

A new bioassay detecting for IGR activity with larvae of *Tribolium freemani* Hinton (Coleoptera: Tenebrionidae)

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Abstract

Tribolium freemani, a sibling species of *T. castaneum*, has larvae that cannot pupate when reared in crowded condition. However, pupation did occur with administration of anti-juvenile hormone agents (AJHAs) such as precocenes. Moreover, the pupation induced with precocenes was inhibited when larvae were simultaneously treated either with juvenile hormones or juvenile hormone analogues (JHA). These results suggest that this insect could be used as a test insect for either screening or assaying new insect growth regulators such as AJHA and JHA. Bioassays using this insect with extracts from 43 tropical plants showed that the extract from sweet flag, *Acorus calamus* promoted pupation of the larvae under crowded condition. The chemical formula of an active substance of this extract was determined as β -asarone.

Introduction

Two species of flour beetles, *Tribolium castaneum* and *T. confusum*, are known not only as pests, but also as experimental animals in the fields of ecology, physiology and genetics. The biology of the genus is covered by Sokoloff (1972, 1974, 1977).

They are also useful test insects for screening of insecticides, since they can be easily handled and mass reared. In the screening of insect growth regulators (IGRs), the growth and development of immature stages, especially the larval stage must be focused on to assess the effectiveness of the chemical tested. The usefulness of *T. castaneum* and *T. confusum* in bioassay of IGRs is limited because of their short larval period and tiny body size. *T. freemani*, a sibling species of *T. castaneum*, may be more useful since its larval period can be artificially manipulated and larvae are sensitive to IGRs such as anti-juvenile hormone agents (AJHAs) and juvenile hormone analogs (JHAs). Although *T. freemani* is rare in the field, it is easy to mass rear and handle.

This paper briefly describes the biology of *T. freemani*, the response of its larvae to IGRs in a bioassay and detection of IGRs in tropical plants.

Biology of *T. freemani*

The first specimen of *T. freemani*, an adult female, was captured at Hispar, Kashmir in India 189(3?). It was named much

later by Hinton (Hinton 1948). However, almost a century had elapsed before the biology of the species was studied, after several specimens were rediscovered at Yokohama, Japan in 1978 and identified by Dr. D.G. Halstead (Nakakita et al. 1981).

From studies of the Yokohama strain, we now have information on *T. freemani* regarding its biology (Nakakita et al. 1981; Imura et al. 1982; Nakakita 1983), food habits (Imura 1991), rearing conditions (Nakakita 1982; Imura and Nakakita 1984), population ecology (Imura 1987; Matsumura and Yoshida 1988), genetics and physiology (Suzuki et al. 1987; 1988; Nakakita 1990; Kotaki et al. 1993). Other studies have been reported in the *Tribolium* information bulletin edited by Sokoloff (vol. 31, 1991 and 32, 1992). Among the characteristics revealed, the most remarkable one is that *T. freemani* can easily produce hybrid progeny by crossing each sex with each counterpart of *T. castaneum* (Nakakita et al. 1981), indicating the genetic closeness of the species.

Thus, most of the beetle's characteristics are very similar to those of *T. castaneum*, including its potential as a pest of stored products. However, there was an exception: the response of *T. freemani* larvae to density was quite different; its larvae could not pupate when they were reared in crowded conditions (Nakakita 1982).

Pupal inhibition of crowded larvae and its mechanism

As densities of the larvae of *T. freemani* increase, there is a threshold at which pupation is inhibited, as shown in Figure 1. In the case of cultures in vials (25 mm dia x 55 mm height), the threshold was 3 larvae per 1 g wheat feed with 10% yeast per vial (Nakakita 1990). Above the threshold, apart from a few sporadic pupations larvae remained as larvae for nearly 6 months. If overcrowded larvae are removed to conditions below the threshold level, they invariably pupate within as short a time as 10 days (Nakakita 1982; Kotaki et al. 1993). Mechanical stimulation by shaking or other means may also inhibit pupation at densities below the overcrowding threshold (Fujii et al. unpublished data).

