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Original Article

Multiple Physiological Activity of an Anti-Juvenile Hormone, Precocene 2 on the Whitebacked Rice Planthopper

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The anti-juvenile hormone, precocene-2, showed multiple biological activity against the whitebacked rice planthopper, *Sogatella furcifera*. When newly hatched nymphs were released on rice plants treated with 500 ppm of precocene-2 and continuously contacted with the chemicals, about half of the insects died within the first stadium (rapid toxicity). The other half survived through the following two or three nymphal stadia and then developed into precocious adults in the fourth or fifth stadium (prothetely). In this case, the insects seem to have been rather strongly affected by the anti-JH activity of precocene-2. On the other hand, when the insects were transferred from the treated rice plants to untreated ones on two, four and six days after the release, a large portion of the surviving insects (41.9% at maximum) developed into supernumerary nymphs in the sixth stadium (metathetely). In this case, the insects were supposed to have been incompletely affected by the activity of precocene-2. A juvenile hormone analog, NC-170, induced metathetely by itself, but none of the natural JH-1, JH-2 and JH-3 showed any morphogenetic effect. In addition, NC-170 rescued the prothetely induced by precocene-2, but the three natural JH's did not.

INTRODUCTION

Anti-juvenile hormone (anti-JH) activity of precocenes was first reported by Bowers[1] against the large milkweed bug, *Oncopeltus fasciatus* in 1976. Up to now, biological activities of the chemicals including precocious metamorphosis, sterilization, inhibition of pheromone production, disturbance of embryogenesis, *etc.* have been demonstrated in a number of insects belonging to different orders, and these studies have shown remarkable usefulness of precocenes not only in basic but applied researches.[2-4]

Planthoppers belonging to sub-order Homoptera include several important pests in rice cultivation throughout Asia. Among this insect group, anti-JH effect and rapid toxicity of precocenes have been reported in the brown planthopper, *Nilaparvata lugens*.[5,6] However, precocious metamorphosis described in the paper[5] was not full premature metamorphosis. Furthermore, rescue effects by natural juvenile hormones (JH's) or juvenile hormone analogs (JHA's), which are intelligible proof of anti-JH effects, were not tested in the study.

In this paper, we demonstrate multiple biological activity of precocene-2 in the whitebacked rice planthopper, *Sogatella furcifera*, and rescue effects to precocene-induced precocious metamorphosis by simultaneous administrations of a JHA, NC-170.

MATERIALS AND METHODS

1. Animals

The whitebacked rice planthopper (WBRP), *S. furcifera*, was collected in paddy fields in Shimane Pref., Japan, in 1987 and has been

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reared on rice seedlings at 25°C, 65%R.H. under long-day (16L/8D) conditions.

2. *Chemicals*

Precocene-2 (P-2) [6,7-dimethoxy-2,2-dimethylchromene] and synthetic juvenile hormone-1 (JH-1), JH-2 and JH-3 were purchased from Sigma Chemical Company. Technical material (purity, >99%) of NC-170, [4-chloro-5-(6-chloro-3-pyridylmethoxy)-2-(3,4-dichlorophenyl)-pyridazin-3(2*H*)-one], was synthesized in Central Research Institute, Nissan Chemical Industries Ltd. (Fig. 1). P-2 and NC-170 were formulated into 10% wettable powder (WP) for contact application method.

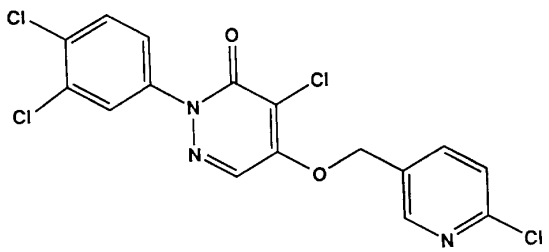

Fig. 1 Chemical structure of a JHA, NC-170.

