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Original Article

Effects of a New Juvenile Hormone Mimic, NC-170, on Metamorphosis and Diapause of the Small Brown Planthopper, *Laodelphax striatellus*

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Effects of a new jevenile hormone mimic (JHM), NC-170 [4-chloro-5-(6-chloro-3-pyridylmethoxy)-2-(3,4-dichlorophenyl)-pyridazine-3(*H*)-one], on the metamorphosis and diapause of the small brown planthopper were examined in comparison with those of Juvenile Hormone(JH)-1,2 and 3. NC-170 had a strong metamorphosis-inhibiting activity with a ID_{50} of 0.42 pg/larva when topically applied to nondiapause mid-4th stadium larvae. The three JHs showed no signs of metamorphosis inhibition not only by single topical application to mid-4th stadium larvae at a maximal dosage (300 ng/larvae), but also by daily repeated topical applications to larvae in the early 4th to 5th stadium (30 ng/day/larva). When the planthoppers were reared at 20°C under short-day (8L/16D) photoperiods throughout the embryonic and larval stages, the development was considerably retarded in the 4th larval stadium, which is apparently the state of diapause. They resumed normal (nondiapause) development when 20-hydroxyecdysone was topically applied. Contact applications of 100 ppm NC-170 through the larval stage prevented larvae from entering diapause, and topical applications of the chemical terminated diapause and induced 4th larval molts about 8 days after treatment. JH-1 also terminated diapause in the same manner at the same dosages as NC-170.

INTRODUCTION

The small brown planthopper, *Laodelphax striatellus*, is one of the most serious pests in rice cultivation in Asia, because of not only its ability to transmit plant pathogens but also direct feeding effects when present in large numbers.[1] In the temperate regions such as Japan and Korea, this species hibernates mainly in the 4th stadium in gramineous weeds grown near paddy fields, and overwintering populations are the major source of notorious population build-ups in spring.[2,3] This species is adapted to sustain adverse environmental conditions in winter with its ability to enter facultative diapause before the onset of such conditions.[4] If it were possible to prevent or terminate the diapause when the conditions are still unfavorable, the overwintering population might be reduced to an insignificant proportion.

Several researchers have succeeded in terminating the diapause of other insects in the adult and larval stages with natural JHs and JHMs, and demonstrated possible new pest management approaches using this unique manner of action.[5-9]

NC-170 is a new JHM of a new chemical class, which is selectively active against leafhoppers and planthoppers. Its strong morphogenetic and sterile activities have shown that it can be a practical new hopper-control agent.[10,11]

In this paper, we report disrupting effects of

NC-170 on the diapause and metamorphosis of the small brown planthopper.

MATERIALS AND METHODS

1. *Animals*

The small brown planthoppers, *Laodelphax striatellus* (Izumo strain) were supplied by Dr. H. Noda at the National Institute of Sericulture and Entomological Science, Tsukuba, Japan, and reared on rice seedlings at 25°C, 70% RH, under long-day (16L/8D) photoperiods (nondiapause or LD conditions).

To produce a diapause generation, eggs 0 to 24 hr-old were kept at 20°C, 70% RH, short-day (8L/16D) photoperiods (SD conditions). About 20 days later, first stadium larvae hatched, which were then reared on rice seedlings under same SD conditions. Rice seedlings were replenished every 10 days. Under these conditions, larval development was considerably retarded, particularly in the 4th stadium (the state of diapause), and some larvae appearing to be in diapause gradually entered the 4th molt, as reported earlier.[4)]

2. *Chemicals*

Juvenile hormone-1,2,3 and 20-hydroxyecdysone were purchased from Sigma. Technical (>99%) and 10% wettable powders (WPs) of NC-170 were produced in the Central Research Institute, Nissan Chemical Industries, Ltd.

