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Original Article

Effects of Insect Juvenile Hormone Active NC-170 on Metamorphosis, Oviposition and Embryogenesis in Leafhoppers

Toshiro MIYAKE, Hiroshi HARUYAMA,* Tomoyuki OGURA,**
Takashi MITSUI and Akira SAKURAI

The Institute of Physical & Chemical Research, Hirosawa, Wako 351–01, Japan
**Shiraoka Research Station of Biological Science, Nissan Chemical Ind. Ltd., Shiraoka-cho, Minami-Saitama-gun, Saitama 349–02, Japan*
***Central Research Institute, Nissan Chemical Ind. Ltd., Tsuboi-cho, Funabashi 274, Japan*

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The activity of a new juvenile hormone analogue, NC-170 [4-chloro-5-(6-chloro-3-pyridylmethoxy)-2-(3,4-dichlorophenyl)-pyridazin-3(2*H*)-one], was evaluated on four species of leafhoppers, *Nephotettix cincticeps*, *N. nigropictus*, *N. virescens* and *Recilia dorsalis*. When NC-170 was topically applied to mid-penultimate larvae, their metamorphosis was strongly inhibited. The compound with the ID_{50}'s of 5.2×10^{-11} to 3.7×10^{-12} g/larva was 10 to 30 times as active as natural JH-1. The critical period of the morphogenetic activity was limited in a 24 hr span before and after the 4th larval molt. The affected insects did not develop into normal adults, but into supernumerary larvae (SL1). About 5 days later, the SL1 could not complete the molt into secondary supernumerary larvae (SL2) and subsequently died. Furthermore, NC-170 showed good sterile effects. When newly emerged female *N. cincticeps* adults were continuously exposed to NC-170, the hatchability of oviposited eggs severely reduced, even at a concentration of 4 ppm. In a field trial, NC-170 showed good foliar persistency in paddy fields and single spray treatment with 100 ppm NC-170 considerably suppressed the population density of *N. cincticeps* for more than 6 weeks.

INTRODUCTION

In the last two decades, much information on the biological activities of juvenile hormone mimics (JHMs) has been accumulated not only in theoretical but also in applied fields. Some JHMs (*e.g.* methoprene, fenoxycarb, *etc.*) have been developed and commercially used in various insect control programs. In leafhoppers and planthoppers, which are notorious pests particularly in Asian paddy fields, however, the physiological and biochemical roles of natural juvenile hormones (JHs) have still remained unclear, and no JHM has shown real promise from a practical viewpoint. Although some papers have fragmentarily reported the morphogenetic and other physiological effects of JHs and JHMs against this insect group,[1-4] further studies with more active chemicals are necessary for a better understanding of the endocrine events and for future hopper control programs involving JHMs or other insect growth regulators (IGR).

NC-170 (Fig. 1), recently discovered by Nissan Chemical Industries Ltd., is a JHM of a new chemical class. This compound has selective JH-like effects on leafhoppers and planthoppers.[5] This paper provides basic information on the morphogenetic and sterile activities of NC-170 to leafhoppers and discusses practical prospects of NC-170 as a new

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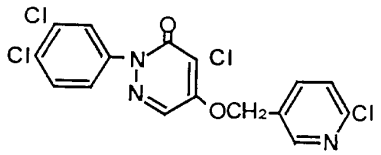

Fig. 1 Chemical structure of NC-170.
[4-chloro-5-(6-chloro-3-pyridylmethoxy)-2-(3,4-dichlorophenyl)-pyridazin-3(2*H*)-one]

leafhopper control agent with JH like activities.

MATERIALS AND METHODS

1. *Animals*

Four species of leafhoppers, *Nephotettix cincticeps*, *N. nigropictus*, *N. virescens* and *Recilia dorsalis* were reared on rice seedlings at 25°C, 70% RH, under long-day (16L/8D) photoperiod regimens. *N. cincticeps* was originally collected in paddy fields in Saitama Pref., Japan, in 1972, and the other three species were supplied by Dr. H. Noda, the National Institute of Sericulture and Entomological Science, Tsukuba, Japan.

2. *Chemicals*

Juvenile hormones 1, 2 and 3 were purchased from Sigma. NC-170 was synthesized and formulated into a 10% wettable powder (WP) in the Central Research Institute, Nissan Chemical Industries Ltd.

