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Metamorphosis and Wing Formation in the Brown Plant Hopper, *Nilaparvata lugens*, After Topical Application of Precocene II

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In the present paper the effects of precocene on *N. lugens* strains obtained by successive selection for adults with specific wing form under highly crowded conditions over 70 generations are reported. When precocene II was applied, macropterous adults developed from presumptive brachypters. Our results also demonstrated the disruptive effects of precocene II on metamorphosis, and we report here the induction of precocious metamorphosis in *N. lugens* in details for the first time. The sensitive periods to precocene II, affecting wing dimorphism and metamorphosis, differed between the two strains.

Key words: juvenile hormone, metamorphosis, wing development

INTRODUCTION

Wing dimorphism giving long-winged macropterous and short-winged brachypterous adults in the brown planthopper, *Nilaparvata lugens*, is known to be caused primarily by population density experienced during the nymphal stage (Kisimoto, 1965). In females, crowding promotes macropterization, while in the males, a moderate nymphal density promotes brachypterization (Kisimoto, 1965).

On the other hand, juvenile hormone has long been presumed to control metamorphosis, and induce wingless or brachypterized wing form when the titer is high (Nijhout and Wheeler, 1982; Hardie and Lees, 1985). Only a few cases of such effects of juvenile hormone on wing polymorphism have been reported (*Aphis fabae:* Hardie, 1981; *Nilaparvata lugens:* Iwanaga and Tojo, 1986; *Gryllus rubens:* Zera and Tiebel, 1988).

As a consequence of the discovery of the pro-allatocidins, better known as precocenes (a name derived from their ability to induce precocious develop-

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ment in insects) by Bowers (1975), it became possible to induce chemical allatectomy. The effect of the precocenes on developmental processes such as sex pheromone production, diapause induction, ovicidal activity, and so on have been reported (Bowers, 1975). Moreover, precocenes have also been reported to promote wing development by lowering JH titers (Mackauer et al., 1979, Kambhampati et al., 1984). Recently, two strains of *N. lugens* predominantly exhibiting a specific wing form, brachypterous or macropterous, at a wide range of densities have been generated by successive selections for adults with specific wing form (Morooka and Tojo, 1992). These special strains enabled us to study genetically based wing polymorphism, effects of precocene II on the molting profile and to establish the "sensitive period" for the control of developmental pathways.

MATERIALS AND METHODS

The experimental insects *(Nilaparvata lugens)* were kept in cages (11 × 12 × 18 cm) containing rice seedlings (variety Reiho) under a 16L:8D photoperiod at 25 ± 2°C (Morooka et al., 1988). The brachypterous and macropterous strains, selected for yellowish brown brachypters and blackish macropters, respectively, produced about 100 percent short-winged and long-winged individuals under a broad range of densities. Hereafter, the former and latter strains are referred to as presumptive brachypters and macropters, respectively.

Precocene II (Sigma Chemical Company, St. Louis, MO) was dissolved in acetone to yield 1 μg/μl stock solutions and stored at –20°C. These were serially diluted with acetone at the time of each application. 2nd, 3rd, 4th, and 5th stadia insects were treated within 6 or 12 h of molt unless otherwise stated, after anaesthetizing with CO_2. After treatment, they were reared on rice seedlings in a glass cylinder (2 cm diameter × 2- cm tall) at a density of 5 insects per plant. Precocene II was applied to the abdomen using a syringe (Terumo) and a micro applicator (Burkard) at the dosage rates of 1, 10, 100, and 1,000 ng per insect dissolved in 0.1 μl of acetone. The molting profile in the treated insects was monitored by visually observing for ecdysis every 6 h. The rice seedlings were changed every 24 h. The wing form of emerged adults and the time to final molt were noted. Treated individuals were described as "precocious adults" when they skipped a nymphal stadium during the course of metamorphosis into the adult stage.

RESULTS

Effects of Precocene II Treatment on Metamorphosis

Topical application of precocene II to the presumptive brachypters produced different responses depending on the nymphal stage at treatment. Table 1 shows induction of precocious metamorphosis when precocene II was applied to 6-h-old 2nd stadium or older nymphs. Starting from the 1 ng treatment level, i.e., the 6-h-old 2nd stadium, presumptive brachypters molted from the 4th stadium straight into the precocious adult form, thereby skipping the 5th nymphal stadium. Although the treatments did not change the duration of the 3rd stadium, the duration of the 4th stadium of these preco-

TABLE 1. Induction of Precocious Metamorphosis by Precocene II Treatment in the Presumptive Brachypterous and Macropterous Strains of *N. lugens**

