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ANTIGONADOTROPIC EFFECTS OF PRECOCENE II: ALLATICIDAL ACTION IN FEMALES OF *NILAPARVATA LUGENS* (STAL)

A.R. Pradeep* and V.S.K. Nair[1]

ABSTRACT

Exposure of day 0, 1, 2 and 3 fifth instar nymphs and newly ecdysed brachypterous females of *Nilaparvata lugens* to different doses of the antijuvenile hormone agent, precocene II (PII) residue inhibited ovarian growth and oocyte maturation, reduced fecundity and induced sterility in a dose-dependent manner. High dose of 5 µg / cm^2 induced complete sterility. Severe histopathological alterations were observed in the ovariole and oocytes of insects treated with high doses. Follicular epithelial cells invaded ooplasm. Uncontrolled multiplication resulted in hyperplasia of the epithelia. Exposure of PII-pretreated nymphs to juvenile hormone analogue (3µg JHA/cm^2) did not enhance fecundity, indicating the requirement of intact corpora allata later in the final instar for pre-ecdysial ovarian growth and maintenance. But JHA treatment to PII-pretreated adults restored normal rate of fecundity, revealing PII-induced JH deficiency as the cause of infecundity, PII induced significant reduction in size of corpus allatum and cellular degeneration in the treated insects. Infecundity, ovarian histopathology and allatal atrophy apparently suggest that the observed anti-juvenile hormone effects of PII in *N. lugens* female are due to its systematic allaticidal activity.

Key words: *Nilaparvata lugens,* precocene II, sterility, anti-allatal effects.

INTRODUCTION

The role of juvenile hormone (JH) in regulating larval development and oocyte maturation in insects is well documented (Koeppe *et al.* 1985). An induced JH deficiency during early development leads to precocious metamorphosis or causes sterility in adults. Precocenes are chromene compounds isolated from the bedding plant *Ageratum houstonianum,* which inhibit JH controlled physiological/morphological processes in certain insect species (Unnithan *et al.* 1977; Bowers 1985; Nair 1993). The anti-JH effects of precocene are ascribed to the selected destruction of parenchymatous cells of the corpus allatum (CA), the source of JH and the consequent JH deficiency. Treatments with precocene induced prothetely during larval development (Sam Mathai and Nair 1984; Bowers 1985; Subrhamanyam and Rao 1987; Pradeep and Nair 1989; 1998), sterility in adults (Bowers 1985; Baldellou and Belles 1986; Brasiliero 1987; Sam Mathai *et al.* 1989) and toxicity and antifeedant effects in few insects (Fridman-Cohen *et al.* 1984; Abraham and Muraleedharan 1990). In this paper, we have established that precocene II acts as a systemic allaticidal

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[1] Department of Zoology, University of Calicut, Kerala - 673 635, India.

* Corresponding author and present address: Physiology Section, Central Tasar Research and Training Institute, Piska Nagri. P.O., Ranchi-835 303, Bihar, India.

agent in *Nilaparvata lugens* (Stal), and induces ovarian dysfunction. Juvenile hormone analogue treatments were also made to examine whether the PII-induced effects are due to JH deficiency or not.

MATERIALS AND METHODS

A laboratory colony of the brown planthopper, *N. lugens* was raised at 27+2°C temperature and 57+3% relative humidity, exposed to natural photoregime as described by Medrano and Heinrichs (1985). Newly ecdysed fifth (final) instar nymphs and brachypterous females were isolated from the stock colony and used for the experiments. Day of molting to fifth instar or adult was considered as day 0 of that stage. The experimental and control insects were released into small test cages made as described earlier (Pradeep and Nair 1989). Precocene II (PII; 6, 7-dimethoxy 2, 2-dimethyl chromene; Aldrich Chem. Co., Germany) and Juvenile Hormone Analogue, hydroprene (ZR 512; ethyl 3, 7, 11-trimethyl dodeca-2, 4-dienoate; gift from Dr. G.B. Stall, Zoecon Corporation, California, U.S.A.) were dissolved in acetone and diluted to obtain various concentrations. The insects were treated for an hour by contact method with a residue of the compound at different concentrations (0.5 µg, 1.0µg, 3.0µg, 5.0µg or 10µg / cm^2) coated in Petri dish with a total surface area of 63cm^2 (Pradeep and Nair 1989). Control insects were released to Petri dishes previously coated with acetone alone for the same period.