3. *Morphogenetic Activity against Nondiapause Larvae*

Single topical application: Mid-penultimate (2 day-old 4th stadium) larvae were anesthetized with carbon dioxide. Technical NC-170 or JHs in 0.03 μl acetone was topically applied to the dorsal thoracic surface with an Arnord automatic microapplicator fitted with a 100 μl syringe and a glass capillary. Control groups were treated with 0.03 μl acetone. The treated larvae were reared on rice stems set in glass tubes (20 cm height × 2 cm diameter) with small amounts of water. Thirty larvae were used for every dosage of chemicals. Metamorphosis-inhibiting activity was observed after the 5th molt (larval-adult molt in normal conditions). Medium metamorphosis-inhibiting dosages (ID_{50}'s) were obtained using the probit analysis method from the morphological analysis data. In this paper, any insects possessing larval pores (see Fig. 1) and unexpanded wing buds in the 6th stadium were classified as supernumerary larvae (SL).

To determine the critical period of the morphogenetic activity, 4th and 5th stadium larvae of various ages were topically applied with 3 ng NC-170, and morphogenetic activity was observed as mentioned above. A diagram was compiled from the relationships between application timing and resulting morphogenetic effects.

Repeated topical application: Thirty nanograms/larva of JH-1,2 and 3 were topically applied to larvae everyday from the 1st day of 4th to the 2nd day of 5th larval stadia. It was applied 6 or 7 times in total depending on the duration of the 4th stadium of each larva. More than 30 larvae were treated with each chemical.

4. *Prevention of Diapause*

Rice plants in 6 to 7 leaf stages were immersed in aqueous suspension of 0 ppm and 100 ppm NC-170 for 30 sec. After being dried, the stems were put into glass tubes. Newly hatched larvae, which had developed under SD conditions, were individually reared on the stems under SD conditions. To determine the effects of NC-170 on larval development under diapause-inducing conditions, each larval and adult molt were checked everyday up to 70 days after hatching. More than 50 larvae were used for each group. The rice stems were replenished each week. Similarly, effects of NC-170 on nondiapause development were evaluated under LD conditions.

5. *Termination of Diapause*

NC-170, the three JHs and 20-hydroxyecdysone in 0.03 μl acetone were topically applied to diapause 4th stadium larvae (about 45 days after hatching). Control groups were treated with 0.03 μl acetone. The larvae were then individually reared on untreated rice stems in glass tubes under SD conditions. The 4th and 5th molts were checked everyday and the morphology of the resulting adults or supernumerary larvae was observed.

RESULTS

1. Morphogenetic Activity

When mid-4th stadium larvae were topically applied with 3 ng NC-170, all of the resulting adults were strongly juvenilized, and appeared to be 'supernumerary larvae' (SL) (Fig. 1). Table 1 shows the detailed morphological criteria of the SL compared with those of normal 5th stadium larvae and adults. The ID_{50} of the metamorphosis-inhibiting activity of NC-170 was 0.42 pg/larva (Table 2). The critical periods of juvenilizing activity concerning each morphological criterion were found in the early 5th stadium, as represented in Fig. 2. On the other hand, the three JHs tested did not show any signs of such juvenilizing effects either by single application or by repeated applications (Table 2).

2. Prevention of Diapause

When newly emerged larvae were continuously reared on rice stems treated with 100 ppm NC-170, the diapause induction under SD conditions was definitely prevented and the larval development proceeded fairly constantly (Fig. 3). On the other hand, 100 ppm NC-170 did not alter the programmed course of non-