3. *Morphogenetic Activity*

Contact application: Rice plants in the 6–7 leaf stage grown in 15 cm diameter pots were sprayed with the aquatic suspension of NC-170 (10% WP) until runoff, and air-dried. Each pot was surrounded by a transparent plastic cylinder (40 cm in height $\times$15 cm in diameter). Thirty mid-penultimate (2 day-old, fourth stadium) leafhopper larvae per pot were released on the rice plants. The pots were kept at 25°C and morphogenetic effects of NC-170 were observed under a vinocular microscope, when needed, 14 days after treatment.

To evaluate the sensitivity of *N. cincticeps* to NC-170 at different developmental stages, fixed age larvae were fed on rice plants treated with 10 or 1 ppm NC-170 (10% WP) for 24 hr, and then transferred onto untreated rice plants. The morphogenetic activity of NC-170 was observed after the 5th molt (usually the transformation into adult). In addition, to evaluate the effects of NC-170 on the duration of the 5th larval stadium, rice stems treated with 100 or 0 ppm NC-170 were put into glass tubes, in which mid-penultimate larvae had been individually reared. The timing of the 4th and 5th molt was checked every eight hours after release.

Topical application: Mid-penultimate larvae were anesthetized with carbon dioxide. Technical NC-170 or natural JHs in 0.03 μl of acetone was topically applied to the dorsal thoracic surface with an Arnord automatic microapplicator fitted with a 100 μl syringe and a glass capillary. The treated leafhoppers were kept on potted rice plants until observed.

The IC_{50} and ID_{50} values were obtained by statistical analysis using the probit technique from the morphological analysis data. In this paper, any larvae possessing setae on their dorsal abdominal terga in the 6th stadium were recognized as supernumerary.

4. *Sterile Activity*

Five pairs of newly emerged of *N. cincticeps* adults were released on potted rice plants sprayed with various concentrations of NC-170 (10% WP), and kept for 15 days (the preoviposition period of this species was about 8 days). After the adults were removed, the plants were kept for another 15 days, and the number of newly hatched larvae were then counted.

In the case of mature adults (10–14 days after eclosion), they were fed on treated plants for 5 days and removed. The effect of NC-170 was observed in the same manner as mentioned above.

In another series of experiments, the number of oviposited eggs on the rice plants were counted immediately after the removal of the adults in order to evaluate the effects of NC-170 on the oviposition.

5. *Ovicidal Activity*

N. cincticeps eggs 0–12 hr-old were collected from rice plants and kept in distilled water. Every 24 hr, more than 50 eggs were transferred on filter papers soaked with an aquatic

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suspension of 10, 1, 0.1, 0.01, or 0 ppm NC-170 (10% WP) to remain wet throughout the experiment. Then, the eggs were kept at 25°C, 70% RH, under long-day photoperiod regimens. Hatching inhibition and developmental stages of embryos were examined under a binocular microscope on the 14th day after oviposition.

6. Field Trials and Residual Activity

The experiment on *N. cincticeps* was conducted in a paddy field in Saitama Pref., Japan, in 1989, where a 'Musashi-kogane' rice variety had been planted in mid-June. Both treated and untreated plots were 80 m^2 in size and each plot was replicated three times. An aquatic suspension of 100 ppm NC-170 (10% WP) was sprayed with a power sprayer (Kyôritsu Co. Ltd., Model HPE-170) at 120 *l*/1000 m^2 on July 18.

N. cincticeps adults in each plot were collected by a sweeping method (10 sweeps with a 30 cm sweep net, repeated four times per plot) every week from July 7 (pre-treatment) to September 12, and the number was counted.

At the same time, five rice plant stumps per plot were taken from the fields treated with NC-170 to the laboratory every week after treatment, and replanted in 25 cm diameter pots. Each pot was surrounded by a transparent plastic cylinder (75 cm in height $\times$22 cm in diameter). Fifty mid-penultimate larvae of *N. cincticeps* or *R. dorsalis* per pot were released. The morphogenetic activity of the chemical was assessed 14 days after release.

RESULTS

1. Metamorphosis-Inhibiting Activity

The effects of NC-170 and natural JHs on the metamorphosis of leafhoppers were evaluated with two different methods (Table 1). NC-170 strongly inhibited the metamorphosis of the four leafhopper species when mid-penultimate larvae were released on rice plants sprayed with it. The sensitivity to NC-170 was almost the same among the three *Nephotettix* species with IC_{50}'s of 0.01 to 0.08 ppm, whereas *R. dorsalis* was slightly less sensitive, with a IC_{50} of 0.3 ppm. Among three natural JHs, JH-1 was the most active against all of the four species, followed by JH-2, and JH-3 was almost inactive as a metamorphosis inhibitor. NC-170 was 10 to 30 times more active than JH-1.