3. *Normal Developmental Pattern in WBRP*

The juvenile stage of WBRP consists of five nymphal stadia. When the nymphs were reared on rice plants (rice stems) in glass tubes (20 cm height×2 cm diameter) at 25°C, 70%R.H. under long-day (16L/8D), a cumulative molting pattern of each stadium was obtained as shown in Fig. 2. On the basis of the result, we designed "transfer tests," in which insects were transferred from P-2 treated to untreated rice stems, or *vice versa* (Fig. 2).

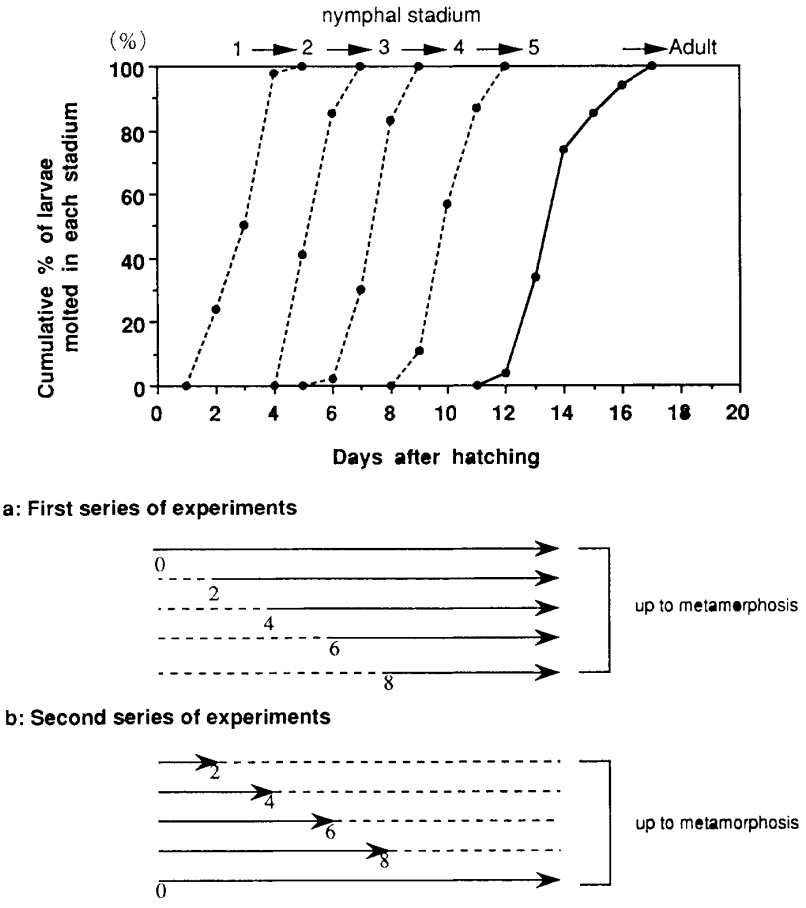

Fig. 2 A normal developmental pattern in juvenile stages of the whitebacked rice planthopper, and the experimental design of two series of "transfer test" for precocene-2.

(a) Reared on untreated rice for the indicated days (– – –) and then transferred to treated rice with 500 ppm precocene-2. (b) Reared on treated rice with 500 ppm of precocene-2 for the indicated days (——) and then transferred to untreated rice.

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4. Metamorphosis-Inhibiting Activity of a JHA, NC-170, and Three Natural JH's

Metamorphosis-inhibiting activity of NC-170 and three natural JH's in WBRP was tested with two different methods.

Contact application: Potted rice plants in six to seven leaf-stage were sprayed with various concentrations of NC-170 (10% WP) aquatic solutions until runoff, and air-dried. Stems were cut from the plants and put into glass tubes with small amount of water. Mid-penultimate (two day-old fourth stadium) nymphs were released into the tubes (five nymphs/tube, six replications in each concentration). Morphogenetic and other biological effects were observed everyday for ten days after release.