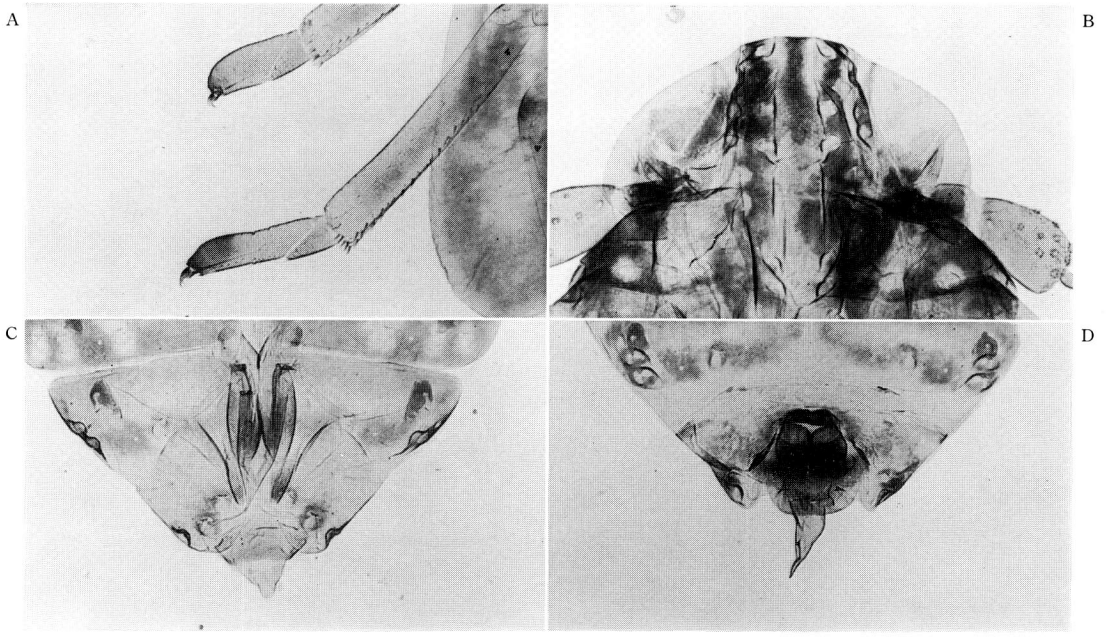

Fig. 1 Morphology of supernumerary larva on the small brown planthopper.
A: Two-segmented tarsus of fore- and mid-legs, B: Larval pores and larval-type frons structure, C: Juvenilized female ovipositor, D: Juvenilized male genitalia.

Table 1 External morphology of the supernumerary larva of the small brown planthopper.

	5th stadium larva	Supernumeral larva	Adult
Wing	Rudimental	Rudimental	Expanded
Tarsus of fore & mid leg	2 segmented	2 segmented	3 segmented
Frons & head[a)]	Larval-type	Larval-type	Adult-type
Larval pore[a)]	Present	Present	Absent
External genitalia[a)]	Not visible	Juvenilized	Developed
Oceri	Absent	Absent	Present

[a)] See Fig. 1.

diapause development under LD conditions (Fig. 4).

3. *Termination of Diapause*

In this series of experiments, 4th stadium larvae which had spent about 45 days after hatching under SD conditions were used as diapause larvae. As mentioned earlier, the diapause 4th stadium larvae spontaneously molted into the 5th stadium even under SD conditions. When the diapause larvae were transferred from diapause-inducing SD conditions to diapause-terminating LD conditions, the larvae molted synchronously to the 5th stadium in about 7 days (Fig. 5).

To investigate whether diapause maintenance and termination were affected by exogenous JHs, diapause larvae receiving 300 ng JH-1, 2 and 3 were kept under SD conditions.

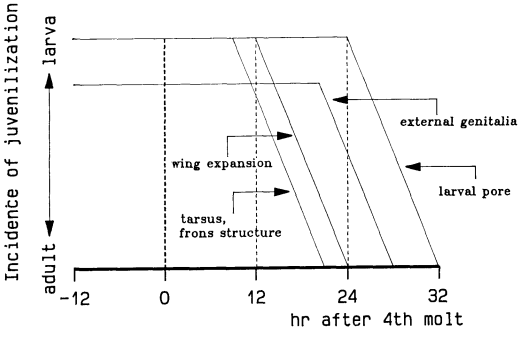

Fig. 2 Diagrammatic representation of the 'critical period' of morphogenetic activity with NC-170 in the small brown planthopper.

