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Acquiring Vitellogenic Competence in the Rice Pest *Nilaparvata lugens* Stal: Effects of a Juvenile Hormone Analogue, Hydroprene

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Though many insecticides are commercially available, development of resistance, pest resurgence and effects on non-target organisms led to the search for alternate insect pest management (IPM) strategy based on larval growth and reproductive fitness. Reproductive potential of insects depends on its acquiring of vitellogenic competence which is under hormonal control. Exogenous application of analogues of JH (JHAs) and ecdysterone could derail normal development and reproduction in insects by manipulating an array of physiological processes. In the rice pest, brown planthopper, *Nilaparvata lugens*, JHA, hydroprene induced metathetely from the fifth (final) instar nymphs in an age-dependent manner. Day 0 of the final instar showed highest sensitivity to induce this abnormal development. Adults emerged from treated day 3 nymphs looked normal. Both the morphotypes were reproductively incompetent and showed partial to complete sterility. Pre-adult exposure of the ovarian tissue to hydroprene suppressed mitotic division of germinal cells and induced abnormalities in the later stages of growth and differentiation of ovary in *N. lugens*. More over the nymphal exposure to hydroprene inhibited patency changes of follicular epithelium and affected competence of the follicles for yolk sequestration. In the absence of ovarian growth and oocyte differentiation, germarium found disintegrated, trophic core regressed and terminal oocytes resorbed. Hydroprene exposure to newly ecdysed brachypterous females did not affect ovarian development and egg production. Proper larval-adult transition appeared as a prerequisite for vitellogenic competence in *N. lugens* for which the ovarian tissues must be exposed to ecdysterone in the internal milieu devoid of JH.

Key words: Hydroprene, Vitellogenic competence, *Nilaparvata lugens*

Introduction

In insects, larval growth, development and reproduction are controlled by endocrine factors under the cascade of events initiated by brain neurosecretory cells. Juvenile hormone (JH) exerts pleiotropic functions in the insect life cycle (Hartfelder, 2000) including reproduction (Dubrovsky *et al.*, 2002; Rauschenbach *et al.*, 2003). Though the role of JH in metamorphosis is well conserved across insect orders, its role in reproduction varied in different insects. On the other hand the steroid moulting hormone, 20-hydroxyecdysone (ecdysterone; 20E) is the master regulatory molecule that triggers ovarian development and follicular epithelium differentiation in many insects. Any disturbance in the hormonal milieu at critical periods could induce abnormal development or reproductive incompetence (Don Wheeler and Engelman, 1991). Inhibition of ovarian development and induction of sterility after JHA treatment of different insects species were reported and reviewed (Rudolph, 1989). The JHA, hydroprene (ZR-512; ethyl-3,7,11-trimethyl-dodeca-2,4-dienoate) disrupts metamorphosis, leading to deformity and sterility in adults that have been exposed during the final nymphal instar (Bao and Robinson, 1990; Short and Edwards, 1992). Recent work on physiological basis for the toxicity and morphogenetic effects of JH/ JHAs has linked these effects with interference with the expression or action of certain genes, particularly the Broad-Complex (BR-C) transcription factor gene, that direct metamorphic change. Therefore, JH is a necessary molecule at certain times in insect development but becomes toxic when present dur-

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ing metamorphosis (Wilson, 2004).

Rice brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae) is a serious pest of paddy, develops resistance to most of the insecticides and causes resurgence in the field. In order to develop an alternative strategy in management of the rice pest, compounds of physiological interest were selected to evaluate its morpho-physiological effects on *N. lugens* (Pradeep and Nair, 1998; Bertuso *et al.*, 2002). Morphological development, vitellogenic competence and reproductive potential of *N. lugens* were examined after exposing variously aged final instar nymphs and newly ecdysed females to the JHA, hydroprene to elucidate the role of JH in ovarian differentiation and oocyte development in this pest.

