

Effects of precocene I and II on the sensory system of antennae and mouthparts of Colorado potato beetle larvae, *Leptinotarsa decemlineata* (Col.: Chrysomelidae)

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Abstract

The chrysomelid species *Leptinotarsa decemlineata* (Say), commonly known as Colorado potato beetle (CPB), is an important pest of potato and other solanaceous crops. The effects of precocene I and II, juvenile hormone inhibitors, on morphological characteristics of the chemoreceptor organs of antennae and mouthparts of *L. decemlineata* larvae were studied in the laboratory. Different doses of precocenes were applied on the CPB second-instar larvae. The results showed that precocene caused changes in the form and number of sensilla of the antennae and maxilla-labial complex. The treatment of second-instar larvae by precocene I and II, considerably changed the cuticular structure of antennae and labio-maxillary palps after the first molt. In addition, other anomalies such as remaining of the previous instar cuticle, degeneration of some or whole sensilla on the second and third antennal segments and on the terminal segment of labio-maxillary palps, and reduction of both receptor cells and their dendrites were observed. The effects of precocenes on the insect sensory system and disturbance of chemical recognition of host plants and environment are discussed.

Key words: Colorado potato beetle, *Leptinotarsa decemlineata*, antenna, maxillary and labial palps, sensory system, sensilla, precocene, juvenile hormone inhibitor

چکیده

سوسک برگ‌خوار سیب‌زمینی، *Leptinotarsa decemlineata* (Say) یکی از آفات مهم سیب‌زمینی و دیگر محصولات خانواده‌ی Solanaceae می‌باشد. تأثیر پریکاسن I و II و مهارکننده‌های هورمون جوانی، روی ویژگی‌های مورفولوژیکی گیرنده‌های حسی شیمیایی شاخک و قطعات دهانی لارو سوسک برگ‌خوار سیب‌زمینی مورد مطالعه قرار گرفت. جهت آزمایش، غلظت‌های مختلف پریکاسن روی لارو سن دوم به کار برده شد. نتایج حاکی از ایجاد تغییرات در شکل و تعداد گیرنده‌های حسی شاخک‌ها و ضمامت قطعات دهانی بود. همچنین، کاربرد پریکاسن روی لاروهای سن دوم، تغییرات مورفولوژیکی در ساختمان کوتیکولی گیرنده‌های حسی شیمیایی شاخک و پالپ‌های آرواره‌ای و لبی را بعد از اولین پوست‌اندازی نشان داد. علاوه‌براین، تغییرات دیگری از قبیل باقی‌ماندن کوتیکول مرحله‌ی قبلی و نیز تحلیل تعدادی و یا تمام گیرنده‌های حسی روی بند دوم و سوم شاخک و بند انتهایی پالپ‌ها، و کاهش تعداد سلول‌های حسی و دندریت‌های آن‌ها مشاهده شد. با توجه به تأثیر پریکاسن روی سیستم حسی حشرات، نقش آن در ایجاد اختلال در اکولوژی شیمیایی و یافتن میزبان مورد بحث قرار گرفته است.

واژگان کلیدی: سوسک برگ‌خوار سیب‌زمینی، *Leptinotarsa decemlineata*، شاخک، پالپ‌های آرواره‌ی پایین و لب پایین، سیستم حسی، گیرنده‌های حسی، پریکاسن، مهارکننده‌ی هورمون جوانی

Introduction

The anti-juvenile hormone, precocene, is a phytochemical that has been identified from two genera of remotely-related plant families. It probably has an important role in plant defences against herbivory because of its anti-feedant property, which interferes with juvenile

hormone-controlled processes (Binder & Bowers, 1992). Precocene, which was originally extracted from floss flowers, *Ageratum houstonianum* Mill (Bowers *et al.*, 1976), has cytotoxic effects on corpora allata of susceptible insect species. Recent studies have shown that precocenes significantly reduce the longevity of last instar larvae, inducing ecdysis of larval cuticle and generating abnormal puparia (Farazmand & Chaika, 2008a).

Many insect growth regulators affect development of chemoreceptor organs causing chemical communication disorders in relation to insects' sexual behavior and migratory activities (Bowers *et al.*, 1976; Dorn, 1982; Polivanova, 1982). The precocene I and precocene II increase larval mortality and developmental period, leading to early formation of adultoids, severe morphological abnormalities and epithelial deformation in midgut (Farazmand & Chaika, 2008a; Farazmand, 2009). The effects of precocenes on the chemoreceptor organs of *Myzus persicae* (Sulzer), *Eurygaster integriceps* Puton and *Archips podana* (Scopoli) have been studied (Polivanova & Triseleva, 1992; Polivanova, 1997; Triseleva, 2007).