Topical application: Mid-penultimate nymphs of WBRP were anesthetized with carbon dioxide. Various doses of technical NC-170 and of the three natural JH's in 0.03 μl of acetone were topically applied to the dorsal thoracic surface. Thereafter, the treated insects were reared on untreated rice stems in glass tubes (five nymphs/tube, six replications in each dose). Morphogenetic and other biological effects were observed everyday for ten days after treatment. In this paper, the insects possessing "nymphal pore (a typical morphological mark of juvenile stages in planthoppers)" and unexpanded wing buds in the sixth stadium were classified as supernumerary nymphs.

5. Morphogenetic Effects of Precocene-2

Continuous contact application: Rice stems sprayed with 500 ppm P-2 (10% WP) were put into glass tubes with small amount of water. First stadium nymphs of WBRP, within 12 hr after hatching, were released into the tubes and were raised to observe the metamorphosis (five nymphs/tube, twenty replications). The treated rice stems were renewed every second day. Mortality, molting and external morphology of dead insects were observed everyday until metamorphosis.

Temporal contact application: Two series of "transfer tests" were conducted for investigating relationships between the duration of contact to residues of P-2 and the resulting morphogenetic responses (see Fig. 2). Insects were transferred from P-2 treated to untreated rice stems, or *vice versa*, on two days (late-first stadium), four days (mid-second stadium), six days (mid-third stadium), eight days (mid-fourth stadium) and ten days (late fourth stadium) after hatch (five nymphs/tube, twenty replications). Treated and untreated rice stems were renewed every second day. Morphogenetic and other biological effects were observed in the same way as mentioned above.

6. Rescue Effects of a JHA, NC-170 and JH-1, JH-2 and JH-3

Effects of NC-170 and the three natural JH's against the precocene-induced precocious metamorphosis were evaluated with two different methods.

Contact application: Various concentrations of NC-170 (10% WP) were mixed into aquatic solutions of 500 ppm P-2. First stadium nymphs, within 12 hr after hatch, were reared on rice stems treated with the mixed solutions. As shown in "RESULTS," newly hatched nymphs of WBRP are rather vulnerable to P-2, and about half of the nymphs die within the first stadium, when continuously contact with 500 ppm residues of the chemical. In this experiment, therefore, only surviving nymphs after the first nymphal molt were used for the evaluation of the rescue effects. Fifty insects in each dose of NC-170 were used.

Topical application: Nymphs were reared on rice stems treated with 500 ppm of P-2 from hatch. When the nymphs developed to the third stadium, various doses of NC-170 and of the three natural JH's were topically applied to the dorsal thoracic surface in 0.015 μl of acetone. Fifty nymphs were used in each dose of the experiment. Observations were made everyday up to metamorphosis.

RESULTS

1. Metamorphosis-Inhibiting Activities of NC-170 and Three Natural JH's

NC-170 of more than 1.0 ppm and that of more than 3.0 pg/nymph showed strong metamorphosis-inhibiting activity by contact application and topical application to mid-penultimate nymphs of WBRP, respectively (Fig. 3). The affected nymphs developed into

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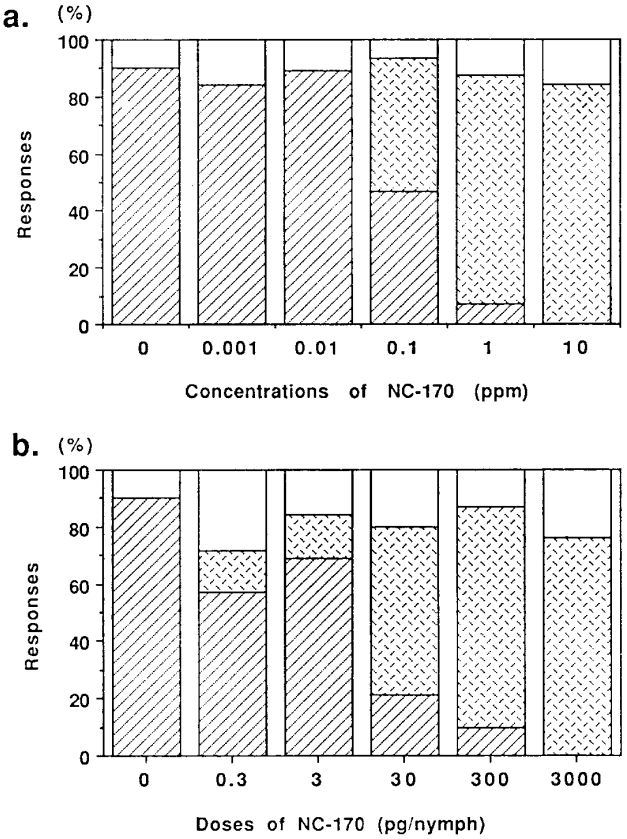