Fourth stadium leafhopper larvae treated with NC-170 molted normally into the fifth (usually the final) stadium with some unusual discoloration. The whole cuticle was strongly melanized at this stage, turning to shiny black in *N. cincticeps* and *N. nigropictus*, and to blackish red in *R. dorsalis*. On the other hand, the epidermis became dark green in *N. virescens* because of the accumulation of green pigments, as shown in Fig. 2.

The mean duration of the final stadium was 5 days on average in *N. cincticeps* males, slightly shorter than that of females (5.7 days). Applications of NC-170 shortened the duration to 4 days in both males and females (Fig. 3-a) in a dose-related manner (Fig. 3-b). They then developed into first supernumerary larvae (SL1). The SL1 survived for about 5 days and molted to second supernumerary larva (SL2). This molt was not completed, however, resulting in death.

2. Morphology of Supernumerary Larva

The morphological characteristics of SL1 and

Table 1 Metamorphosis-inhibiting activity of NC-170 and natural JHs to four species of leafhoppers.

Species	IC_{50} (ppm)[a]	ID_{50} (g/larva)[b]			
	NC-170	NC-170	JH-1	JH-2	JH-3
N. cincticeps	0.08	7.65×10^{-12}	2.29×10^{-10}	2.85×10^{-8}	$>1\times10^{-6}$
N. nigropictus	0.01	3.70×10^{-12}	8.5×10^{-11}	5.2×10^{-8}	$>1\times10^{-6}$
N. virescens	0.03	1.5×10^{-11}	1.85×10^{-10}	7×10^{-8}	$>1\times10^{-6}$
R. dorsalis	0.30	5.2×10^{-11}	4.2×10^{-10}	1.08×10^{-7}	$>1\times10^{-6}$

[a] Contact application, [b] Topical application.

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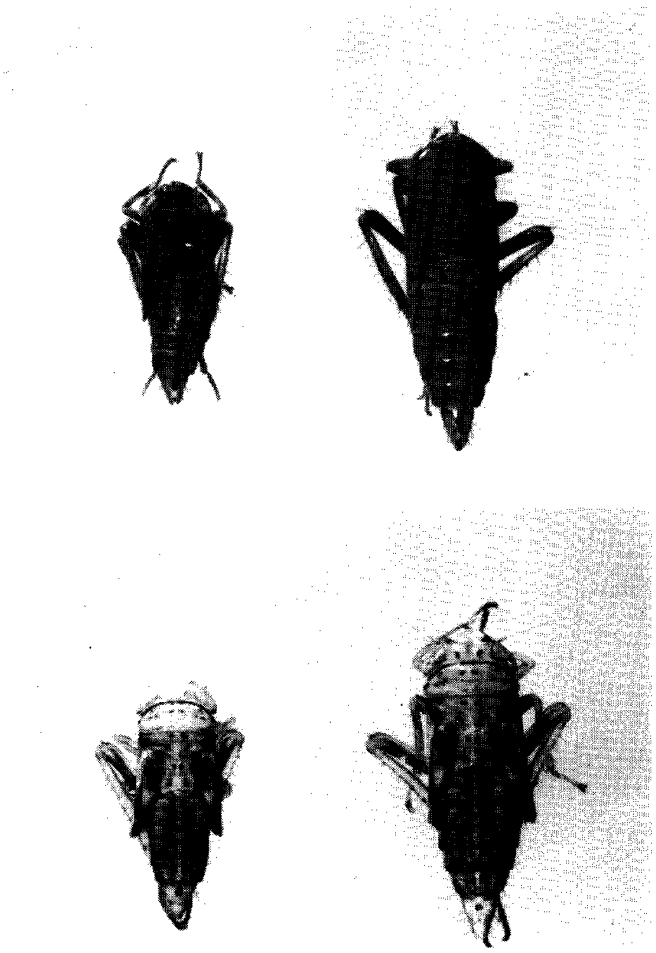

Fig. 2 General appearance of 5th stadium larvae and SL1 of leafhoppers affected with NC-170.