The chrysomelid Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is a destructive pest of potato and other solanaceous crops. Both CPB larvae and adults feed on foliage, which can result in complete defoliation of potato if no control measure is undertaken (Gelman *et al.*, 2001). Recent studies on the fine structure of sensory receptors of antennae, maxillary and labial palps of CPB larvae have identified 11 sensilla on the antenna (3 trichoid, 2 basiconic and 1 styloconica on the apex of the third segment; 2 trichoid, 2 basiconic and 1 conical on the distal part of the second segment) (Farazmand & Chaika, 2011), 21 sensilla on the maxillary palp (16 basiconica, 4 trichoid and 1 digitiform) and 11 sensilla on the each labial palp (Farazmand & Chaika, 2008b).

The increasing attention to insects' sensory mechanisms and chemo-communication issues underlies the feeding behaviour and host-plant selection of herbivorous insects. The chemoreceptor organ of holometabolous insect larvae is a candidate model for analysis of the effects of biological compounds as far as the number of sensilla is consistent for all larval stages. The objective of this study is to examine the effects of precocene on the sensory system of antennae and mouthparts of CPB larvae.

Materials and methods

The CPB eggs were collected from potato fields around Moscow, Russia. The eggs were transferred to the laboratory ($25 \pm 1^\circ\text{C}$, $65 \pm 5\%$ R.H. and a photoperiod of 16: 8 (L: D) h)

where the colony was established on fresh potato foliage. A total of one hundred CPB larvae (2nd instar) were treated with precocene I (7-methoxy-2, 2-dimethylchromene) and precocene II (6, 7-dimethoxy-2, 2-dimethylchromene) (Sigma-Aldrich company), at a sublethal dose of 10 µg (3 replicates) using topical application. Larvae were then reared in plastic boxes and foliage was resupplied daily. After molting, the larvae were fixed in 75% alcohol and 2.5% glutaraldehyde and their head capsules rinsed by 75%, 90%, 100% ethanol and acetone. The air-dried samples were, later secured on aluminum stubs coated with approximately 200 Å of gold by vacuum evaporation to be examined and photographed, using a Hitachi S-405A scanning electron microscope (SEM).

Using transmission electron microscopy (TEM), each head capsule was prefixed in 2.5% glutaraldehyde, mixed in phosphate buffer solution at a PH of 7.3 at 4°C for 4 hours and post-fixed with 2% osmium tetroxide in phosphate buffer at room temperature for 2 hours. The tissue was dehydrated with ethanol and embedded in Epon. Serial sections with a thickness of approximately 70-80 nm were made using an ultramicrotome with a diamond knife mounted on Formvar coated copper grids and then stained with 2% uranyl acetate in 50% ethanol and examined using a JEM-100B microscope.

Results

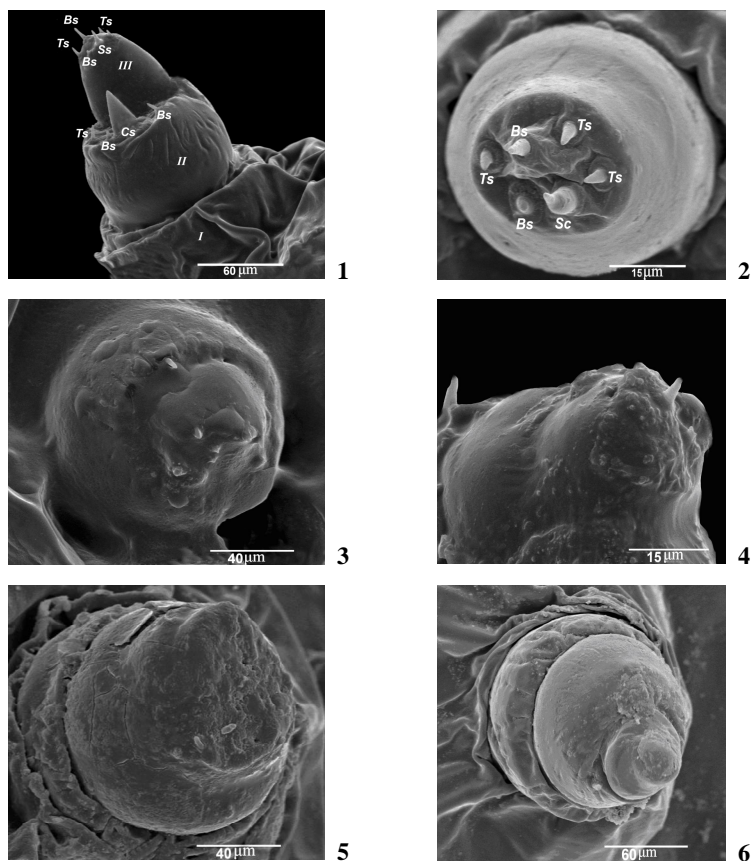
Antennae

The precocene I (1 µg) treated larvae, showed disorder in development of the sensory system of antennae, where the cuticular parts of terminal sensilla in some larvae were not expressed or had only 2-3 sensilla. The sensilla on the second antennal segment were slightly developed or heavily reduced. The disorder in molting led to the strongly modified antennae, in which the separation of antennal segments was not distinctive. The remaining old cuticle also surrounded the sensilla of new instar larvae. Although there was no significant changes in antennal receptor construction of the larvae treated with precocene II (1 µg), they showed striking deformation of the cuticle of integument and head appendages (table 1).