Strain	Stage at treatment	Dosage of precocene (ng)	% of precocious metamorphosis
Presumptive brachypters	6 h after molting to 2nd stadium	0	0
		1	100
		10-100	100
	6 h after molting to 3rd stadium	0	0
		1-10	0
		100-1,000	100
	6–12 h after molting to 3rd stadium	0	0
		1-10	0
		100-1,000	0
Presumptive macropters	6 h after molting to 2nd stadium	0	0
		1-1,000	0
	12 h after molting to 3rd stadium	0	0
		1	0
		10-1,000	100
	6–12 h after molting to 4th stadium	0	0
		1-10	0
		100-1,000	100

*Precocene II was dissolved in 0.1 µl acetone, and topically applied to each animal at the stages noted. Control insects were treated with 0.1 µl acetone. Twenty-five animals were treated at each dosage. Five animals were enclosed in a test tube (2 × 20 cm height) with a rice seedling, and reared at 25 ± 2°C under 16 h L:8 h D photoperiod. Molting was checked every 6 h.

ciously metamorphosed animals shortened with increasing dosage; by 16 h at the 1,000 ng dosage level. As a result, precocene treatment led to a reduction in the average number of hours required for the insects to emerge as adults by about 24–36 h (data not shown). Similar results were obtained for the presumptive brachypters treated within 6 h of the 3rd instar and 6- to 12-h-old 3rd instar nymphs, although no precocious metamorphosis was observed in the latter treatment despite a progressive prolongation of the 3rd instar duration. The insects could not be induced to molt into the adult stage directly from the 4th instar even when the dosage was increased to 10 µg; high mortality rates made further dosage increase impractical. In the experiments reported here, survival rate varied between 80–96%. No effects were observed on metamorphosis when the presumptive brachypters were treated in the 1st, 4th, or 5th stadia.

The stages sensitive to precocene II treatment differed considerably in the presumptive macropterous hoppers. No effects were observed on metamorphosis when presumptive macropters were treated during the 2nd stadium. By contrast, the presumptive macropters treated with 1 ng precocene II within 6–12 of molting to the 3rd nymphal stadium exhibited a prolonged 3rd instar period with a corresponding shortening of the 4th instar stadium (Table 1).

The same results were obtained when 6- and 12-old 4th stadium nymphs of the presumptive macropters were treated with precocene II, although a larger dose was required in the latter treatment.

Effect of Precocene II Treatment on Wing Form

When the presumptive brachypterous hoppers were treated with precocene II during the 2nd stadium, macropterous wing forms were induced as shown in Figure 1a and b; the same results were obtained when the presumptive brachypters were treated in the 3rd stadium. In these stadia, precocene II treatment caused a gradual reduction in the percentage of the hoppers that emerged as brachypters, i.e., more presumptive brachypterous hoppers emerged as macropters in a dose-dependent manner. In all the treatments (1–1,000 ng), the males were more sensitive, as more males emerged as macropters than the females. Precocene treatments of the presumptive brachypters in the 1st, 4th, and 5th stadia shortly after ecdysis did not induce any macropterous adults. Moreover, there were no differences between the treated hoppers and the control in relation to wing form.

DISCUSSION

Our results demonstrate the induction of precocious metamorphosis in both strains of *N. lugens* used in this study, and also macropterization of presumptive brachypterous hoppers by topical application of precocene II. In the pre-