Fig. 3 Metamorphosis-inhibiting activity of NC-170 in the whitebacked rice planthopper.

(a) Contact application. (b) Topical application. □: death without specific morphological abnormalities, ⊠: supernumerary nymph, ▨: normal adult.

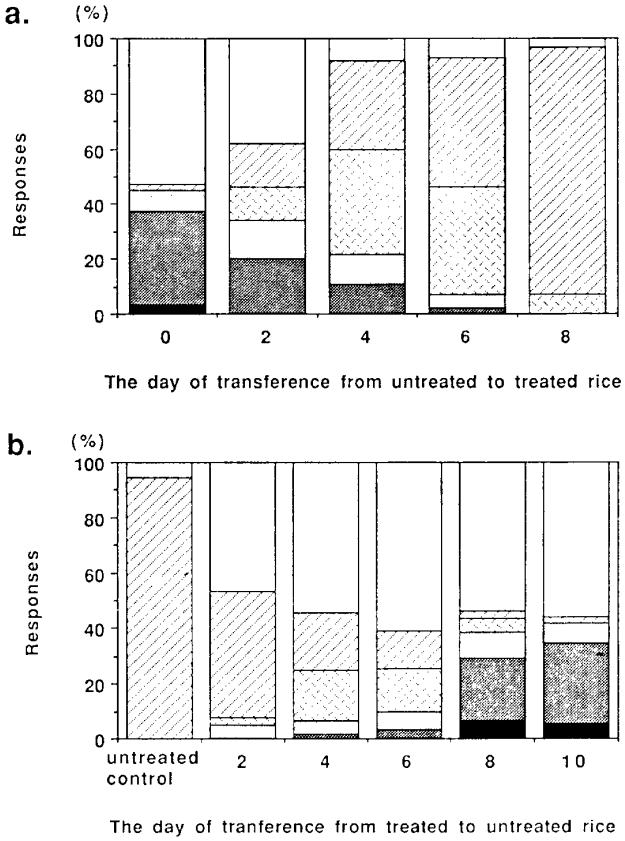

Fig. 4 Morphogenetic effects of precocene-2 in the whitebacked rice planthopper.

(a) First series of experiments (transferred from treated rice with 500 ppm precocene-2 to untreated rice). (b) Second series of experiments (transferred from untreated rice to treated rice with precocene-2). □: death without specific morphological abnormalities, ▨: normal adult, ⊠: supernumerary nymph, □: imperfect fifth stadium precocious adult, ▩: perfect fifth stadium precocious adult, ■: imperfect fourth stadium precocious adult.

supernumerary nymphs (nymphal-adult intermediates, morphologically) after the fifth molt. Females of them were died during the fifth molt by molting-inhibition, but the morphological characteristics of the dead insects beneath the old exuviae appeared to be those of supernumerary nymphs. Males of them succeeded to molt into supernumerary nymphs and survived for about five days and then, tried to molt again. The sixth molt, however, was not completed, resulting in death. On the other hand, none of the three natural JH's showed any biological effect against the metamorphosis by topical application even at the maximum dose, 300 ng/nymph (data not shown).