Horizontal line represents the initial time of application, and perpendicular line the incidence of juvenilization of each morphological charactor in resulting SL or adults.

Figure indicates, for example, that external genitalia is not completely juvenilized even if NC-170 is applied in the 4th stadium, and the sensitivity is completely lost about 30 hr after the 4th molt.

Table 2 Metamorphosis-inhibiting activity of NC-170 and JHs on the small brown planthopper.

Chemicals	Single application[a] ID_{50}/larva (95%CI)	Repeated application[b]
NC-170	0.42 pg (0.31–0.58 pg)	
JH-1	Inactive	Inactive
JH-2	Inactive	Inactive
JH-3	Inactive	Inactive

[a] Single topical application on the 2nd day of 4th stadium larvae. 'Inactive' signifies that 300 ng a.i./larva caused no inhibitory effect.

[b] Repeated topical applications of 30 ng a.i./larva/day from the 1st day of 4th stadium through the 2nd day of final stadium.

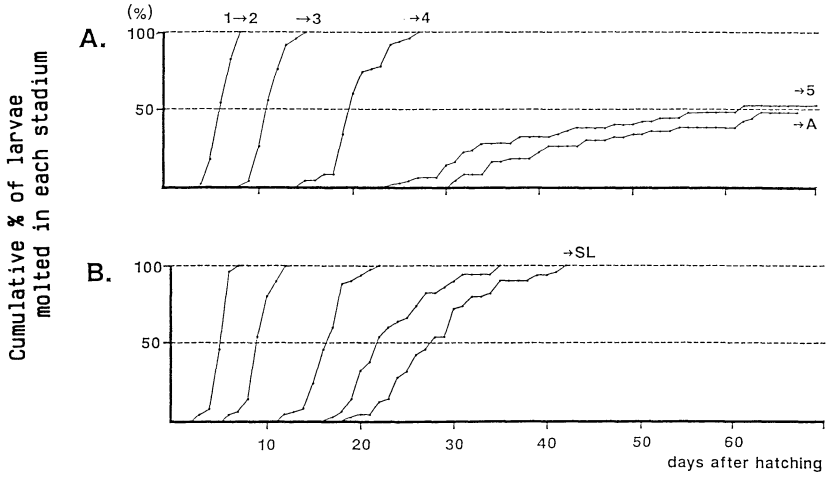

Fig. 3 Cumulative percentage of molted individuals under short-day conditions (20°C, 8L/16D).

A: untreated, B: treated with 100 ppm NC-170.

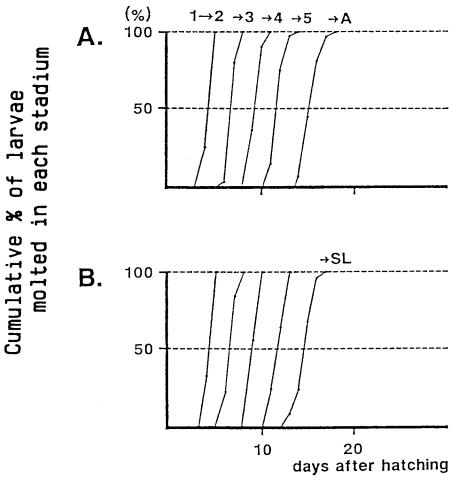

Fig. 4 Cumulative percentage of molted individuals under long-day conditions (25°C, 16L/8D).
A: Untreated, B: Treated with 100 ppm NC-170.