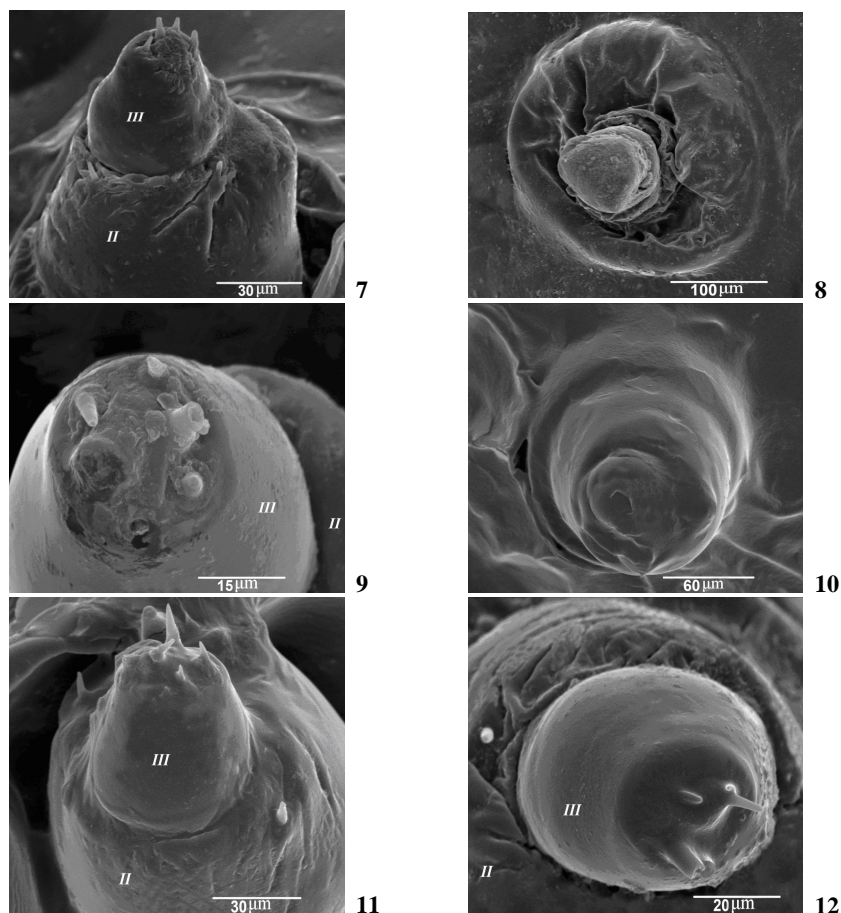
The treatment of second-instar larvae with precocene I (10 µg) caused considerable changes in cuticular structure of sensory system of antennae of the third-instar larvae, notably, the fusion of the segments II and III, leaving the cuticle of the previous instar, and heavy reduction of sensilla (0-4) (figs 3-4). The treated larvae with precocene II (10 µg) had only three sensilla on the apical antennal segment in comparison with the control larvae which had six sensilla. The conical sensillum on the second antennal segment was strongly reduced or

degenerated (table 1) (fig. 7), but there was no significant reduction of basiconic sensilla (figs 11-12).

The treated larvae with precocene I (20 μ g, twice for the second and third instars), showed a range of reduction of sensilla, absence of conical sensillum of the segment II and strongly reduced terminal sensory platform of the antennae (fig. 5).



Figures 1-6. 1-2, SEM micrograph of antenna of CPB larvae (control): 1. chemoreceptor organs on antenna, 2. sensilla at the apex of the antenna; 3-6. SEM micrographs of antenna of CPB larvae treated with precocene I (I = 1st segment; II = 2nd segment; III = 3rd segment; Bs = basiconica sensilla; Cs = conical sensillum; Ss = styloconica sensilla; Ts = trichoid sensilla).



Figures 7-12. SEM micrographs of antenna of CPB larvae treated with precocene II (I = 1st segment; II = 2nd segment; III = 3rd segment).

The larval treatment with precocene II (20 μ g, twice) led to the remaining of old cuticle on the antenna, distortion of normal form and dimensions of antennal segments, frequent mergence of the second and third antennal segments and also reduction of cuticular sensilla. (table 1) (figs 8-9).