2. *Morphogenetic Effects of Precocene-2 by Continuous Contact Application*

When newly hatched nymphs contacted continuously with P-2, 52.8% of them died within the first stadium (Fig. 4(a), the first column from left). Surviving nymphs developed through the second and third nymphal stadia as normal. In these two stadia, no morphological abnormality was observed. After the ensuring third molt, however, small number of them (7.1% of surviving nymphs) prematurely metamorphosed into miniature precocious adults (4th PA's). But all of the 4th PA's observed in our study were incomplete nymphal-adult intermediates, which had partly expanded wings, diminutive external genitalia and sometimes three-segmented tarsi in their fore and middle legs (Fig. 5). The 4th PA's survived for a couple of days and under-

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Fig. 5 Imperfect fourth stadium precocious adult (female), ventral view.

went an additional (fifth) molt, although they died in its course, being unable to shed off their exuviae. In this case, the forth and fifth nymphal stadia were apparently skipped.

Almost all the remaining survivors (morphologically normal fourth stadium nymphs) developed into precocious adults in the following fifth stadium (5th PA's). Of the 5th PA's, 81.9% were morphologically perfect precocious adults, and they did not molt any more (Fig. 6), and the other 18.1% were incomplete nymphal-adult intermediates. Only 4.2% of surviving insects developed into morphologically normal adults in this test.

3. *Morphogenetic Effects by Temporal Contact with Precocene-2*

In the first series of tests, newly hatched nymphs were released on untreated plants and then transferred to P-2 treated plants on two, four, six and eight days after hatch (Fig. 4(a), from the second to the fifth column from left). In these tests, the rapid toxicity was drastically reduced when nymphs were reared for more than 4 days on untreated plants and were then, transferred to P-2 treated plants (transferred on the fourth, sixth and eight day). The mortality was only less than 10% in these cases. Incidence of precocious metamorphosis gradually decreased as the transference was later, while incidence of normal metamorphosis increased.

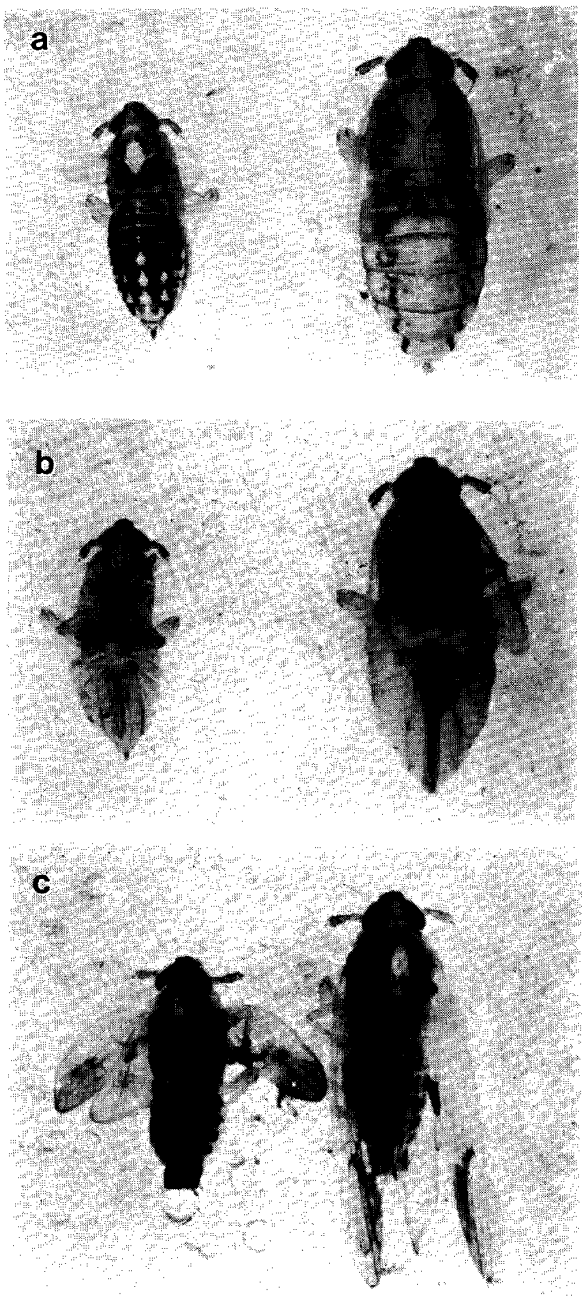

Fig. 6 Precocious adults induced by precocene-2 in the whitebacked rice planthopper.