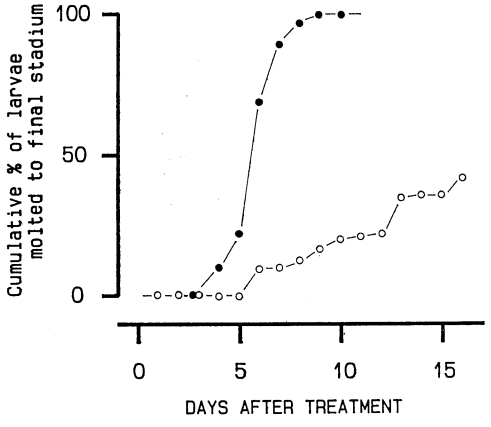

Fig. 5 Cumulative percentage of molted diapausing 4th stadium larvae, transferred into long-day conditions (25°C, 16L/8D).
○, control, kept under short-day conditions (20°C, 8L/16D); ●, transferred into long-day conditions (25°C, 16L/8D).

According to the analysis of the accumulated molting curve patterns, only JH-1 accelerated diapause termination (Fig. 6). JH-1 was effective in accelerating it even at rather low dosages (3 ng, 300 pg and 30 pg/larva) (Fig. 7-A). NC-170 also terminated diapause in the same manner to the same degree of magnitude as JH-1 (Fig. 7-B). Treated 5th stadium

Table 3 Metamorphosis-inhibiting activity of NC-170 and JHs on diapause 4th stadium larvae.

Chemicals	Dosage	Metamorphosis inhibition (%)
NC-170	30 ng	100
	3 ng	100
	300 pg	70.2
	30 pg	61.5
	3 pg	9.5
JH-1	300 ng	0
JH-2	300 ng	0
JH-3	300 ng	0

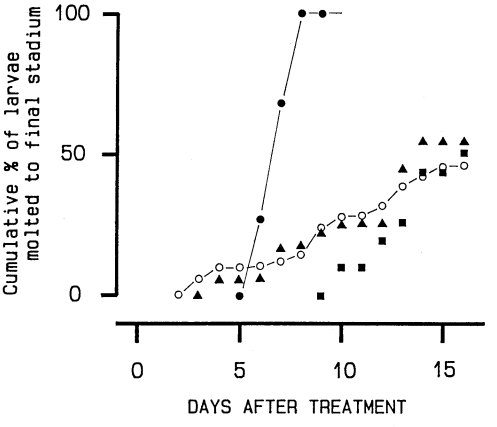

Fig. 6 Effects of JH-1, 2 and 3 on diapause.
○, control (0.03 μl acetone/larva); ●, 300 ng JH-1/larva; ■, 300 ng JH-2/larva; ▲, 300 ng JH-3/larva.

larvae molted into adults or SL about 6–10 days after the 4th molt, which is nearly the normal duration for larvae reared at 20°C under long-day photoperiods. Then, the incidence of juvenilization of each resulting adult was observed. NC-170 of more than 30 pg caused considerable juvenilizations, but the three JHs showed no effects as observed in nondiapause insects (Table 3).

Similarly, effects of 20-hydroxyecdysone were examined, since molting hormone is considered to be essentially involved in the termination of larval diapause in other insects.[12] The activity of 20-hydroxyecdysone is not usually evaluated by topical application because of its low permeability to insect cuticles.

