Hormonal Control of Inhibition of Pupation Caused by Crowding Larvae of *Tribolium freemani* HINTON (Coleoptera: Tenebrionidae)

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The hormonal control of the inhibition of pupation in *Tribolium freemani* larvae caused by crowding has been investigated using several types of anti-juvenile hormone agents (AJHAs); precocenes, imidazoles, ETB and FMeV. The precocenes were the only chemicals which could induce the pupation by releasing the inhibition, although FMeV seemed to have weaker effect, compared to them. An exogenous juvenoid (S31183) administrated along with the precocene antagonized the action of the precocene to the larvae. These results suggest that the corpora allata of the larvae reared under crowded conditions secrete high levels of juvenile hormone. The characteristics observed in the beetle suggest that this insect could be applied as a test animal for either screening or assaying new insect growth regulators.

INTRODUCTION

*Tribolium freemani* is a very close species of *T. castaneum*: it produces sterile progenies when crossed with either sexes of the letter (NAKAKITA et al., 1981). *T. castaneum* is a cosmopolitan stored product insect and also well known as an experimental insect as well as *T. confusum* (SOKOLOFF, 1972). However, *T. freemani* is a very rare species to find, since only two instances have been reported; one female in Kashmir, India in 1893 (?), as cited by HINTON (HINTON, 1948) and several individuals in Yokohama, Japan in 1978 (NAKAKITA et al., 1981).

Using the strain of Yokohama, some of the biological characteristics on *T. freemani* have been analyzed (NAKAKITA et al., 1981; NAKAKITA, 1982; IMURA et al., 1982; IMURA and NAKAKITA, 1984; IMURA, 1987; SUZUKI et al., 1987; SUZUKI et al., 1988). Since *T. freemani* is a sibling species of *T. castaneum*, its major characteristics were very similar to those of *T. castaneum*. However, it was observed that the mature larvae of the 6th instar of *T. freemani* repeated stationary molts and could not pupate when they were reared under crowded condition (NAKAKITA, 1982). As it was speculated that the physical contacts of the larvae reared under crowded conditions may stimulate the corpora allata to secrete high titre of juvenile hormones, the present study was conducted to elucidate the phenomenon using various anti-juvenile hormone agents (AJHAs).

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MATERIALS AND METHODS

Insect. *T. freemani* used in the experiment was the strain described in a previous report (Nakakita et al., 1981). Larvae of the insect were reared on wheat feed medium containing 5% yeast powder at a high density (more than 5 larvae per 1 g diet) at 30°C and 70% RH. Larvae fully matured (older than 2 months after hatching) were used for the experiments.

**AJHAs and JHA.** Four different types of AJHAs were tested: 1. precocene I (7-methoxy-2, 2-dimethyl-chromene) and II (6, 7-dimethoxy-2, 2-dimethylchromene), purchased from Sigma Chemical; 2. imidazoles, KK 22 (1-citronellyl-5-phenylimidazole) and 42 (1-benzyl-5-(E)-2, 6-dimethyl-1, 5-heptadienyl imidazole) supplied by Dr. E. Kuwano, Kyushu University; 3. ETB (ethyl 4-[2-(tert-butylcarboxyloxy)butoxy]-benzoate); 4. FMev (tetrahydro-4-fluoro-methyl-4-hydroxy-2H-pyran-2-one); 3 and 4 were gifts from Dr. G. B. Staal, Zoecron.

As a juvenile hormone analog (JHA), S31183 (2-[1-methyl 1-2-(4-phenoxy-phenoxyl)epoxyl]pyridine) was used (supplied by Sumitomo Chemical).

**Application of chemicals.** Each anti-JH was dissolved in acetone to give appropriate concentrations, and added either in the diet or applied by contact to the larvae. For the dietary test, 10 larvae were reared in a vial (25 mm dia × 55 mm hight) containing 1 g of the diet treated with the chemicals. For the contact test, 5 larvae were placed in an empty vial in which the bottom was coated with the chemicals tested. The experiments were carried on for 60 days in a room conditioned at 25°C and 70% RH.

For the tests of precocene II on different insect densities, 3–10 larvae were reared on 1 g diet containing the chemical at 1,000 ppm per vial and kept for 30 days at 30°C and 70% RH.