Upper left: *N. cincticeps*, 5th stadium larva, Upper right: *N. cincticeps*, SL1, Lower left: *N. virescens*, 5th stadium larva, Lower right: *N. virescens*, SL1.

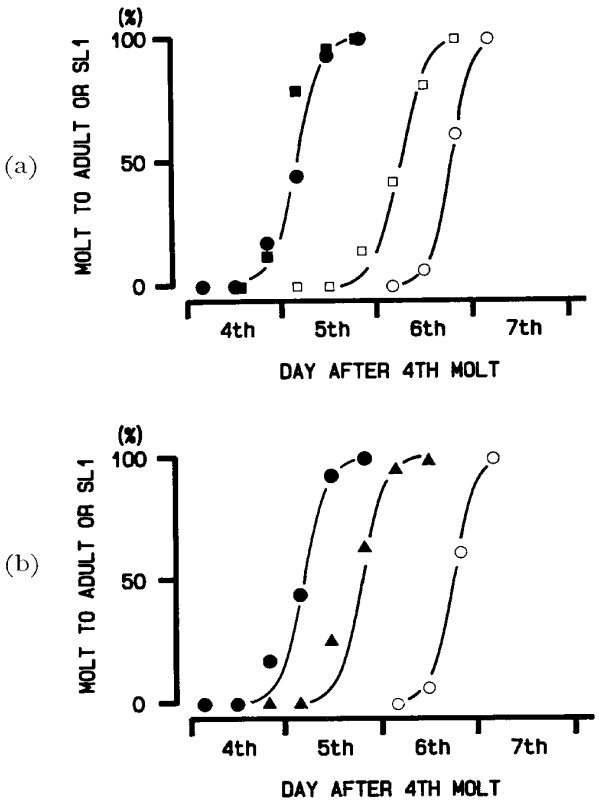

Fig. 3 Effect of NC-170 on the duration of 5th larval stadium in *N. cincticeps*.

Each mark indicates a chronological increase in the percentage of molted insects to adults (untreated larvae) or SL1 (larvae treated with NC-170).

●: female treated with 100 ppm NC-170, ■: male treated with 100 ppm NC-170, ▲: female treated with 1 ppm NC-170; ○: untreated female, □: untreated male.

SL2 are summarized in Table 2, compared with those of normal larvae and adults. Except for the size and the color, SL1 and SL2 were almost the same in general external shape as 5th stadium larvae (Fig. 2). However, they had adultoid characteristics such as three-segmented tarsi, adult-type setae on pygofer and rudimental genitalia, and oceli.

3. *Sensitivity in Different Stage*

The sensitivity of *N. cincticeps* to NC-170 increased as the larvae advanced in stadium, reaching maximum at the late (3 day-old) 4th stadium, and decreased rapidly thereafter (Fig. 4). The individual rearing revealed that the sensitivity was lost in the period from 24 hr before the fourth larval molt to 24 hr after the molt at least in male (Fig. 5), which is the so-called 'critical period' of the morphogenetic activity in this species. From this finding, the assumption was led: the switchover of commitment from larva to adult occurs in this period.

4. *Sterile and Ovicidal Activities*

When five pairs of newly emerged *N. cincticeps* adults were released on a rice plant sprayed with NC-170, there was no observed effect in mating behavior, ovary maturation and oviposition during 15 days after emergence (Table 3). The hatchability of laid eggs, however, was severely suppressed by NC-170.

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Table 2 External morphology of supernumeral leafhopper larvae.

	Larva	SL1	SL2	Adult
Wing	Rudimental	Rudimental	Rudimental	Expanded
Tarsus	2 segmented	3 segmented	3 segmented	3 segmented
Setae on abdominal tergite	Present	Present	Present	Absent
Setae on pygofer	Larval type	Larval type & adult type	Larval type & 2 series of adult type	Adult type
External genitalia	Rudimental	Rudiment with adult setae	Rudiment with 2 series of adult setae	Developed
Oceli	Absent	Present	Present	Present

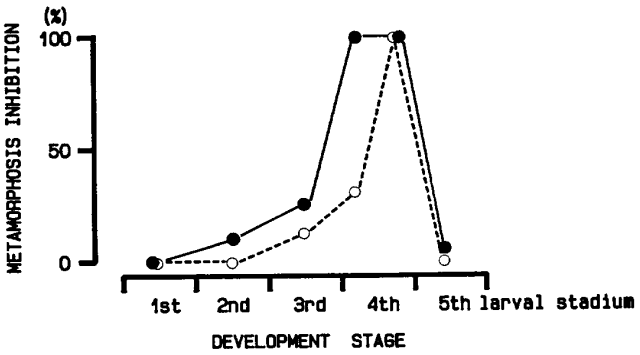

Fig. 4 Sensitivity of *N. cincticeps* in different stages to NC-170.