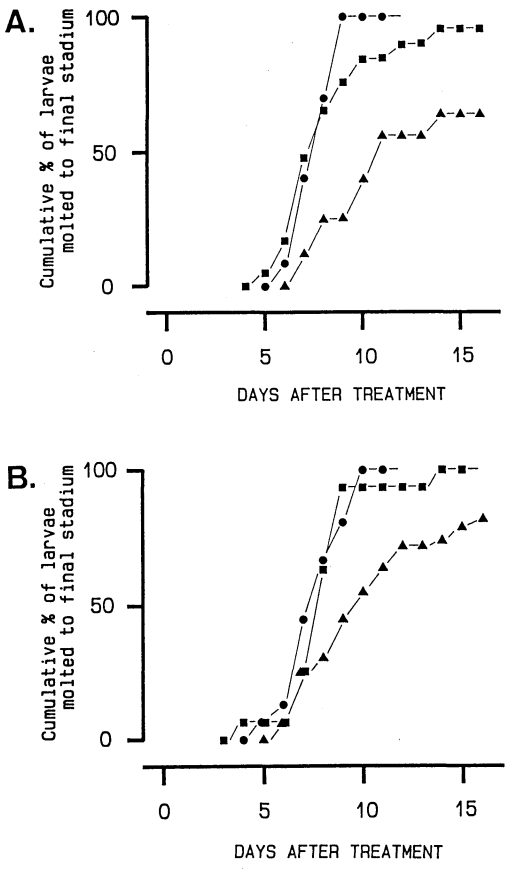

Fig. 7 Effects of JH-1 and NC-170 on diapause.
A: JH-1, B: NC-170.
●, 3 ng/larva; ■, 300 pg/larva; ▲, 30 pg/larva.

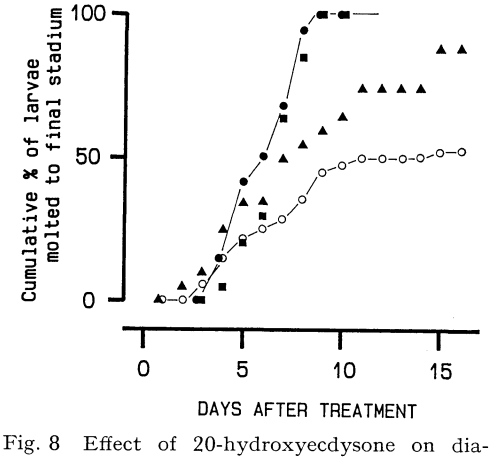

Fig. 8 Effect of 20-hydroxyecdysone on diapause.
○, control (0.03 μl acetone/larva); ●, 30 ng/larva; ■, 3 ng/larva; ▲, 300 pg/larva.

However, some positive results[13,14] encouraged us to conduct a qualitative evaluation using this method. The hormone also terminated diapause in a dose-dependent manner, although the required dosages were relatively high (more than 300 pg) (Fig. 8).

DISCUSSION

In our previous paper[11] we reported that exogenous JH-1 exhibited a strong metamorphosis-inhibiting activity against four species of leafhoppers, whereas JH-2 was less potent and JH-3 was almost completely inactive. NC-170 was about 30 times more active than JH-1 and essentially mimicked the effects of JH-1 as observed in the morphology of the affected insects. The critical period of the morphogenetic effect of NC-170 was found to exist in a 24 hr span before and after the 4th larval molt. Hence, the chemical appears to be a so-called JH mimic.

To the small brown planthopper, however, three JHs tested were completely inactive as metamorphosis inhibitors, whereas NC-170 was as potent as observed on leafhoppers. Observations of the SL produced by NC-170 revealed that apparent juvenilizations of morphological characteristics occurred, and the critical period of the morphogenetic effects was in the early final stadium. Based on the accumulated knowledge of the morphogenetic effects of JHs and JHMs on many other insects,[15] these results indicate that the endocrinological basis of metamorphosis in this species is also under the rule of 'classical scheme' of metamorphic-controlling functions of JH in insects,[16] and that 'a juvenile hormone' primarily regulates the metamorphosis, although the three JHs tested were inactive. NC-170 is thus presumed to be a mimic of the hypothetical juvenile hormone which is supposed to possess a morphogenetic activity on the small brown planthopper.

In addition, of notable interest are the effects of JH-1 and NC-170 on larval diapause of this species. Although no information on the endocrinological basis of the diapause is available at this time, some observations have shown the following basic diapause patterns: the diapause is caused by short-day photoperiods in concert with low temperatures

during embryonic and larval development, and can be easily canceled by certain favorable environmental factors at any stage in diapause.[4] In other words, this diapause is a kind of 'quiescence,' in which only an expected 4th larval molt is postponed and 'diapause development' is unnecessary to complete the diapause. Therefore, a molting hormone (ecdysteroid) titer in haemolymph is likely to be of key importance to regulate the diapause as shown in the larval diapause of other species.[12] Our finding that a 20-hydroxyecdysone application can solely terminate the diapause suggests that a lack of an ecdysteroid surge (inducing the ensuing molt) results in quiescence.