(a) Left: perfect fifth stadium precocious adult (female), dorsal view. Right: normal adult (female), dorsal view. (b) Left: perfect fifth stadium precocious adult (female), ventral view. Right: normal adult (female), ventral view. (c) Left: perfect fifth stadium precocious adult (male), dorsal view. Right: normal adult (male), dorsal view.

In addition to the prothetely, metathetely was also observed in these tests. Of the surviving nymphs 19.4, 41.3, 41.9 and 10.3% developed into "supernumerary nymph"

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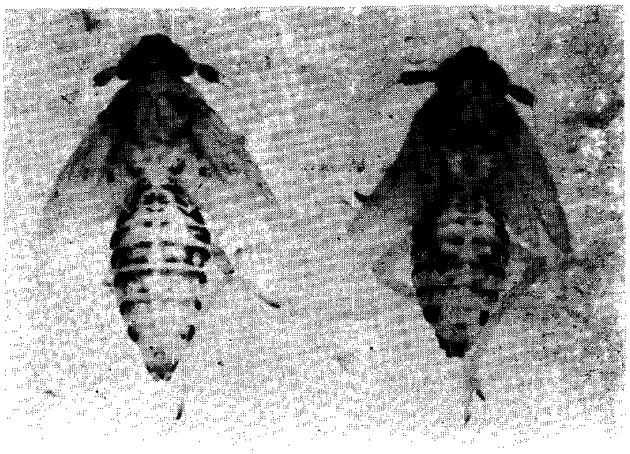

Fig. 7 Male supernumerary nymphs of the whitebacked rice planthopper.

Left: induced by a JHA, NC-170. Right: induced by precocene-2.

after the fifth molt, when the nymphs were transferred on the second, fourth, sixth and eighth day, respectively. Of course, metathetely is not a result of anti-JH activity but a result of JH-mimicking activity. Yet observations under a binocular microscope clearly demonstrated that they shared same morphological characteristics with supernumerary nymphs induced by treatment of the JHA, NC-170 (Fig. 7).

In the second series of tests, newly hatched nymphs were released on P-2 treated plants and then, transferred to untreated plants. In any of these cases, about half of the nymphs died within the first stadium due to the rapid toxicity of P-2 against young nymphs. When the nymphs were reared on treated plants for eight and ten days and then, transferred to untreated plants, almost all the surviving nymphs developed into precocious adults as observed in the test of continuous contact application. Again, under moderately affected conditions (transferred on the fourth and sixth day), distinct portion of the nymphs developed into supernumerary nymphs (Fig. 4(b)).

4. Rescue Effect by NC-170 and Natural JH's

When NC-170 was mixed into 500 ppm P-2 and simultaneously treated to nymphs, incidence of precocious metamorphosis decreased as the concentrations of NC-170 increased. Ten ppm of NC-170 completely prevented the precocious metamorphosis (Fig. 8 (a)). NC-