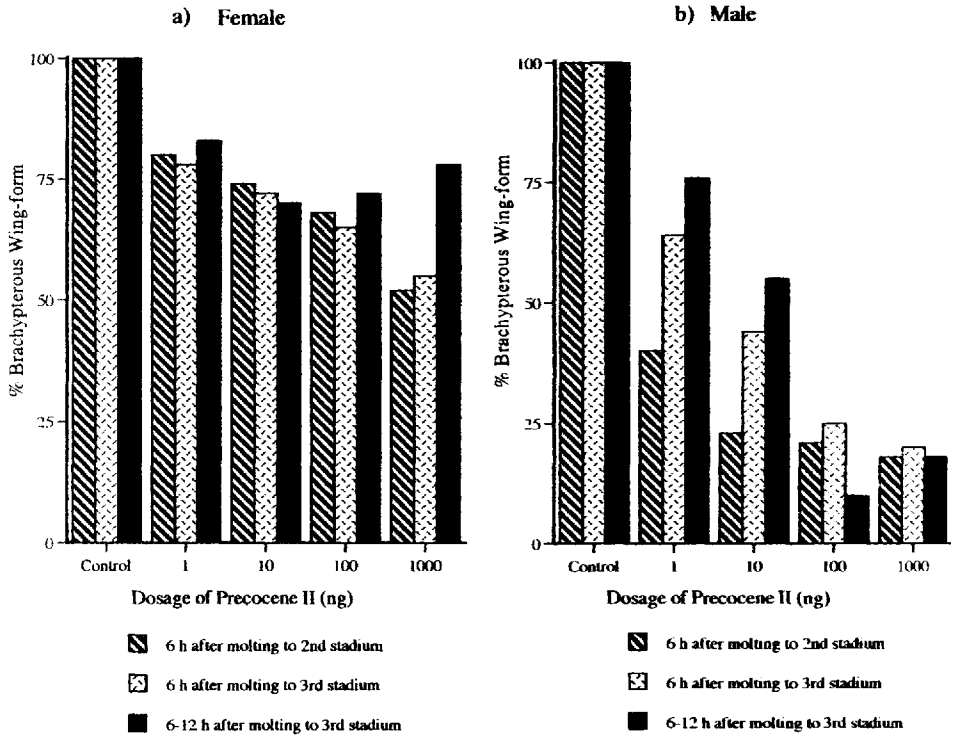

Fig. 1. Induction of macropterous wing-form by precocene II treatment in the presumptive brachypterous strain of *Nilaparvata lugens*. Precocene II dissolved in 0.1 µl acetone was topically applied to respective nymphs at the stages noted.

sumptive brachypterous strain, the formation of macropterous wing-form was enhanced more prominently in males, than in females, with increasing dosage from 1 to 1,000 ng of precocene II, in all cases when the nymphs were treated at 6 h of 2nd stadium, 6 h or 12 h of 3rd stadium (Fig. 1a and b). No effects were observed when nymphs in other developing stages were treated (data not shown). Although the mechanism of juvenile hormone titer reduction during these periods, especially corpora allata activity around the 3rd instar, is a question for further research, we speculate that a high juvenile hormone titer above a threshold level shortly before the last nymphal ecdysis determines the induction of brachypterous wing-form, and that precocene II works to induce long-wing forms by lowering the titer under a threshold level during this period. Also, the difference in potency of precocene II in the males and females may be a result of difference in penetration through the cuticle or difference in metabolic breakdown between the sexes, although this remains to be proven in further studies.

According to Riddiford (1976), precocious metamorphosis results when ecdysone is released in the absence or during sub-threshold level of juvenile hormone early in the feeding period of the penultimate larval instar. Present results show that in the presumptive macropters, 100 ng of precocene II treatment to the 4th stadium nymphs was effective to induce precocious metamorphosis. On the other hand, in the 4th stadium nymphs of presumptive brachypters, no indications of disruption of metamorphosis, either by way of prolongation of instar duration or by skipping of instar(s) to form a precocious adults, were observed by precocene II application even when dosage was increased to 10 μg (data not shown). These results suggest that the brachypterous strain possesses such a high activity of corpora allata for juvenile hormone secretion at the early penultimate nymphal instar, which could not be suppressed even by large doses of precocene II. Clarifying this supposition forms the focus of our ongoing experiments aimed at verifying if the effects of exogenously applied precocene can be rescued by successive application of juvenile hormones or juvenile hormone agonists and vice versa, when applied sequentially and/or simultaneously at the precocene II-sensitive stadia including the penultimate instar.

Precocene II application caused the treated presumptive brachypter hoppers to skip an instar, i.e., precocious metamorphosis, when treated in the 2nd or early 3rd nymphal stadium, while in the presumptive macropters, precocene II caused precocious metamorphosis by the treatment to the 3rd or 4th nymphal stadia. The dosage necessary to disrupt metamorphosis increased as the hoppers were treated in later nymphal stage (data not shown). Thus, the sensitive periods to precocene II for disruption of metamorphosis differed between the two strains, supporting also the hypothesis that the endocrinological events proceed differently between the two strains even during the 2nd and 3rd instars.

Alate progeny has been shown to be induced from apterous adults of several aphid species prenatally treated with precocene I, II, or III (Mackauer et al., 1979; Delislie et al., 1983; Kambhampati et al., 1984; Hardie, 1986, 1987). In these aphids, potencies of precocene analogues for induction of alate do not coincide with those for precocious metamorphosis; these results have raised doubts about the presumption that precocene promotes wing devel-

opment because it decreases juvenile hormone titers (Hardie, 1986, 1987). The effects of precocene in these aphids seem to differ from those in *N. lugens*, where precocene II exhibited both effects to promote long wing formation and disrupt metamorphosis in a dose-dependent way with 1 ng, providing an effective dose in some cases. Moreover, the present paper describes precocene II effects on an individual, while the above-mentioned studies on aphids observed effects on progeny born to the treated insect.

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