For the tests of JHA, an acetone solution of S31183 diluted to appropriate concentrations was mixed with the diet and applied to a larva isolated from the larvae reared under crowded conditions. The diet mixed with both JHA (0.01–100 ppm) and precocene II (1,000 ppm) was also tested to the larvae at different densities (1–10 larva per 1 g of the diet) at 30°C and 70% RH for 30 days.

All the experiments were replicated 5 times.

RESULTS

**Effects of AJHAs**

As shown in Table 1, effect of AJHAs in the dietary test for induction of pupation in the *T. freemani* larva reared under crowded conditions differed markedly among the types of the chemicals. Stimulative effects were obtained by precocene I and II which caused the earliest pupation of the larvae after the treatment, although precocene II was more effective than precocene I. While a slight positive effect was observed with the FMev treated diets, no effect was observed with the other chemicals.

In contrast, none of those chemicals gave any stimulative effect on the larvae for pupation in the contact test, and higher concentrations of all chemicals tended to exert lethal effects (Table 2).

**Effect of precocene II to larvae reared at different densities**

Since the dietary administration of the precocene II (1,000 ppm) was the most
Table 1. Effects of various AJHAs in the dietary test on *T. fremani* larvae reared under crowded conditions\(^a,b\)

<table>
<thead>
<tr>
<th>AJHAs</th>
<th>Pupation (%)</th>
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<tbody>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>precocene I</td>
<td>0</td>
</tr>
<tr>
<td>precocene II</td>
<td>0</td>
</tr>
<tr>
<td>KK-22</td>
<td>0</td>
</tr>
<tr>
<td>KK-42</td>
<td>0</td>
</tr>
<tr>
<td>ETB</td>
<td>0</td>
</tr>
<tr>
<td>FMev</td>
<td>0</td>
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</table>

\(^a\) Ten larvae were reared in a vial and given 1 g diet containing AJHAs.
\(^b\) The cumulative % of pupation was obtained during 60 days at 25°C and 70% RH.

Table 2. Effects of various AJHAs in contact test on *T. fremani* larvae reared under crowded conditions\(^a,b\)

<table>
<thead>
<tr>
<th>Pupation and mortality (%)</th>
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<td></td>
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<tr>
<td>100</td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>precocene I</td>
</tr>
<tr>
<td>precocene II</td>
</tr>
<tr>
<td>KK-22</td>
</tr>
<tr>
<td>KK-42</td>
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<tr>
<td>ETB</td>
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<tr>
<td>FMev</td>
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</table>

\(^a\) Five larvae were kept in a vial coated with AJHAs on the bottom.
\(^b\) The numbers in parentheses are % of mortality obtained for 60 days at 25°C and 70% RH.

Fig. 1. Effects of dietary administration of precocene II (1,000 ppm) on the larvae of *T. fremani* at different densities. O, on diet without precocene II; ●, on diet with precocene II. The values are averages with standard deviations (5 replicates) as cumulative % of pupation for 30 days at 30°C and 70% RH.

Effective to promote pupation of crowded larvae, the effects of precocene II at this concentration in the diet were further analyzed on the larvae reared at various densities, as shown in Fig. 1. In the media without the chemical a complete inhibition of pupa-
Fig. 2. Effects of dietary administration of S31183 (JHA) on the larvae of T. freemani. ○: JHA was singly administrated to the isolated larva, ●: JHA was administrated with precocene II (1,000 ppm) to the larvae reared at density of 5 per vial. The values are averages with standard deviations (5 replicates) as cumulative % of pupation for 30 days at 30°C and 70% RH.