Distorted antennae and widely reduced chemoreceptors occurred when the larvae treated with precocene I (30 μ g and 50 μ g applied on 1st, 2nd and 3rd instars) (fig. 6). The larvae treated with precocene II (50 μ g) showed anomalies such as strong deformation of antennae,

Table 1. Effect of precocene I & II on sensilla of antennae, maxillary and labial palps in larvae of CPB*.

	Treated area (Length in μm)	Control	Treatments					
			Precocene I		Precocene II			
			10 μg	20 μg	30 μg	10 μg	20 μg	30 μg
Antennae	Length	65.07 \pm 1.28a	42 \pm 1.21c	40 \pm 1.68cd	57.33 \pm 1.50b	38.18 \pm 2.51de	20.04 \pm 0.89f	35.17 \pm 2.13e
	Number of sensilla	5 \pm 0.0a	0.5 \pm 0.5c	0d	0.83 \pm 0.41c	2 \pm 0.89b	0.5 \pm 0.54c	0d
	Length of conical sensillum	31.75 \pm 0.97a	NA	NA	NA	7.30 \pm 1.50b	NA	NA
Third segment	Length	50.63 \pm 1.11a	21.33 \pm 2.67c	24.25 \pm 1.15c	23.50 \pm 1.0c	35.34 \pm 3.76b	15.55 \pm 0.45d	12.46 \pm 0.47e
	Number of sensilla	6 \pm 0.0a	0.8 \pm 0.44d	0.6 \pm 0.54d	0e	3.67 \pm 0.55b	2 \pm 1.73c	0e
	Length of basiconic sensilla	12.82 \pm 0.20a	4.17 \pm 0.85b	6.27 \pm 0.23b	NA	6.52 \pm 1.96b	4 \pm 0.14b	NA
Second segment	Length	64.04 \pm 0.99a	39.9 \pm 0.55b	27.4 \pm 2.09d	42.02 \pm 1.32b	35 \pm 1.15c	22.10 \pm 1.58e	39.16 \pm 1.70b
	Number of sensilla	4 \pm 0.0a	2.8 \pm 0.84bc	2.25 \pm 0.50bc	0.75 \pm 0.96e	3.25 \pm 0.96b	1.75 \pm 1.5d	0.17 \pm 0.41e
	Length	75.04 \pm 0.57a	45.08 \pm 1.56b	33 \pm 1.78d	44.67 \pm 0.82b	29.33 \pm 1.75e	37.13 \pm 1.21c	32.25 \pm 1.41de
Maxillary palps	Number of basiconic sensilla	16 \pm 0.0a	5.67 \pm 1.21d	0.5 \pm 1.22e	0.17 \pm 0.41e	10.83 \pm 0.41b	6.08 \pm 0.67b	0.33 \pm 0.52e
	Length of basiconic sensilla	3.44 \pm 0.12a	3.05 \pm 0.58ab	2.92 \pm 0.10c	1.83 \pm 0.42d	2.70 \pm 0.08bc	1.57 \pm 0.21de	1.32 \pm 0.42e
	Length	47.96 \pm 0.54a	39.31 \pm 0.57d	40.17 \pm 2.04cd	30.55 \pm 0.83e	44.75 \pm 1.08ab	43.5 \pm 1.76bc	34.33 \pm 1.51e
Labial Palps	Number of basiconic sensilla	11 \pm 0.0a	6.50 \pm 1.87b	6.16 \pm 1.90b	3.33 \pm 2.65d	5.17 \pm 0.96c	4.83 \pm 0.75c	1.33 \pm 1.50e
	Length of basiconic sensilla	4.55 \pm 0.13a	4.42 \pm 0.22a	4.08 \pm 0.26a	3.28 \pm 0.27a	4.38 \pm 0.20a	4.02 \pm 0.41a	2.75 \pm 0.68a

*Means within column followed by the same letter not found significant ($P < 0.05$; DMRT).

NA = Not Applicable.

slightly developed conical sensillum and reduction of several apical sensilla on the antennae (fig. 10).

In larvae treated with precocene I and II (50 μg), disorders in cuticular (endocuticle) and hypodermal layers, such as breakages and lacunae in cuticle of the antennae, were seen. There were also numerous lacunae in the lumen of the antennae while sensilla were almost invisible. The dendrites of receptor cells of sensilla were not observed (fig. 26).

Maxillary palps

When precocene I (1 μg) was applied, the old cuticle remained on maxillary palps while in molted larvae, various ranges of reduction of sensilla on a sensory platform of terminal segment of maxillary palps occurred. The application of precocene II (1 μg) slightly changed the structure and number of receptors on maxillary palps. However, larvae were holding the remains of sensory platform on their old cuticle.