Ovaries of treated/control insects were dissected out at various intervals in insect Ringer's solution and length of the ovariole was taken. Ovary and CA tissues were fixed in Bouins' fluid and processed for routine histological studies. Volume of terminal oocyte was calculated considering it as a prolate spheroid using the formula 4/3 $\prod ab^2$ where "a" is half the length and "b", half the width of the spheroid. Area of CA was computed considering it as a spheroid using the formula 4 $\prod r^2$ where "r" is the radius. Fecundity index was calculated according to Saxena (1969) as given below:

$$\text{Fecundity index} = \frac{\text{Number of eggs laid}}{\text{Preoviposition period}}$$

Statistical analysis was done using ANOVA and Students' *t*-test.

RESULTS

Antigonadotropic effects

Control fifth (final) instar nymphs molted into adults within 3.8±0.38 days. Adult females initiated egg laying within 2 days after emergence and laid on an average 290 eggs in 12 days. Variously aged (day 0, 1, 2 and 3) fifth instar nymphs were treated with 3 or 5µg PII/cm^2. The adults resulted from PII treated day 0 and day 1 nymphs survived for 2 to 3 days only and did not lay any eggs. On the other hand, adults formed from PII-treated day 2 nymphs survived for 7 to 8 days and laid on an average of 58 eggs. Those formed from treated day 3 nymphs survived for 8 days but were completely sterile. For counter action experiments, day 2 nymphs pretreated with 3µg PII were exposed to 3µg JHA/cm^2 on day 3 or on day 0 of adults formed from the nymphs treated with PII. Both these treatments did not enhance

fecundity (Table 1). Treatments of newly ecdysed adults with various doses of PII extended preoviposition period to 4 days. Fecundity was conspicuously reduced due to the treatments, in a dose-dependent manner (Table 2). High doses induced complete sterility in majority of the treated insects. Fecundity indices were also small. In order to study whether the decreased fecundity was due to PII - induced JH deficiency, PII-treated day 0 females were exposed to 3µg JHA/cm^2 on day 1, i.e. 24h after PII treatment. These adults laid on an average 116 eggs, thus partially restored the rate of fecundity (Table 2).

Telotrophic ovary of control *N. lugens* female was completely differentiated at the time of adult emergence. Post emergence growth of ovarioles was sudden. By 36h after adult eclosion, first terminal oocyte became completely mature and oviposition commenced within the next 12h. PII treatment to day 2 fifth instar nymphs significantly ($P<0.005$) reduced the size of terminal oocytes in the adults (Table 3). Growth of ovarioles as well as terminal oocytes was retarded after treatments of day 0 adults with PII (Fig. 1 & 2). Statistical analysis (ANOVA) showed significant ($P< 0.001$) variation among volumes of the terminal oocytes of treated and control females, during different intervals. Fat body was not utilized, unlike in that of controls. Nymphal and adult treatments with PII induced conspicuous histopathological alterations in the germarium as well as vitellarium. Germarium contained few disintegrated trophocytes interspersed with dark droplets (Fig. 3). Numerous oogonial cells were clumped in the posterior region of the germarium. In the vitellarium, follicular epithelial layer was multilayered and invaded into the ooplasm (Fig. 4). Ovarioles were slender and transparent in 48h old treated females. Vitellogenic oocytes were absent. Ooplasm appeared smooth and without any yolk granules in all the terminal oocytes (Fig. 5). Large vacuoles enclosing cellular debris were observed in the oocytes of 72h old treated females. Ooplasm-follicular epithelium interface became indistinct (Fig. 6). Follicular epithelium did not exhibit any potency-related morphological changes, such as changes in shape and size of the cells observed in control insects (Pradeep 1994). In advanced stage of resorption, ooplasm appeared darkly stained (Fig. 7) and the oocytes were surrounded by degenerated follicular epithelium. In control females, ovarioles showed normal development and egg maturation (Fig. 8).

Allaticidal effects

Size of the CA was significantly ($P< 0.001$) reduced in 24 h old females treated with 5 µg PII/cm^2. Area of the CA of 24 h old females resulted from PII pre-treated day 3 nymphs was $2.51\pm0.14 \times 10^{-3}$ mm^2 and that of the female treated on day 0 was $2.98\pm0.023 \times 10^{-3}$ mm^2 whereas that of control female of the same age was $5.68\pm0.535 \times 10^{-3}$ mm^2. Histologically, CA showed atrophy after treatment with PII. Cells were arranged more peripherally, leaving cell free areas in the disintegrating matrix. Cytoplasm was apparently degenerated and less distributed and nuclei became pycnotic. Darkly stained granular bodies were observed in the matrix (Fig. 9). In the CA of control females, the parenchymatous cells were normal in appearance and uniformly distributed (Fig. 10).