On the other hand, the dietary administration of AJHAs such as precocene I (7-methoxy-2, 2-dimethylchromone) and II (6, 7, dimethylxy-2,2-dimethylchromone) can cause successful pupation of the crowded larvae at densities above the crowding threshold (Fig. 1). As far as we know, *T. freemani* is the first beetle species on which precocenes have been shown to act, although these chemicals are known to affect a comparatively wide range of insect taxa, including Homoptera, Heteroptera, Dictyoptera, Orthoptera, Isoptera, Lepidoptera and Diptera (Staal 1986).

Moreover, JHAs such as methoprene and pyriproxyfen, and JHs, completely suppressed the pupation of either the crowded larvae treated with the precocene (Nakakita 1990) or the isolated larvae (Kotaki et al. 1993).

On the basis of the observed phenomena, the mechanism of the crowding effects and the action of IGRs such as precocene and JHA can be illustrated by models as shown in Figure 2. The crowded larvae (Fig. 2a) must contain a high titre of JH,

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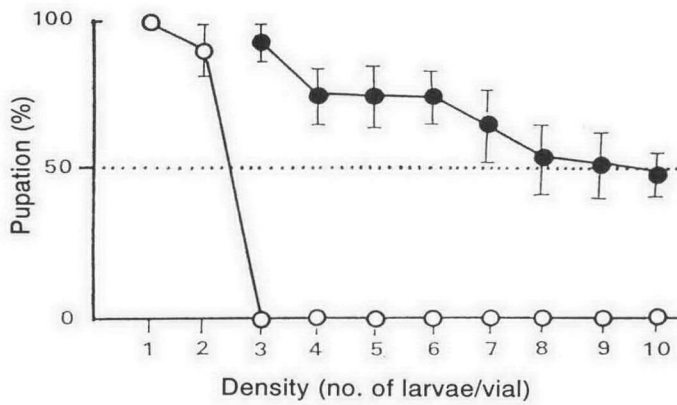


Fig. 1. Crowding effect of *T. freemani* larvae and its release by precocene: ○, on diet without precocene; ●, on diet with precocene II (1000 ppm).

resulting from activated corpora allata through the brain, which detect signals caused by the external irritation of contact with other individuals. In the larvae administered with precocenes (Fig. 2b) pupation of the crowded larvae is enhanced because of the interruption of the corpora allata and blocking of the secretion of JH, as described by Mansner et al. (1979) and Bowers (1983). JHA co-treated with precocenes (Fig. 2c) again inhibits pupation, since precocenes do not act on exogenous JH but directly on the corpora allata.

Bioassay for IGR activity using *T. freemani* larvae

The sensitivity of *T. freemani* to IGRs, such as both types of AJHAs and JHAs presents a useful tool for bioassay of IGR activity of materials. Figure 3 shows the bioassay procedure: the larvae must be prepared under crowded conditions (density over 5 larvae/1 g wheat flour with 10% dried yeast) in

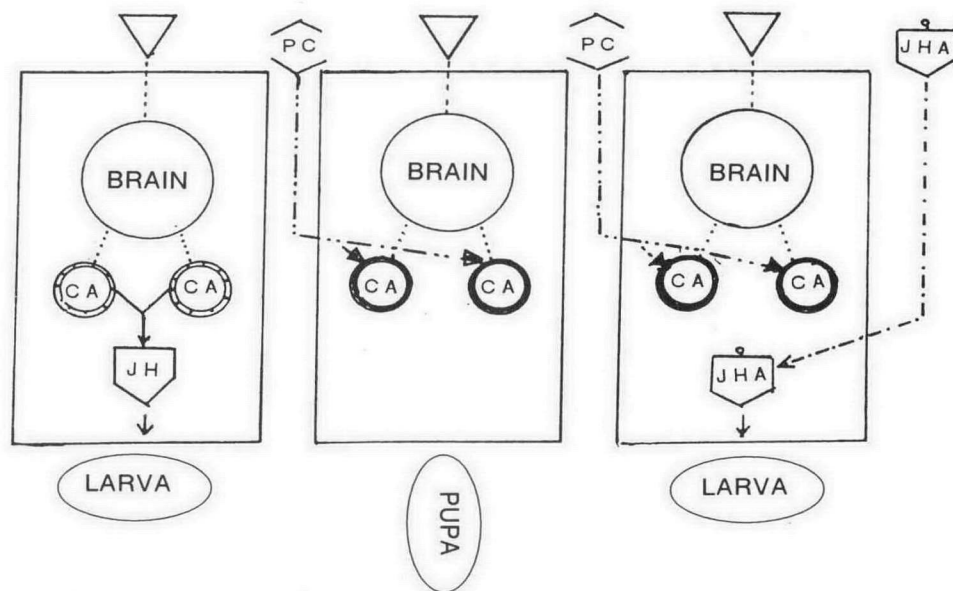
glass jar cultures at 30°C and 70%r.h. The fully mature larvae (older than 2 months after hatching) are used for the bioassay. One gram of the diet mixed with test material to give appropriate concentrations is put in each vial. For detecting AJH activity, 5–10 larvae from the jar are placed on 1 g of the test diet in a vial. After 3–4 weeks, if the diet promoted pupation, the material tested may have AJH activity. On the other hand, for detecting JH activity, a larva isolated from the crowded condition is placed on 1 g diet mixed with a test material. If the diet interrupts the pupation for even two weeks, the material may have JH activity.