Fig. 3. Scheme*: Bioassay for the detection of IGR activity with the larvae of T. freemani. a) Procedure for the scheme. For detecting AJH activity, 5–10 larvae are placed on one g diet mixed with a test chemical. As a result, if the diet could promote the pupation, the chemical may have AJH activity. On the contrary, for JH activity, a larva isolated from the crowded condition is placed on one g diet mixed with a test chemical. If the diet interrupt the pupation, the chemical may have JH activity.

tion was induced when the larvae were reared at densities exceeding 3 per vial. However, the inhibition was markedly released by the addition of precocene II with increase of the larval density; 70% pupation occurred at a density of 6, while less than 50% at a density of 10, 30 days after the treatment. The time required for the commencement of pupation was about 2 weeks for the larvae treated with the precocene and 1 week for those reared at densities less than 3.
Effect of JHA to the larvae co-treated with precocene II

Figure 2 shows the dose-dependent effects of JHA (S31183) on the crowded larvae which were simultaneously treated with precocene II (1,000 ppm), and on the isolated larvae. While isolation of the crowded larvae allowed them to pupate, 1 ppm of JHA completely suppressed the pupation in the isolated larvae. When the precocene was also administrated, JHA of 1 ppm or higher completely suppressed the pupation of the larvae reared at crowded conditions. Thus, it was shown that the effect of precocene II on the larvae could be counteracted by the simultaneous administration of JHA.

DISCUSSION

The pupation of *T. freemani* was greatly inhibited by increasing larval population density (Nakakita, 1982). The inhibition was suggested to be caused by the external irritation due to contact with other individuals which promoted the activation of the corpora allata resulting in high titre of JH (Nakakita, 1982).

The results presented here strongly suggested that high titre of JH in the larvae reared under crowded condition is responsible for the inhibition of pupation, because precocenes which have been known to directly interrupt the corpora allata and block the secretion of juvenile hormone (Masner et al., 1979; Bowers, 1983), enhanced pupation of the crowded larvae, and JHA co-treated with precocene II inhibited the action. Thus, the inhibitory effect of pupation under crowded conditions seemed to be similar to the larval diapause in other species; in the diapausing larvae the corpora allata secrete a high titre of JH (Yagi and Fukaya, 1974; Chippendale, 1977).

The cancellation of precocene effect to induce pupation by JHA can be understood to be caused by its rescue or antagonistic effect to precocious action of precocene: precocene does not act on either JH secreted in the hemolymph or other enzymes like JH esterase, lower activity of which is associated with diapause in some insects (Bean et al., 1982), but directly on the corpora allata, as described above.

It is well known that precocene induces precocious adults when applied to insects of many species at larval stages (Staal, 1986), and also known that it exerts an effect in the termination of diapause in a few species (Bowers, 1983). In the codling moth, *Laspeyresia pomonella*, diapause induced by short day exposure was associated with a high titre of JHs (Hansen and Harwood, 1968). The injection of precocene II to the diapausing larvae caused pupation within 10 days (Sieber and Benz, 1980). Similar mechanisms both for diapause termination in the codling moth and enhancement of metamorphosis in *T. freemani* by the precocene are expected to function, although details remain unsolved.

Higher densities of the larvae gradually reduced the effect of precocene II in *T. freemani* (Fig. 1). This finding seems to indicate that the corpora allata in the larvae is activated in a density dependent manner, and so the feeding of 1,000 ppm of precocene II is insufficient to completely inhibit the JH secretory function of the corpora allata in the larvae reared at densities over 3 per vial. As to other types of AJHAs tested, only FMev showed a weak effect on blocking the inhibition of pupation by crowding. These differences in the antagonistic function among AJHAs presumably reflect the selectivity of the chemicals specific to some insect orders, because precocenes exhibit functions on the insects covering comparatively wide range of taxa; Heteroptera, Homoptera, Orthoptera, Dictyoptera, Isoptera, Lepidoptera and Diptera, while other chemicals only
on a very few species mostly belonging to Lepidoptera (Staal, 1986).

As far as the authors know, this is the first report to indicate that precocenes act on Coleoptera; although the effect of precocenes on the Colorado potato beetle, Leptinotarsa decemlineata had been reported (Bowers et al., 1976), it was disproved later (Khan et al., 1982).

The present findings indicate that T. fremani is a useful material to study the hormonal regulation of density-dependent metamorphosis. Furthermore as shown in the scheme (Fig. 3), the physiological character of the beetle reared under high population density would be also useful to identify and assay the insect growth regulators like AJHA, JHA, etc., since the mass production of T. fremani is comparatively easy (Nakakita, 1983).

ACKNOWLEDGMENTS

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REFERENCES


Hormonal Control of *Tribolium fremani*