Age of each treated group: 2nd day of 1st, 2nd and 3rd stadium; 1st and 3rd day of the 4th stadium; 2nd day of the 5th stadium.
●: NC-170 10 ppm, ○: NC-170 1 ppm.

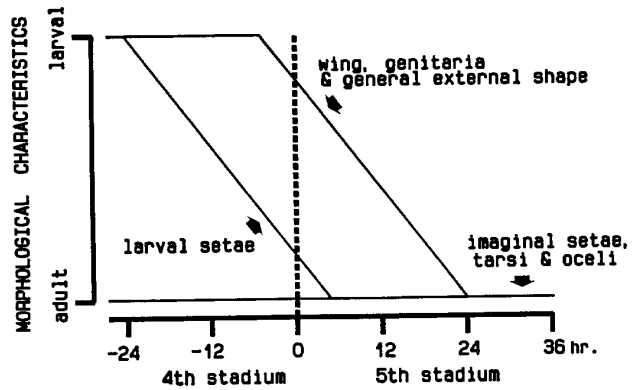

Fig. 5 Diagrammatic representation of the 'critical period' of morphogenetic activity with NC-170 in male *N. cincticeps*.

Horizontal line represents the initial time of application, and perpendicular line the character of detailed morphology in resulted SL1 or adults. Figure indicates, for example, that the critical period of larval setae on abdominal tergite is from 24 hr before the 4th larval molt to 5 hr after the molt.

Table 3 Effects of NC-170 on the oviposition of *N. cincticeps*.

Conc. (ppm)	Mean No. of oviposited eggs (±SD)/5 females	
	Newly emerged adults[a)]	Matured adults[a)]
500	105.1 (24.4)	362.3 (33.1)
100	111.1 (21.4)	319.7 (18.6)
Untreated check	103.9 (17.8)	314.8 (24.0)

[a)] See MATERIALS AND METHODS.

Table 4 Sterile activity of NC-170 to *N. cincticeps*.

Conc. (ppm)	Mean No. of hatched larvae (±SD)/5 females	
	Newly emerged adults[a)]	Matured adults[a)]
500	0^{A}	$17.5\ (12.2)^{A}$
100	0^{A}	$71.3\ (25.2)^{A}$
20	0^{A}	$204.3\ (75.6)^{B}$
4	$11.1\ (\ 9.4)^{A}$	$253.8\ (43.2)^{B,C}$
0.8	$58.9\ (16.1)^{B}$	$321.8\ (33.0)^{C}$
Untreated check	$82.5\ (19.8)^{C}$	$320.5\ (23.7)^{C}$

[a)] See MATERIALS AND METHODS.
Means in a culumn followed by the same letter (A, B or C) are not significantly different ($p=0.05$; Duncan's multiple range test).

No larvae hatched when rice plants were treated with NC-170 higher than 20 ppm, and the number of newly hatched larvae was substantially decreased even at 4 ppm (Table 4). On the other hand, when mature adults were released and fed on treated plants the hatchability of the oviposited eggs were not so

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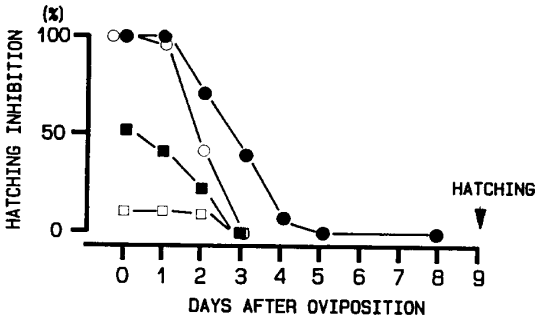

Fig. 6 Ovicidal activity of NC-170 to *N. cincticeps*.

●: NC-170 100 ppm, ○: NC-170 10 ppm, ■: NC-170 1 ppm, □: NC-170 0.1 ppm.