Using the above-mentioned bioassay, we have tested nearly 50 species of tropical plants growing in Thailand (Nakakita et al. 1991). As a result, we identified crude oil by hydro-distillation of sweet flag, *Acorus calamus*, which promoted pupation of larvae under crowded conditions. β -asarone was determined by gaschromatogram (Fig. 4) and mass-spectrogram (Fig. 5) as the active substance in the oil. When we administered diet containing 1000 ppm of pure β -asarone (purchased from Sigma Chemical Co.), the chemical induced pupation of crowded *T. freemani* larvae as shown in Figure 6.

The effects of the oil and β -asarone from *A. calamus*, on mortality and sterility in insects have been already reported by, for example many researchers such as Saxena and Mathur (1976), Saxena et al. (1977), Virginia et al. (1986), Schmidt et al. (1991) and Smet et al. (1992). However, our findings may shed new light on the effects of β -asarone on the endocrine system of insects. The bioassay using *T. freemani* indicates that this simple method will be useful for detecting IGR activities from a large number of substances of natural origin or from intermediate chemicals of industrial origin.

Acknowledgment

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Figs. 2a, b, c. Mechanism of crowding effect of *T. freemani* larvae and the action of precocene and JHA.

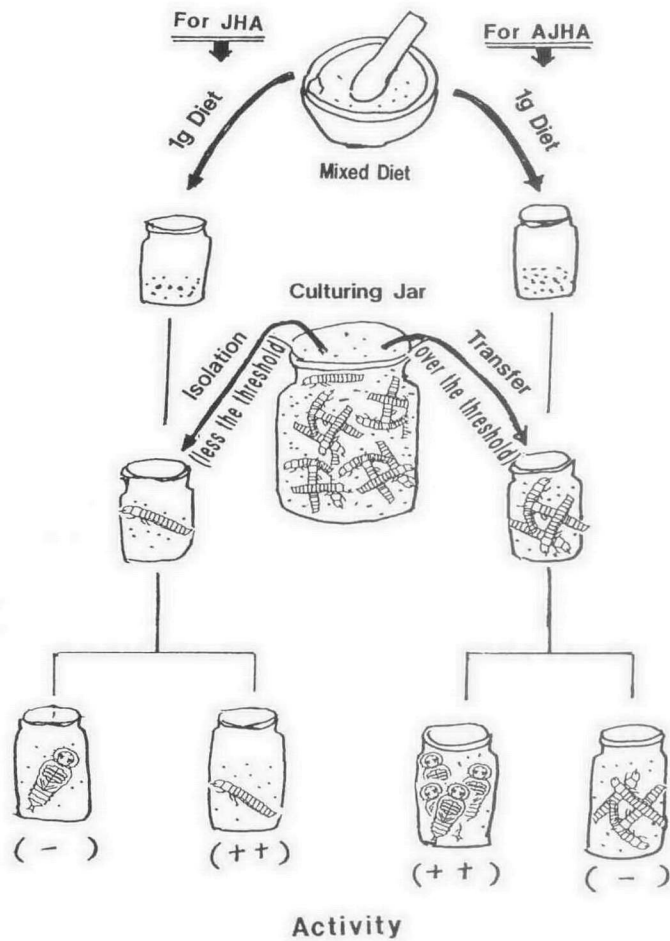


Fig. 3. Detection of IGR activity with larvae of *T. freemani*.