The larvae treated with precocene II (10 μg) were characterized by their remaining old cuticle on maxillary palps that in some larvae covers all sensory platform of terminal segment. Otherwise, the sensory organs on this platform were partly reduced, for example 11 sensilla instead of 16 sensilla (table 1).

Treatment with precocene I (20 μg , twice) effectively prevented the larval molting. Therefore, the cuticular part of sensilla was not visible at the apex of maxillary palps (fig. 15) and the dimensions of sensory platform were much smaller than normal larvae. In addition, the cuticular part of sensilla, located on this platform, was either weakly expressed or widely reduced. The three-time-treated larvae with precocene had their sensory platform at the apex of maxillary palp invisible (fig. 15). In larvae treated with precocene II, 10 μg (two or three times), the sensory organ of maxillary palps changed and in most of them, the cuticular part of sensilla on the terminal segment reduced (table 1) (figs 17-18).

The rate of reduction of larval sensory organs was proportional to the frequency of the treatments rather than their concentrations. The larvae treated with precocene I (50 μg) showed the same anomalies as occurred when lower doses of precocene applied (fig. 16). The examination of ultrastructure of such larvae did not show considerable changes in receptor organ as they were visible and their dendrites were accessible in cuticular part of sensilla. Apparently, the formation of cuticular structure of palps had been largely affected by the application of precocene with such doses.

Labial palps

The remains of the old cuticle on the second-instar in addition to crack on labial palps of most of third-instar larvae was visible when precocene I (1 μg) applied. In some larvae the number of sensilla decreased to seven and the rest showed weak developments of cuticular parts (fig. 22).

In the larvae treated with precocene I (10 μg), reduction of sensory platform was visible. Otherwise, a weak expression of sensilla occurred. A similar change in the three-time-treated larvae was seen as well (fig. 21). In larvae treated with precocene II (10 μg) the old cuticle remained on labial palps. In some larvae, more than nine sensilla on sensory platform of labial palps were counted (fig. 24). The application of precocene II (20 μg , twice) reduced the cuticular parts of sensilla. Similar anomalies in development of the sensory organs discovered when the larvae treated with precocene II (30 μg) three times. The presence of sensilla in cavity made their counting difficult.

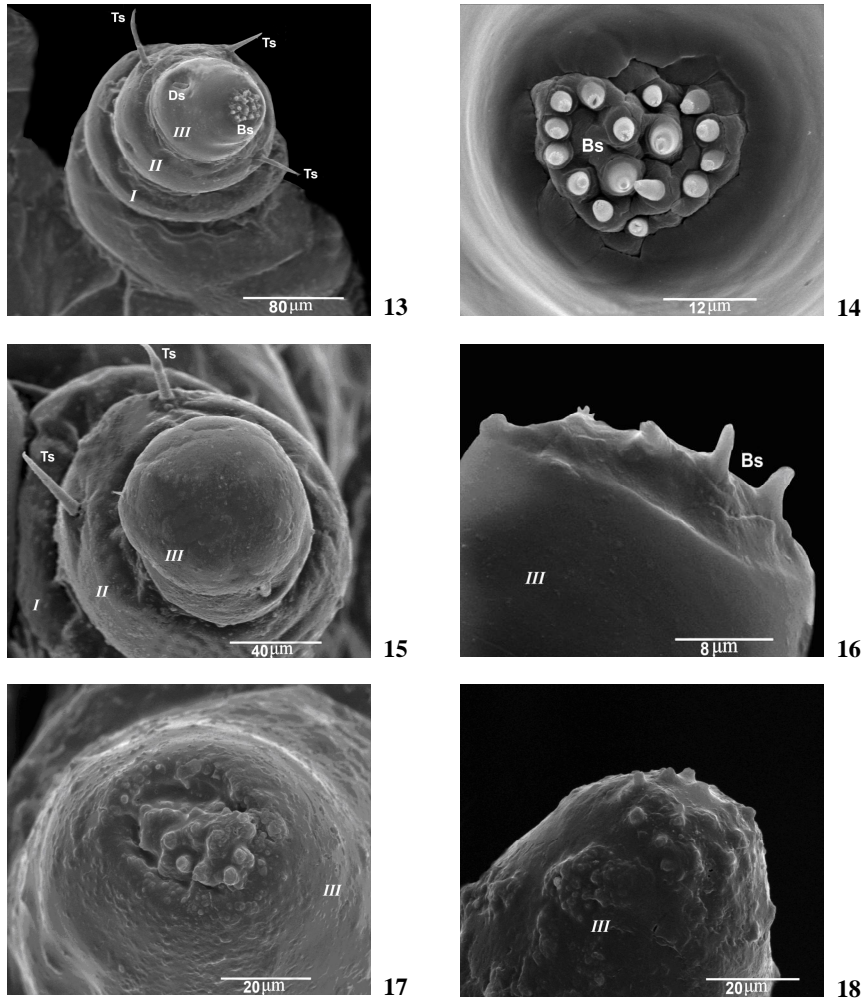
The detailed examination of larvae treated with precocene I (50 μg) was made possible by TEM which helped to discover the deep degeneration of structures in the receptor organs of labial palps. The structure of lumen of the palp did not exist and the cuticle of labial palp had many breakages. The cuticular structures of sensilla and the dendrites of receptor cells were not present (figs 28 & 30).