JH-1 also terminated the diapause in a dose-dependent manner. The physiological roles of JHs in larval diapause have been most extensively studied in several lepidopterous insects in which JHs are likely to execute regulatory capacity over diapause by several types of interactions with the molting hormone: In the Asiatic rice stem borer, *Chillo suppresalis*,[17] the south-western corn borer, *Diatraea grandiosella*[18] and the slug moth, *Monema flavescens*,[19] the larval diapause is primarily induced and maintained at a moderate titer of JH (slightly lower than that required for a larval-larval molt) and is terminated when the JH titer drops to a level which permits pupal differentiation to occur. In these insects, JH and JHM treatments on diapause larvae result in a prolongation of diapause. On the other hand, the JH involvement is limited to diapause induction in the European corn borer, *Ostrinia nubiralis*[20] and the codling moth, *Laspeyresia pomonella*.[21] In these cases, JH and JHM treatments on diapause larvae do not alter the programmed pattern of diapause so drastically.

In addition to the above, termination of diapause with JHM treatment has been reported on two species: the pink bollworm, *Pectinophora gossypiella*[9] and the stem borer, *Sesamia inferens*.[22] However, the JHM dosages used in these experiments were too large to demonstrate essential physiological functions of JH involved in their diapause. Therefore, the effects of NC-170 on the small brown planthopper are likely to be classified in the third category mentioned above.

However, we must point out a unique physiological situation caused in the diapause of the small brown planthopper by the limited action of JH-1. For instance, all of the JHs and JHMs used in the studies cited above also exhibited some morphogenetic effects: producing supernumerary larvae, larval-pupal intermediates, and so on. In these insects, therefore, an identical JH exerts different roles (in diapause and metamorphosis) during each critical period or at each critical titer in haemolymph. On the small brown planthopper, however, exogenous JH-1 did not show any morphogenetic effect but only terminated the larval diapause, and JH-2 and 3, which are closely related to JH-1 in chemical structure, showed no effects in either case. This leads us to speculate that more than two different JHs are assigned to different functions in the postembryonic development of the species. NC-170 exhibited JH-like effects on both metamorphosis and diapause.

Although further studies are needed in order to elucidate the physiological basis of the diapause, the results obtained this time suggest that the diapause is primarily under the regulation of the molting hormone, and exogenous JH-1 and NC-170 would activate the prothoracic glands (or the brain-prothoracic gland system) to release the molting hormone, by which the diapause is subsequently terminated. Analytical work to clarify the ecdysteroid fluctuation after JH-1 and NC-170 applications is now in progress.

From the practical standpoint, NC-170 with the dual biological activities in concert with its strict selectivity may open a new management approach for control of populations of the small brown planthopper.

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要　　約

幼若ホルモン様物質，NC-170 のヒメトビウンカの変態および休眠に対する影響

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ピリダジノン環を有する新規 JHM, NC-170 は，ヒメトビウンカの変態を強く阻害し，その活性は4齢幼虫に対する局所施用法で ID_{50}=0.42 pg/larva であった．さらに，NC-170 は，短日，低温条件により誘起される本種の幼虫休眠を阻止し，また，休眠幼虫に対しては，その休眠を打破する作用を示した．本種の休眠は，脱皮ホルモンの処理によって打破されることが確かめられたことから，NC-170 は，内因性脱皮ホルモンの分泌を刺激し，その結果，二次的に休眠打破を引き起こした可能性がある．一方，天然の JH-1, 2, 3 について同様の検討を行なったところ，JH-1 のみが，NC-170 と同等の休眠打破活性を示したものの，変態に関してはいずれの JH もまったく影響を与えなかった．したがって，本種においては，NC-170 は，JH-1 のみならず，変態生理に関与する別種の JH homologue の mimic として機能しているものと想像された．