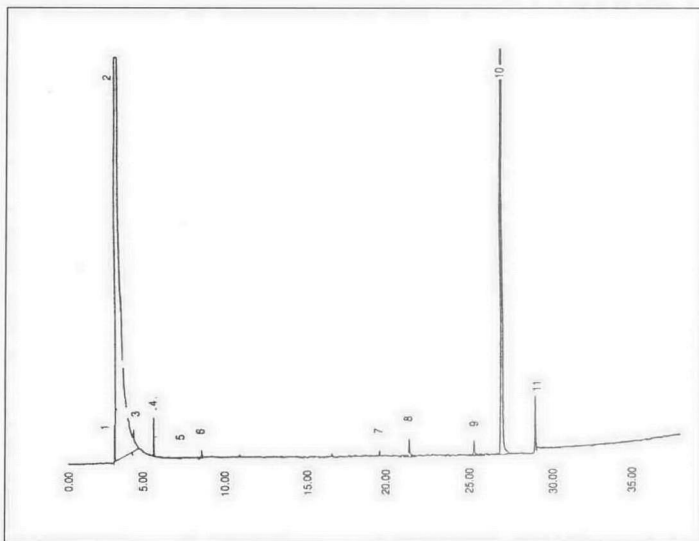


Fig. 4. A gas chromatogram of the active fraction of *Acorus calamus*.

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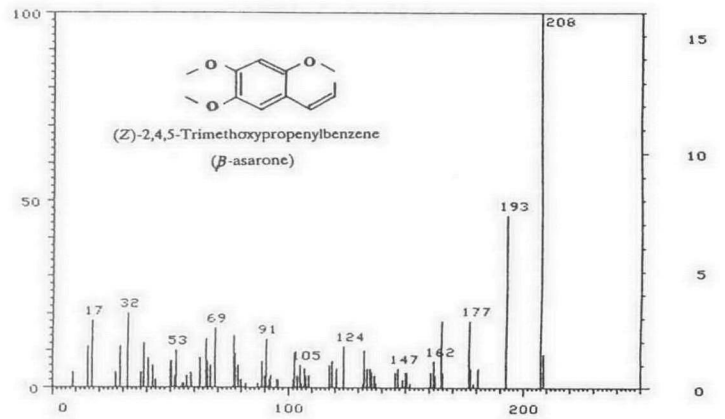


Fig. 5. Mass-spectra of the active fraction of *Acorus calamus*.

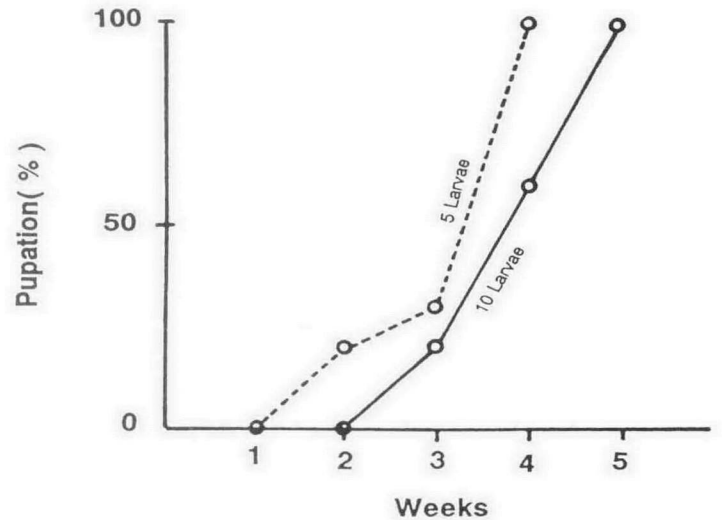


Fig. 6. Effect of dietary administration of β -asarone (1000 ppm) on the larvae of *T. freemani*.

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