DISCUSSION

Both nymphal and adult treatments with PII induced dose-dependent reduc-

Table 1. Effects of treatments of day 2 fifth instar nymphs with PII and JHA on the preoviposition period and fecundity.

Treatments	Dosage (μg/cm²)	n	% Mortality	No. of females which laid eggs	Preoviposition period (days) (Mean±SD)	Average number of eggs laid during 8 days
PII	3.0	40	6.78	18	4±1	58
PII	5.0	40	26.80	0	-	-
PII* + JHA	3.0 3.0	45	12.60	22	4±1	49
PII** + JHA	3.0 3.0	42	9.80	19	4±1	52
Control	-	30	-	30	2±0	160

* Day 2 nymphs were treated with PII. These nymphs were exposed to JHA on day 3.
** Day 2 nymphs were treated with PII. The ecdysed adults were exposed to JHA on day 0.

Table 2. Effects of treatments of day 0 brachypterous females with PII and JHA on fecundity.

Dosage (μg/cm²)	n	No. of survivors	% of females which laid eggs	Preoviposition period (days) (Mean ± SD)	Average number of eggs laid during 12 days	Fecundity index
0.5	50	46	94.0	4 ± 0	244	61.00
1.0	50	40	95.0	4 ± 0	190	47.50
3.0	72	54	87.0	4 ± 0	23	5.75
5.0	45	30	66.7	4 ± 0	7	1.75
10.0	20	0	-	-	-	-
2.0 (PII) ± 3.0 (JHA)	48	22	45.8	7 ± 1	116	16.57
Control	30	30	100.0	2 ± 0	290	145.00

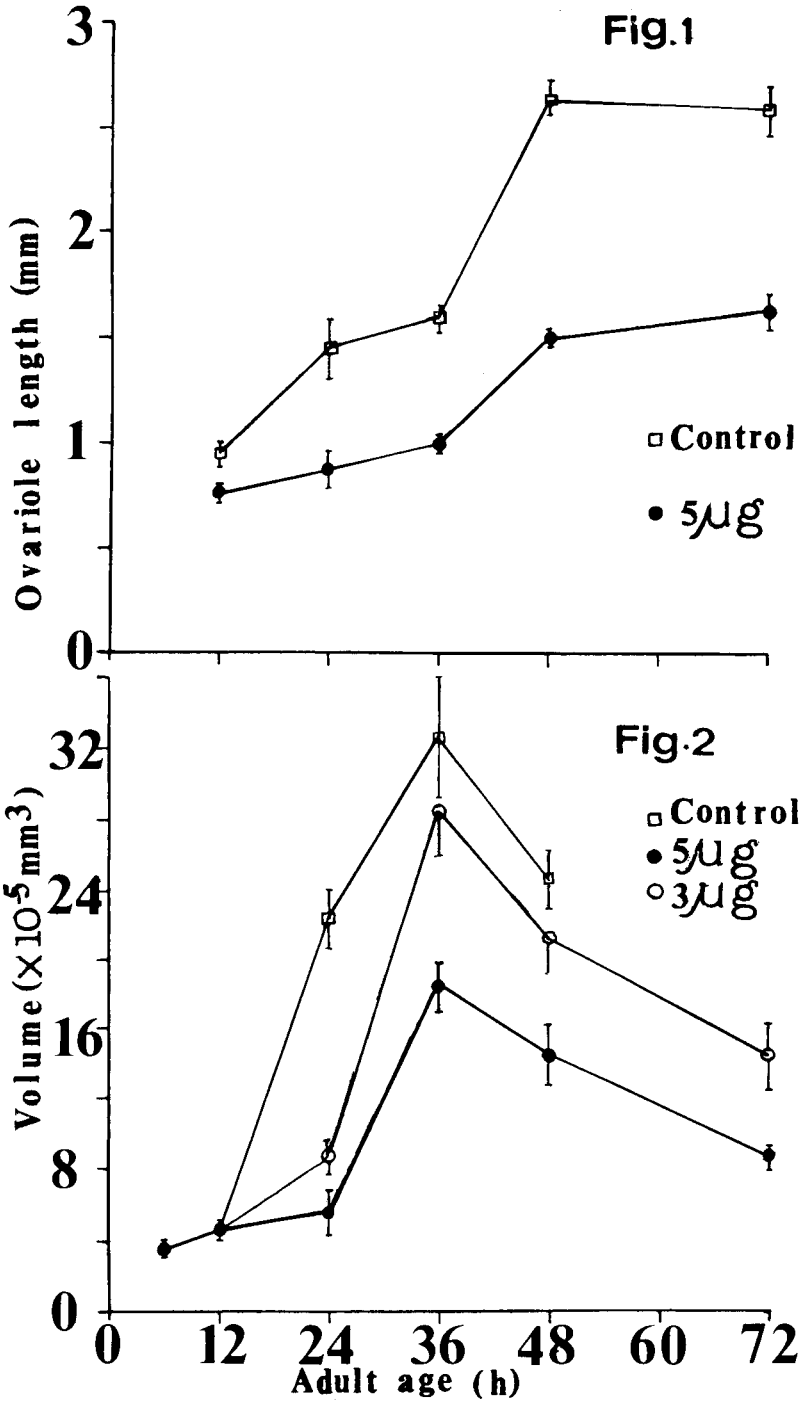
Table 3. Effects of treatments of day 2 fifth instar nymphs with PII on the growth of terminal oocyte.