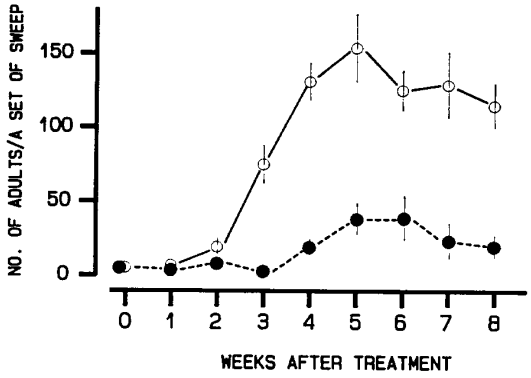

Fig. 7 Field trial of NC-170 on *N. cincticeps*.

●: NC-170 100 ppm, ○: untreated check. Perpendicular bars on each mark show SD.

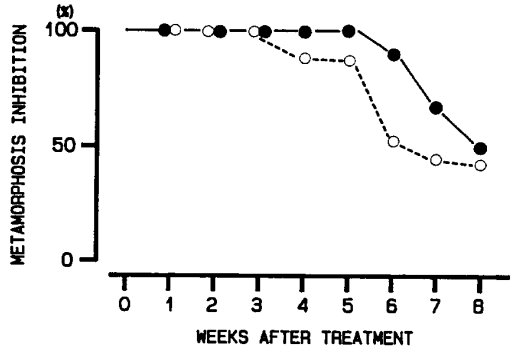

Fig. 8 Residual activity of NC-170 in *N. cincticeps* and *R. dorsalis*.

●: *N. cincticeps*, ○: *R. dorsalis*.

drastically suppressed compared with the case of in newly emerged adults (Table 4).

The ovicidal effect of NC-170 was clear in the experiment, as shown in Fig. 6, where the chemical was applied directly to eggs. The eggs 0 to 3 days old were very sensitive to NC-170, but after day 5 they completely lost the sensitivity.

5. *Field Trial and Residual Activity*

Practical applications of NC-170 to *N. cincticeps* were tested in a field. A single treatment of 100 ppm NC-170 in mid-July suppressed the population density for more than 6 weeks (Fig. 7).

The simultaneous evaluation of residual activity in the field showed that the metamorphosis-inhibiting activity of NC-170 remained high for more than 6 weeks not only to *N. cincticeps* but also to less sensitive *R. dorsalis* (Fig. 8).

DISCUSSION

The morphogenetic activity of JHMs is in general unattractive for use in agricultural pest controls, since the destructive phase in almost all agricultural insect pests is the larval stage. The control with the activity of JHMs is only after the larval phase. However, many field studies with JHMs conducted over the last two decades have shown that in some special cases aimed at some special targets, it is possible to use morphogenetic controls.[6,7] Homopterous insects such as aphids, whiteflies, psyllids, scale insects, are typical representatives. Some positive aspects in these insects for JHM control are: economic damage often requires a population build-up through several generations with a short life cycle, most life stages are usually well exposed, and they are often sedentary. Even though the ecological characteristics of leafhoppers are not so different, they have thus far not received much attention as targets of JHMs, and little information is available in this field at present. One of the most practical reasons was the lack of promising chemicals.

There are reports on the morphogenetic activity of JHMs to *Draeculacephala crassicornis*[1,3] and *N. cincticeps*,[2] but the dose rates required do not appear to be very promising. Even 'kinoprene,' one of the most active JHMs against homopterans, had to be higher than 625 ppm to disturb the metamorphosis of *N. virescens*.[4] Commercially used JHMs, methoprene, fenoxycarb and pyriproxyfen, were also almost completely inactive against

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leafhoppers in our experiments (data not shown).

This study demonstrates that NC-170 has excellent morphogenetic activity to all of the four species of leafhoppers examined by both contact and topical applications, and that it is by far the most active among the existing JHMs such as methoprene, kinoprene, fenoxycarb. When the mid-penultimate *N. cincticeps* larvae were treated with NC-170, a shortening of the duration of the ensuing final stadium was initially observed. This syndrome appears to be closely related to the following metamorphosis inhibition. For instance, the duration of the final stadium in this species is obviously longer than that of four previous stadia, and it is only in the final stadium in which a sexual difference can be observed (which is possibly a reproductive strategy).[8] These characteristics indicate important features of the special 'final stadium,' within which transformation from larva to adult occurs. In the final stadium of the affected larva, however, the duration was shortened to about 4 days regardless of sex; and in addition, a 4 days period is almost the same as that of a penultimate stadium. This observation suggests that the affected final stadium larvae had maintained certain physiological characteristics of the penultimate stadium. Thereafter, the metamorphosis was inhibited and the larval cycle was repeated. Similar observations were reported in a hemipterous bug, *Oncopertus fasciatus*, in which a direct activation of the prothoracic glands by JHMs was believed to have shortened the duration.[9,10]