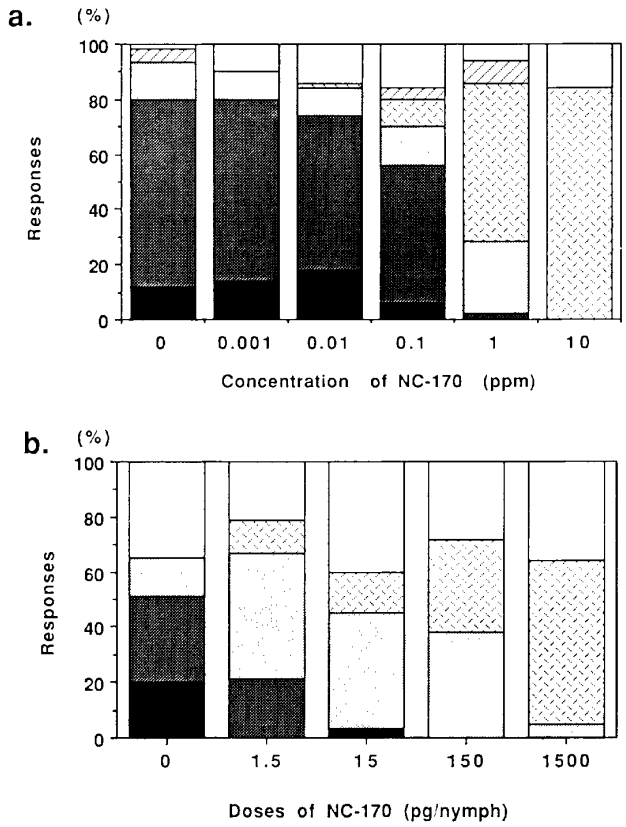

Fig. 8 Rescue effect by NC-170 on precocious metamorphosis in the whitebacked rice planthopper.

(a) Contact application (see text). (b) Topical application (see text). The pattern of each column represents the same response as shown in Fig. 4.

170 inhibited the precocious metamorphosis even by topical application in a dose-dependent manner (Fig. 8(b)), but none of the three natural JH's rescued the precocious metamorphosis at all. They caused slight increase of mortality at the highest dose (150 ng/nymph) (data not shown).

DISCUSSION

Since Bowers discovered precocenes in 1976,[1] many studies have demonstrated that the chemicals showed rapid toxicity in addition to anti-JH effects on several insect species. Therefore, the expected anti-JH effects could have been often concealed by the lethal effect, particularly when high doses of precocenes were administrated at one time by topical applications, injections *etc.* Praddep & Nair[5] topically applied P-2 to the third,

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fourth and fifth stadium nymphs of the brown planthopper, *N. lugens* in their study. All the treated nymphs, however, resulted in death. With "residual coating method," on the other hand, they obtained some precocious adults (PA's) but the PA's were not perfect but some incomplete nymphal-adult intermediates. In addition, as mortality was still high even with this method, it was rather difficult to observe the details of precocene-induced morphogenetic effects. This result strongly suggests that such temporal administrations of precocenes are not suitable for assessing the anti-JH effects at least in insects like planthoppers.

Continuous contact application used in our study was a very useful method in that context. Newly hatched nymphs of WBRP were certainly vulnerable to P-2 even under our test conditions: half of the insects released on the residues of 500 ppm P-2 died within the first stadium. However, almost all the surviving half were not in death in the following nymphal stadia but developed into PA in the fourth and fifth stadium. The temporal contact application tests clearly showed that only the first stadium nymphs are specifically sensitive to P-2's rapid toxicity. We could easily obtain morphologically perfect PA's with this method.

One of the interesting results we obtained in this study was the JH-mimicking effect of P-2. Some of the nymphs that might be moderately affected by P-2 did not develop into precocious adults but developed into supernumerary nymphs in the sixth stadium. In the course of the metathetely, females of them died during the fifth extra-nymphal molt by molting-inhibition, whereas males succeeded in molting to supernumerary nymphs and survived for about five days and then, died during the ensuring molt. This curious difference between sexes is the same phenomenon as observed in metathetely induced by a JHA, NC-170. In addition, external morphology of the supernumerary nymphs is not distinguishable from those induced by NC-170. At the present time, only JH's or JHA's are known to induce metathetely in insects. If P-2 act as a JHA, longer exposure to P-2 would have induced higher incidence of metathetely, and have never induced prothetely. Therefore, the metathetely observed here was not a result of typical JH-mimicking activity but presumably a result of endogenous JH excess caused by P-2.