Materials and Methods

Rice brown planthopper, *N. lugens* was reared as described earlier (Medrano and Heinrichs, 1985) on the susceptible rice variety T(N)1. When the larvae were moulted to penultimate (fourth) instar, they were selected and reared separately. On the day of moulting to the final instar (day 0), the nymphs ($n = 15$ each with five replications) were collected and exposed for two hrs, to 5 μg/cm^2, 10 μg/cm^2 and 20 μg/cm^2 of the JHA, hydroprene (gift from Dr. G. B. Stall, Zoecon Corporation, Palo Alto, CA) smeared on a Petri dish, by contact method (Pradeep and Nair, 1989). Day 1, day 2 and day 3 fifth instar nymphs and day 0 normal mated brachypterous females were also exposed to the same concentrations of hydroprene. Control insects of the same age were exposed to a smear of the solvent, acetone for the same period. Experimental and control insects were reared separately. Mortality and moulting were recorded every 24 hrs. On moulting to the adult/ metathetelic adult, the insects were fixed in Bouins' fluid at 0 hr, 24 hrs, 48 hrs and 72 hrs. The whole body sections were stained with Heidenhains' haematoxylin - eosin and were examined under a Carl Zeiss Universal microscope and photographed.

Results

Effects on morphogenesis

Exposure of variously aged fifth instar *N. lugens* nymphs to different concentrations of hydroprene were less toxic but induced significant ($P < 0.001$) prolongation (4.715 ± 0.053 days) in instar duration when compared to the control final instar nymphal duration of 3.311 ± 0.046 days. Hydroprene treatment resulted in disruption of development, moulting and metamorphosis (Fig. 1). Large proportion of the treated day 0 nymphs were moulted into metathetelic (juvenilized) adults having nymphal characteristic abdominal white patches and short expanded transparent wings but without bristles. The female juveniles showed presence of ovipositor and the male forms had aedeagus. Few remained as permanent nymphs and died later without attempting a moult. Proportion of these juvenilized adults reduced with increase in age at treatment (Fig. 1). Most of the treated day 2 and day 3 nymphs moulted into normal looking adults. Control nymphs moulted into normal adults along with untreated ones.

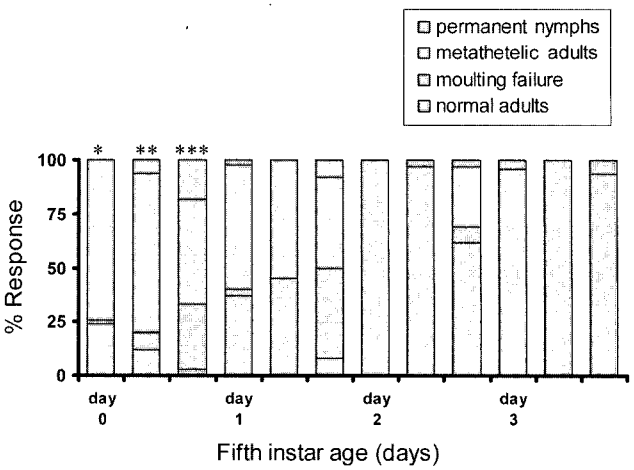

Fig. 1. Percentage of responses of variously aged fifth instar nymphs of *N. lugens* to 5 μg/cm^2 (*), 10 μg/cm^2 (**) and 20 μg/cm^2 (***) of hydroprene in induction of morphogenetic effects.

Effects on ovariole and oocyte development

Acetone treated control females showed a preovipostion period of 2 ± 0 days and laid a total of 244 ± 6 eggs in 12 days. Metathetelic adults did not lay any eggs during its survival of five days after eclosion. Ovarioles of 48 hrs old metathetelic adults measured 1.092 ± 0.138 mm whereas in the control, it measured 2.653 ± 0.143 mm in length (Fig. 2). The ovarioles of metathetelic adults and normal looking females showed histopathological changes in the germarium and vitellarium (Fig. 3). Cellular divisions of the germ cells were inhibited and mitotic figures were markedly lacking among the loosely arranged cells. Compound egg chambers appeared by atypical clustering of oogonial cells in the lower zone of the germarium. The oogonia had exceptionally large nucleus with darkly stained cytoplasm and were ensheathed by thick membrane. Few previtellogenic oocytes were observed in the terminal end of the ovary of the metathetelic adults. Follicular epithelium was columnar around the terminal oocytes in 48 hrs old metathetelic females and disorganized by 72 hrs after eclosion. Inter-