The increase of precocene II dose to 50 μg did not produce more anomalies (fig. 23). In larvae treated with precocene, the cuticle of the previous instars remained, the number of sensilla decreased to six; and in some cases, all sensilla were reduced. The examination of sections through labial and maxillary palps of precocene-treated larvae found a reduction in receptor cells and their dendrites (fig. 28). The cuticular structure of treated larvae significantly differed from the control and in many cases the both epicuticle or exocuticle were absent.

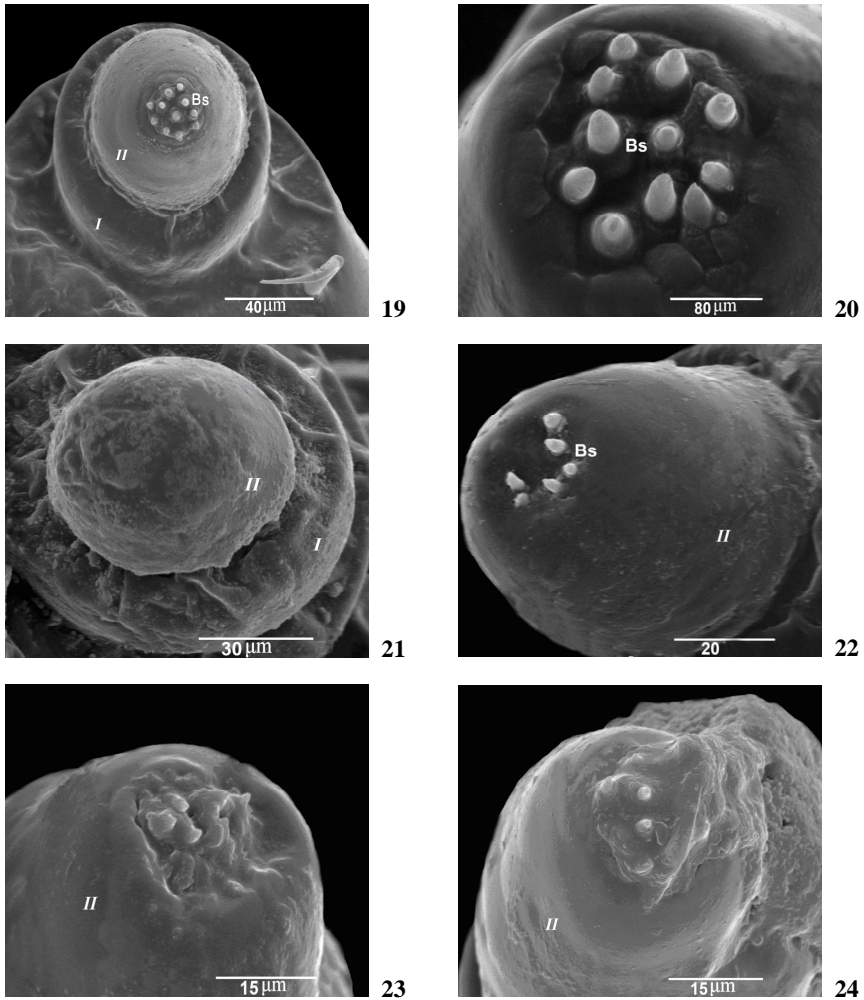
In general, segment size, number and length of antenna and palps sensilla in the control were different from the treatments. The reduction effect of precocene II was similar to precocene I. The results showed that reduction rate was not correlated with treatment doses (table 1).