Dosage	Variables (mm)	Size of terminal oocyte of adults of age		
($\mu g/cm^2$)	(Mean ± SE)	12 h	24 h	48 h
3.0	Length	0.078 ± 0.003	0.079 ± 0.0070*	0.111 ± 0.003*
	Width	0.027 ± 0.004	0.033 ± 0.0006*	0.044 ± 0.001*
5.0	Length	0.048 ± 0.005	0.076 ± 0.0020*	0.093 ± 0.005*
	Width	0.023 ± 0.003	0.032 ± 0.0006*	0.040 ± 0.002*
Control	Length	0.076 ± 0.002	0.115 ± 0.0020	0.134 ± 0.003
	Width	0.032 ± 0.002	0.059 ± 0.0010	0.058 ± 0.001

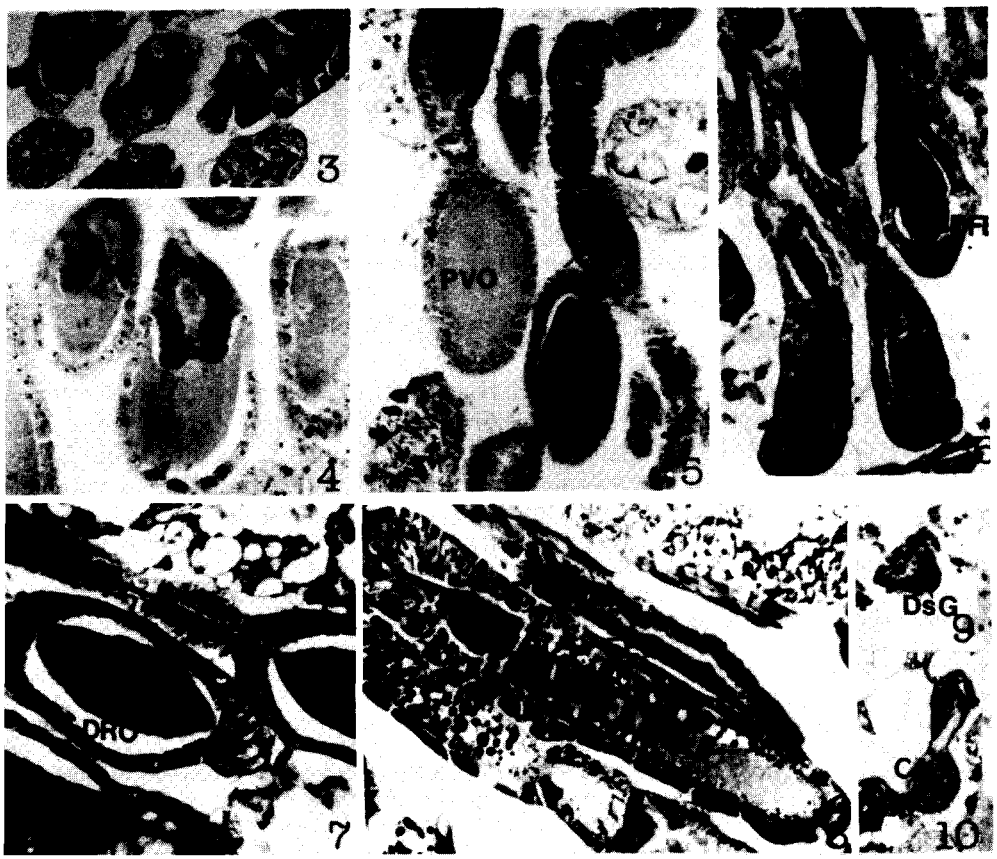
* Significantly different from control at $P<0.005$