The resulting SL1 is not a perfect supernumerary larva in morphological sense but a mosaic individual possessing both larval and adult characteristics. Almost all SL1 died during the subsequent molt because of their morphological and possible physiological imbalance. The mode of metamorphosis inhibition by NC-170 observed in leafhoppers in our study followed the rule of the typical pattern reported in other homopterans such as aphids[11-13] or psyllids.[14]

Another remarkable benefit of this chemical is its long residual activity not only in greenhouse[5] but also in field: it persists longer than 6 weeks on rice plants. Such persistency is indispensable in reducing the insect population below the level of economic damage regarding the slow-acting morphogenetic control, since life cycle of leafhoppers is usually about 4 weeks and its developmental stage in the field population is often unsynchronous.

In addition, the sterile effect of NC-170 should be noted, because it contributes to the field effect in concert with the above-mentioned morphogenetic activity. Leafhoppers generally deposit eggs in rows beneath the epidermis of rice stems, hence direct ovicidal effects of chemicals are frequently haltered. However, NC-170 is taken up by adult females and transferred into the ovaries and oviposited eggs, where the embryogenesis is disrupted, since the difference of its sterile activity between in newly emerged adults and mature adults was observed.

Although the precise mechanism of the ovicidal action remains unclear, our observations suggest that NC-170 attacks early physiological event(s) before the katatrepsis stage (4th day of embryogenesis), disturbing the eclosion of the first stadium larvae. Morphological observation should be done in detail, since the morphogenesis programs of larvae marked by the beginning of embryogenesis would be disturbed as observed in the metamorphosis.

The effect of JHM on insect population is the criterion by which the control potential of the chemical in question can be evaluated. Precise field evaluations of JHM, however, are usually rather difficult, particularly in migratory insects like leafhoppers, since migration would easily obscure all effects on insect populations in small plots. Taking this negative aspect into consideration, the effect of NC-170 described in this paper is deemed to be satisfactory even from the practical standpoint. The increase in the population density observed in treated plots through the 4th to 7th weeks after treatment is to be recognized as a result of immigration from the control plots or the adjacent paddy fields. If the experimental plots had been large enough and the adult migration had been ignorable, the result would have been much clearer. In order to make certain the practical performance of

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NC-170 in various situations, further field trials are now in progress.

The previous paper described that NC-170 had not exhibited any signs of JH-like activity to 15 nonhopper species.[5] Although further studies about negative effects on beneficial or nontarget organisms are to be required, NC-170 has showed no adverse effects on some spiders in paddy fields which are the most important predators of leafhoppers (K. Naba, per. comm.). It is conceivable that the specificity together with the new chemistry and the mode of action is the most significant benefit of NC-170 as a new class IGR. NC-170 may thus be applicable in further integrated pest management programs for paddy fields.

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要　　約

幼若ホルモン様物質 NC-170 のヨコバイ類の変態，産卵，胚子発生に対する影響

三宅敏郎，春山裕史，小倉友幸
満井　喬，桜井　成

ピリダジノン環を有する新規 JHM，NC-170 は，ツマグロヨコバイをはじめとする 4 種のヨコバイ類の変態を強く阻害し（$ID_{50}=5.2\times10^{-11}\sim3.7\times10^{-12}$ g/larva），その活性は JH-1 の 10～30 倍であった．更に，NC-170 は，ツマグロヨコバイ成虫の産卵には影響を与えないが，4 ppm 以上の茎葉散布で産下された卵の孵化を強く阻害する．これは，卵内に取り込まれた NC-170 が胚子発生の初期の生理現象を撹乱し，その結果，孵化が阻止されるものと想像された．NC-170 の JH 様作用がツマグロヨコバイの野外個体群に与える影響を調べたところ，100 ppm の茎葉散布により，6 週間以上にわたってその密度を低く抑え，NC-170 の防除剤としての可能性が示唆された．この効果は，NC-170 の稲体上での長期の残効性に，大きく依存しているものと考えられた．

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