Several researchers[7-9] have reported that P-2 and/or 7-ethoxy P-2 showed the dual morphogenetic effects (anti-JH plus JH-mimicking effect) in *Locusta migratoria*. They speculated that moderate amounts of precocenes might have temporarily activated inactive corpora allata, resulting in an outburst of JH biosynthesis and/or release before the glands were completely inhibited or destroyed by precocenes. If the abnormal outburst of JH occurs during the final nymphal stadium, the metamorphosis would be inhibited and metathetely would be induced. However, the detailed mechanism of action controlling these two opposite effects by precocenes has not been fully elucidated yet.

From the practical viewpoint, Fridman-Cohen & Pener[7] discussed that precocene-induced corpora allata activation must be a key point for considering possible development of precocenes (and related chemicals) to pest control agents. Our finding in this study is the first demonstration of the dual effect of precocenes in species other than Locusts and in economically important agricultural pests and supplies additional information to study further the practical performance of precocenes. The multiple biological activity of P-2 against WBRP is summarized in Fig. 9.

Miyake *et al.*[10,11] have reported that the JH

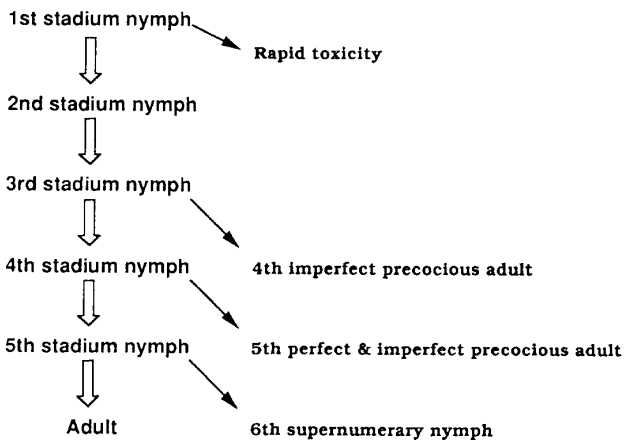

Fig. 9 Summary of multiple biological activity of precocene-2 in the whitebacked rice planthopper.

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mimic, NC-170, showed strong metamorphosis-inhibiting activity against both of leafhoppers and planthoppers but JH-1, JH-2 and JH-3 were active only against leafhoppers. In this study, NC-170 strongly inhibited the metamorphosis of WBRP with topical and contact application, but none of the three natural JH's caused any morphogenetic disturbance by topical application. In addition, NC-170 rescued the precocious metamorphosis induced by P-2 on a dose dependent manner, but the natural JH's showed no effect. At present, we don't have any information about the authentic JH('s) of planthoppers. Yet these results may provide a circumstantial evidence that NC-170 acts as a JH mimic probably at the target sites of hypothetical endogenous JH('s) in planthoppers which might be different from any of the three natural JH's.

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要　　約

プレコセン2のセジロウンカに対する三種の異なった作用

三宅敏郎，満井　喬

抗幼若ホルモン活性物質として知られるプレコセン2のセジロウンカに対する生物活性を検討した．その結果，プレコセン2は，処理時のウンカのステージ，処理期間（取り込み量）を変えることにより 1) 速効的な殺虫活性，2) 早熟変態 (prothetely, 抗幼若ホルモン活性)，3) 過剰脱皮 (metathetely, 幼若ホルモン様活性) という3種の異なった作用を引き起こすことが認められた．このうち，早熟変態については幼若ホルモン様活性物質である NC-170 の同時処理により作用の発現が打ち消されたが，天然の JH-1, JH-2, JH-3 の同時処理では打ち消されなかった．一方，抗幼若ホルモンであるプレコセン2が，なぜ metathetely を誘起するのかは不明であるが，今回の実験結果における"処理期間と metathetely 発現との関係"，および，過去，バッタ類で観察された同様の結果から推測すると，虫体に取り込まれたプレコセン2の量が不十分であると，アラタ体を完全に不活性化できず，逆に幼若ホルモンの生合成/分泌を一時的に促進する効果があるものと思われる．

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