Discussion

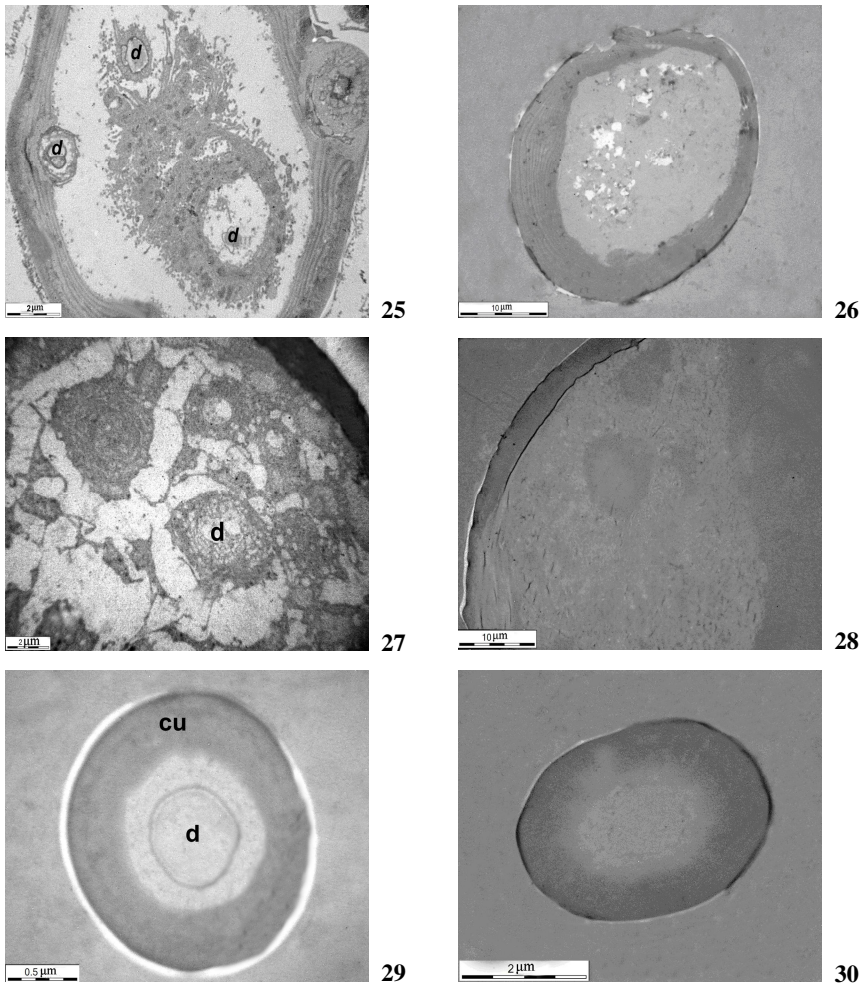
Most coleopteran larvae have extensively developed sensory organs. There are various types of sensilla on antennae and palps of beetle larvae which underscore the role of contact gustatory stimulants. The number of sensilla in all instar-larvae are consistent. The



Figures 13-18. 13-14, SEM micrograph of maxillary palp of CPB larvae (control): 13. chemoreceptor organs on maxillary palp, 14. sensilla on the distal apex of third segment; 15-16. SEM micrographs of maxillary palp of CPB larvae treated with precocene I.; 17-18. SEM micrographs of maxillary palp of CPB larvae treated with precocene II (I = 1st segment; II = 2nd segment; III = 3rd segment; Bs = basiconic sensilla; Ds = digitiform sensillum; Ts = trichoid sensilla).



Figures 19-24. 19-20, SEM micrograph of labial palp of CPB larvae (control): 19. labial palp, 20. sensilla distal apex of second segment; 21-22. SEM micrographs of labial palp of CPB larvae treated with precocene I.; 23-24. SEM micrographs of labial palp of CPB larvae treated with precocene II (I = 1st segment; II = 2nd segment; Bs = basiciconica sensilla).



Figures 25-30. TEM micrographs of antenna and labial palp of CPB larvae: 25. cross-section through 3rd segment of the antenna (control), 26. cross-section through 3rd segment of the antenna (treated with precocene I), 27. cross-section through base of basiconic sensillae at the ciliary region of labial palp (control), 28. cross-section through base of basiconic sensillae at the ciliary region of labial palp (treated with precocene I), 29. cross-section of basiconic sensillum with a thick wall on distal end of labial palp (control); 30. cross-section of basiconic sensillum with a thick wall on distal end of labial palp (treated with precocene I) (cu = cuticle; d = dendrite).

chemoreceptor sensilla on mouthparts of CPB larvae are concentrated on terminal platforms of distant segment of maxillary and labial palps which is commonly observed in ground beetles (Carabidae) as well. (Sinitsina & Chaika, 2003).

The other feature of the chemoreceptor organs of larvae is the conical sensillum on antennae which is present in most of beetle larvae (Zacharuk *et al.*, 1977; Chaika & Tomkovich, 1997; Tomkovich & Chaika, 2001). The sensilla of lateral sides of maxillary palps are digitiform but in CPB larvae, the digitiform sensilla are located only on maxillary palps. The digitiform sensilla can be found on the labial palps of the larvae of the family Elateridae (Honomichl & Guse, 1981) that underlines their orientating function. In particular, these sensilla respond to tactile and vibratory stimulants (Zacharuk *et al.*, 1977).