tion in fecundity. This may be either due to less penetration of the compound in lower doses or due to dose-dependent inactivation of CA by PII (Wennauer *et al.* 1989). Disintegration of the germarium reduced oocyte production as well as supply of nutritive materials required for maturation of oocytes in the vitellarium, thus caused the infecundity. Treatment of JHA to PII-pretreated final instar or to adults resulting from PII-treated final instar nymphs of *N. lugens* did not enhance fecundity. Unlike nymphal treatment, JHA treatment to PII-pretreated adult females restored to a certain extent normal egg-laying. These observations indicate requirement of intact CA in the later stages of final instar for proper pre-ecdysial differentiation and maintenance of the ovary. Presence of a JH surge late in the final instar nymphs of the hopper was suggested earlier (Iwanga and Tojo 1986) which is presumed to be crucial for the pre-ecdysial growth and maturation of the ovariole. Chemical allatectomy by PII might have altered the JH titre late in the final instar of *N. lugens*. Moreover, present observations on CA of the treated insects showed severe degeneration in the matrix. Retarded growth of ovariole and resorption of oocytes are apparent symptoms of JH deficiency (Bell and Bohm 1975; Abu-Hakima *et al.* 1977). In *Aedes aegypti,* early allatectomy prevented the pre-vitellogenic growth of follicles (Gwadz and Spielman 1973). Abnormal multiplication of follicular epithelial cells and absence of distinct oocyte-follicular epithelial interphase are due to loss of control over cellular division and egg maturation processes. Nymphal as well as adult treatments of precocenes induced infecundity and antigonadotropic effects in a few sensitive insect species (Bowers 1985; Alrubei 1986; Brasiliero 1987; Sam Mathai *et al.* 1989).

Histopathological effects observed in the CA of PII-treated insects clearly showed that PII acts as a systemic allatocidin in *N. lugens* adults unlike that reported in a Japanese strain (Ayoade *et al.* 1996), where PII treatment only suppressed the CA activity. The variation in responses is attributed to the dose of PII treated. Low-doses of PII (Ayoade *et al.* 1996) might become detoxified and excreted before reaching CA tissues, caused a subthreshold concentration of PII that was only able to suppress activity of JH. Moreover the lower doses of PII induces less intense response and reflects a slower effect on CA cells (Schooneveld 1981) which results in slower decrease of effective JH titer. High doses of PII elicited the intensity of de-



Figures 1-2. Effects of treatments of day 0 brachypterous females of *N. lugens* with precocene II on ovariole length (1) and terminal oocyte volume (2) at different intervals (n=10 each). Vertical bars represent standard error of the mean.



Figures 3-10. Histological sections: **3-6:** Effects of treatment of day 3 fifth instar nymphs with 5µg PII cm^2 on ovarian development and oocyte maturation: degenerated germarium of 24 hour old female **(3),** invasion of follicular epithelium (FEI) in the resorbing oocytes (RO) at 36 hours **(4),** terminal previtellogenic oocytes (PVO) at 72 hours **(5)** and resorbing oocytes with degenerated follicular epithelium (DFE) and pycnotic epithelial cells (PsC) at 72 hours **(6). 7:** terminal oocytes of 8 day old female pretreated with 5µg PII/cm^2 on day 0 showing darkly stained ooplasm (DRO) and large gaps in between the ooplasm and follicular epithelium **8:** vitellogenic oocytes of 24 hour old control female. **9:** disintegrating corpus allatum of day 1 female pretreated with 5µg PII/cm^2 on day 0 showing darkly stained granular bodies (DsG) in the matrix. **10:** corpus allatum of day 1 control female.

generation of allatal cells (Schooneveld 1981; Bitsch and Bitsch 1984; Piulachs *et al.* 1989) as observed in the present study. Precocene exhibited selective cytotoxic activity and induced cellular death in the CA of *Oncopeltus fasciatus* females and in many other insect species (Bowers 1985; Bowers and Martinez-Pardo 1977; Unnithan *et al.* 1977). The anti-allatal effect of precocene is due to the oxidative bioactivation of the compound in the allatal tissues, mediated through local formation of a highly reactive 3, 4-epoxide (Brooks *et al.* 1979; Soderlund *et al.* 1980) catalyzed by cytochrome P450-linked monooxygenase enzymes (Pratt *et al.* 1980, Soderlund *et al.* 1980).

Present investigations indicated the requirement of CA and/or JH for the pre- and post-ecdysial ovarian growth, maintenance and egg maturation in *N. lugens*. The antigonadotropic effects observed are unambiguously due to a sharp decline in the synthetic activity of CA under the PII influence.

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