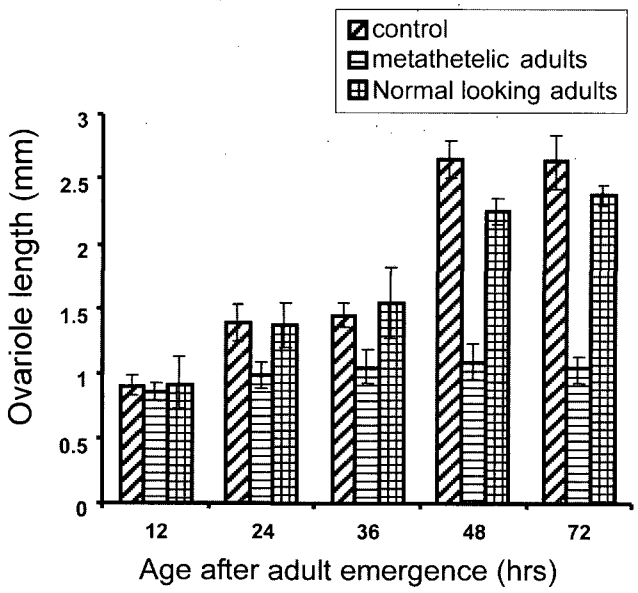

Fig. 2. Variation in length (mean ± S.D) of ovariole of metathetelic adults and normal looking brachypterous adults resulted from final instar nymphs of *N. lugens* exposed to 20 μg/cm^2 of hydroprene and of control brachypterous adults of *N. lugens*.

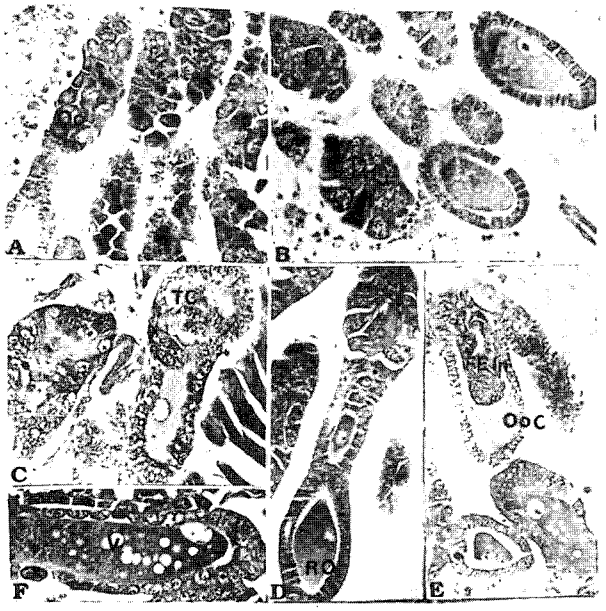

Fig. 3. Section through 48 hrs old metathetelic adults (A - B) and 24 hrs old (C - D), 48 hrs old (E) and 72 hrs old (F) normal looking females resulted from final instar nymphs of *N. lugens* exposed to 20 μg/cm^2 hydroprene. Note the presence of clustered oogonial cells (Oo), accumulation of cellular debris in the trophic core (TC), hyperplasia of follicular epithelium and formation of ooplasmic cap (Ooc), resorbing oocyte (RO) and vacuolated ooplasm (V). (magnification for A, B, C, D, E and F are × 476, × 603, × 480, × 573, × 540, × 667, respectively).

cellular boundary became indistinct and necrotic. The follicular epithelium did neither under go change in shape and size nor developed intercellular spaces. Ooplasm appeared smooth and vacuolated and chromatin material clumped to form highly stainable granules in nucleus. In control females, follicular epithelium developed intercellular spaces that facilitate yolk incorporation during vitellogenesis. The normal looking females showed significantly ($P < 0.004$; Student's *t*-test) less fecundity. The females resulted from the day 3 nymphs exposed to 5 μg and 20 μg hydroprene laid 196 ± 15 eggs and 112 ± 21 eggs respectively. Ovariole length did not vary significantly in these insects from that of control (Fig. 2). The effects on ovary were highly manifested after 72 hrs of emergence. Follicular epithelium became multi layered resulting in hyperplasia. Follicular cells invaded the resorbing oocytes and the ooplasm formed a cap like structure (Fig. 3). In the four days old females, ovarioles were empty without any oocytes. Degenerated cellular debris accumulated in trophic core and nutritive cords disappeared. Female adults treated with hydroprene on day 0, laid 254 ± 21 eggs and did not show any histopathological changes in the ovary.

