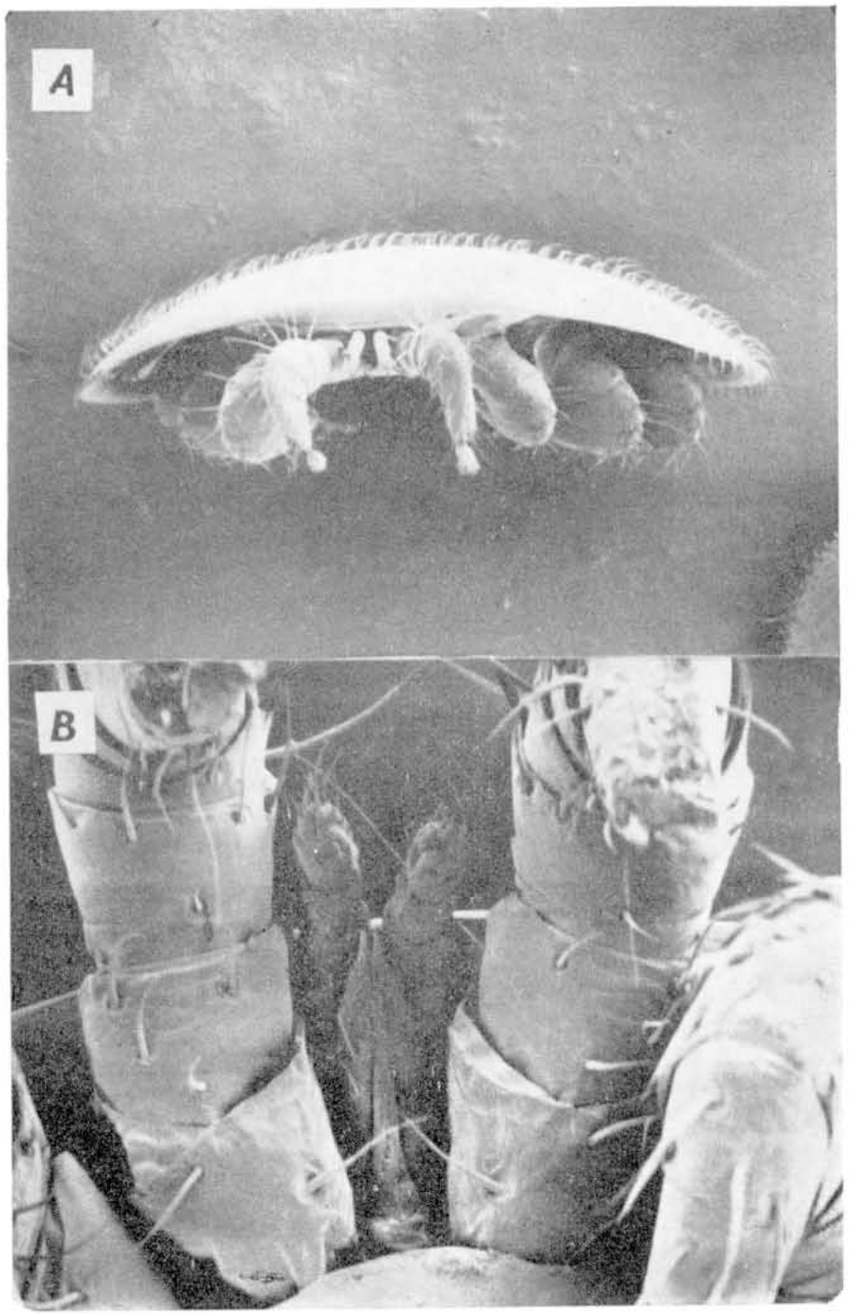


Adult female of the bee mite, *Varroa jacobsoni*: A — dorsal view (×200), B — ventral view (×200).



Adult female of the bee mite, *Varroa jacobsoni*: A — side-frontal view (×200), B — the detail of mouth part (×500).