Int. J. Indust. Entomol. Vol. 10, No. 2, 2005, pp. 137~141

International Journal of
Industrial Entomology

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

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(Received 24 January 2005; Accepted 3 June 2005)

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.

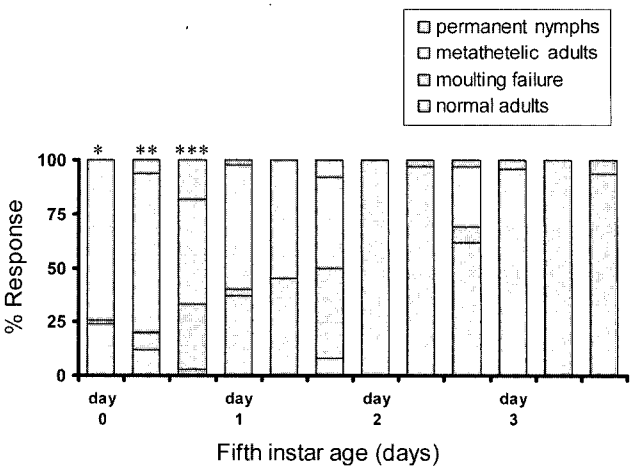

Fig. 1. Percentage of responses of variously aged fifth instar nymphs of *N. lugens* to 5 μg/cm^2 (*), 10 μg/cm^2 (**) and 20 μg/cm^2 (***) of hydroprene in induction of morphogenetic effects.

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-