The ultrastructure examinations showed that basic gustatory sensilla on maxillary and labial palps of larvae were basiconica. The innervation of basiconica sensilla by several receptor cells is related to their probable specialization for different alimentary stimulants. The digitiform sensilla of third segment of maxillary palps can be a mechanoreceptor, similar to trichoid sensilla of second and third segment of maxillary palps (Farazmand & Chaika, 2008b).

Application of precocenes as a tool for chemical allatectomy can prove the effect of the juvenile hormone on morphogenesis without surgical removal of corpora allata. This is the reason that precocenes are regarded as prospective fourth-generation insecticides. The precocenes can have different effects on insect developmental stages depending on the type of metamorphosis (complete and incomplete) that the target species undergoes. Studies on the effect of precocenes on sensory systems have been performed mainly on the insects with incomplete metamorphosis. However, this chemical can impair the ability of host-plant searching by larvae and interfere with mating of adults.

In studies on Homoptera and precocene-synthesizing plants, serious disturbances in the structure of sensory organs have been observed. For example, *Trialeurodes vaporariorum* (West.) (Hem.: Aleyrodidae) showed a decrease in the amount of powdery wax and changes in the shape of sensilla (Polivanova, 1991) and in the aphid pest *M. persicae* led to the deformity of antenna and reduction of the number of sensilla while the rhinaria appeared on wrong antennal segments (Polivanova, 1997).

There is little information about the effect of precocenes on the development of sensory organs in insect with complete metamorphosis. In the noctuid *Spodoptera mauritia* Lawn, the treatment of larvae with precocene II resulted in the formation of pupae with disturbances in

the timing of differentiation of definitive mouthparts, wings and eyes (Mathai & Nair, 1984). The larvae of *Spodoptera litura* F. treated with precocenes I and II developed into adultoids, i.e. defective adult insects with deformed wings, legs and antennae that hatched from externally normal pupae (Srivastava & Kumar, 1997).

The test of precocene I and precocene II on second-instar larvae of *L. decemlineata* resulted in the increase of mortality and their developmental period, leading to early formation of pupal characteristic on larvae and formation of adultoids. The larvae also showed morphological abnormalities, such as size reduction, body segmentation disturbance, strong deformation or reduction of wings and elytra, and remaining of previous cuticle (Farazmand & Chaika, 2008a).

In this study, the treatment of CPB larvae with precocenes caused disturbances in the sensory organs of antennae and mouthparts. The disturbance mainly occurred on basiconic sensilla of the palps and on all sensilla of antennae. The juvenile hormone is involved in the regulation of chemical communication and sensitivity of sensory organs.

The experiments on desert locust, *Shistocerca gregaria* (Forsk.) showed that the adequate response of olfactory neurons in the antennal lobe to the aggregation pheromone was juvenile-hormone dependent and individuals without corpora allata, the organ that synthesizes this hormone, failed to perform their aggregating behavior (Ignell *et al.*, 2001).

Studies on the regulatory role of the juvenile hormone on the development and differentiation of sensory systems in insects are few and largely based on insects with incomplete metamorphosis. It has been particularly shown that juvenile hormone and its analogs cause a noticeable decrease in the number of sensilla on the seventh abdominal tergite of male *Blatta germanica* Linn. (Wheeler & Gupta, 1988) and on the antennae of male *Leucophaea maderae* (F.) (Schafer & Sanchez, 1974). The antennal sensory organ of *E. integriceps* is highly sensitive to juvenile hormone deficiency (Polivanova & Triseleva, 1988) that is indicative of changes in the hormonal level and juvenile hormone deficiency due to any disturbances that disrupt their natural developments and sensory organs differentiation.

Our results show that disturbances in the sensory system of CPB larvae can be the effect of juvenile hormone deficiency caused by the treatment of larvae with precocene. Furthermore, successful development of larvae before metamorphosis suggests that the toxic effect of precocene on corpora allata that could sharply inhibit their synthetic activity. In addition, the little impact of precocenes on structural disturbances in the sensory organs of

antennae and mouthparts, supports our hypothesis that juvenile hormone affects the development of sensory systems in CPB larvae.

The effectiveness of all investigated biologically active substances on receptor structures is correlated to the dosage of the chemical applied on larvae. However, it is certain that consecutive treatments of the larvae cause notable changes in chemoreceptors of mouthparts. Similar effects of precocenes on the receptor organs lead to the reduction of cuticular parts of sensilla and reduction of innervating neurons of the sensilla.

This study, for the first time, shows that biologically-active substances can suppress the differentiation of sensilla and affect morphogenetic degeneration of epithelial cells in sensory organs of Holometabola. The juvenile hormone inhibitor precocene has a property to affect the formation of cuticular parts of sensilla and here is proved first that these substances have qualities to destroy the receptor cells, innervating sensory organs. Consequently, the treatment of larvae with sublethal doses of biologically-active substances can disturb processes of chemical recognition of host plants and environment.

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