Discussion

JH analogue, hydroprene induced delay in moulting, formation of metathetelic adults as well as moulting aberrations in an age-dependent manner when treated on the final nymphal instar of *N. lugens*. Such failures in metamorphosis had been attributed to low titre of moulting hormone, ecdysteroid (Delbeque and Slama, 1980), induced by a direct or indirect effect of JHA on prothoracic glands (Balamani and Nair, 1992). At critical period, when the endogenous JH titre is low, JH application hampered metamorphosis resulting in disproportionate growth of different parts and caused ecdysial failures. The hydroprene treatments elicited age-dependent juvenilization in the ensuing adult moult with day 0 and day 1 as the most sensitive periods for inducing metathetely. In the final stadium, the sensitive period of JH occurs before ecdysial peak of ecdysone which is characterized by the absence of JH. During this period, the peripheral target tissues receive hormonal instructions and become committed to metamorphic program (Slama, 1985). In the presence of hydroprene during this critical period, ecdysone elicited a metathetelic moult and induced formation of juvenilized adults. After the critical period, JHA failed to produce any morphogenetic effects since the cells committed for adult differentiation become irreversibly programmed for the completion of differentiation (Sehnal, 1985). Presence of

adult type sclerotized external genitalia in the metathetelic forms indicated that in *N. lugens,* commitment of primordial cells of genital appendages for metamorphosis had already been taken place, before the final instar as noticed in some hemipterans (Sehnal, 1985). Thus when the ecdysone elicited the metathetelic moult, these primordial cells formed normal genital appendages in the metathetelic forms.

Nymphal treatment of hydroprene induced partial and complete sterility respectively in the resulting normal and metathetelic adults. Significant retardation in linear growth of ovary, disintegration of germarium, inhibition of cellular division among prefollicular cells, hyperplasia and atypical clustering of oocytes are due to lack of proper differentiation of follicular epithelium as excessive JHA prevents normal cell division (Mathai and Nair, 1990; Meola *et al.,* 2001). Since mitotic division and oogonial differentiation are dependent on critical concentration of ecdysteroids (Parlak *et al.*, 1992), it can be presumed that reduction of ecdysterone titre due to JHA is responsible for suppression of mitotic activity.

In control *N. lugens* females, follicular epithelium surrounding the maturing oocytes underwent patency changes, which facilitated incorporation of yolk from the haemolymph into the ooplasm through the intercellular spaces (Telfer *et al.,* 1981). In the metathetelic adults, none of the oocytes exhibited the patency changes in follicular epithelium or signs of yolk sequestration and therefore no mature eggs were produced. In few insects, JHA treatment on larvae induced early development of ovary (Kono and Ozeki, 1987) and appearance of yolk protein, vitellogenin (Koopmanschap *et al.,* 1992) but JH treatment alone was insufficient to induce complete ovarian development or incorporation of yolk into oocytes. These findings support the observations in *N. lugens* that preexposure of JH early in the final instar could not induce proper sequence of ovarian development and oocyte maturation. JH not only governs the synthesis of yolk protein in the fat body (Wyatt, 1980) but also mediates the uptake of yolk protein into the ovary (Davey *et al.*, 1993). But before the uptake, ovary must acquire competence for vitellogenesis, which develops during the final instar (Don Wheeler and Engelmann, 1991). Bell and Sam (1975) observed that larval- adult transition as a prerequisite for acquiring the vitellogenic competence, for which the ovary must be exposed to a milieu of ecdysteroid in the absence of JH. Lack of patency changes in the follicular epithelium and yolk sequestration into the oocytes of metathetelic adults are signs of absence of vitellogenic competence. JHA treatment on day 0 could hinder the increase in ecdysteroid titre and curtailed production of vitellogenin (Engelmann, 2002). On the other hand, JHA exposure on the last day (day 4) of final instar did not inhibit oocyte production but exerted delayed ovicidal effects. In the normal looking females, hydroprene induced sterility was due to regression of the germarium and tropharium which blocked supply of nutrients to the developing oocytes. Moreover, hydroprene treatment to newly ecdysed females did not affect egg production. Since female adults utilize JH for oocyte maturation, the treated doses of hydroprene may be insufficient for derailing oocyte development. Presumably, presence of ecdysterone surge in the final instar *N. lugens* nymphs could have elicited normal nymphal-adult transformation of ovarian tissue and initiated the oogonial differentiation and JH regulates vitellogenesis after adult eclosion.

In *N. lugens*, tissue commitment for ovarian differentiation and development occur on day 0 in the final instar. In the absence of JH, ecdysterone induces cellular division in the germarium and competence in the follicular epithelial cells for yolk incorporation. Once the ovarian differentiation is completed, JH regulates the vitellogenesis, leads to normal egg formation after adult eclosion.

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