variation is maintained within a population is an interesting subject for future studies.

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REFERENCES


Effects of a Juvenile Hormone Analogue, Fenoxycarb, on Larval Growth of the Silkworm, Bombyx mori (Lepidoptera: Bombycidae)

Manabu Kamimura

Department of Sericulture, National Institute of Sericultural and Entomological Science, 1–2, Ohtsuki, Tsukuba 305, Japan

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Key words: Bombyx mori, silkworm, fenoxycarb, juvenile hormone analogue, dauer larvae

Fenoxycarb, O-ethyl N-(2-(4-phenoxypyrenoxo)-ethyl) carbamate is one of the most potent juvenile hormone analogues (JHAs) against a variety of insect species (MASNER et al., 1981; DORN et al., 1981). Although this chemical has been used as an insect growth regulator (IGR) in orchards and vineyards in Europe since the 1980’s, there has been a great deal of concern because of its harmful effects on useful insects and natural enemies (reviewed in GRENIER and GRENIER, 1993). In fact, a non-spinning syndrome of the silkworm, Bombyx mori, was observed in Italy and suggested to have been caused by fenoxycarb (ARZONE et al., 1989).

A similar syndrome was observed in 1991, when fenoxycarb was first used as a pesticide in Japan. Although a direct relationship was not proved, fenoxycarb has been suspected as the cause. Some reports showed that fenoxycarb disturbed the normal growth of B. mori (CAPPELLOZZA et al., 1990; PLANTEVIN et al., 1991; DEDOS et al., 1993). But the maximum non-effective dose at each instar and the difference in sensitivities among the instars had not yet been clarified. Thus, the present study has attempted to clarify these points.

MATERIALS AND METHODS

Insects and chemicals. Silkworm larvae of a racial F1 hybrid between C145 and J140 were reared on an artificial diet (Yakuruto Co., Tokyo) under a 12 h light : 12 h dark photoperiod at 25±1°C in disposable plastic containers (24×18×3 cm).

Technical grade (98.5% purity) fenoxycarb, supplied by Sankyo Corporation (Tokyo, Japan), was dissolved in acetone, and diluted solutions were stored at −20°C.

Topical application of fenoxycarb. In the 1st instar, groups of 20 larvae within 24 h after hatching were topically treated with 0.2 to 20 µg of fenoxycarb in 10 µl acetone using a micropipet. Assuming that each larva absorbed equal amounts of the solution applied, doses of fenoxycarb were 0.01 µg to 1 µg/larva.

During the 2nd to 5th instar, 20 larvae within 24 h after ecysis received individual topical treatment with 1 pg to 100 µg of fenoxycarb in 5 µl acetone. Control larvae were treated with acetone only. Fifth instar larvae which continued feeding for more than 20 days and did not begin spinning were defined as dauer larvae. Experiments were replicated 4 to 6 times.

Statistical analysis. A MANN-WHITNEY U test was employed for statistical analysis between the treated and control groups.

Table 1. Effects of fenoxycarb on spinning and pupation of B. mori larvae

<table>
<thead>
<tr>
<th>Stage of treatment</th>
<th>% Spinning (% Pupation)</th>
<th>Dose/larva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1 pg</td>
</tr>
<tr>
<td>1st</td>
<td>98 (88)</td>
<td>—</td>
</tr>
<tr>
<td>2nd</td>
<td>96 (93)</td>
<td>—</td>
</tr>
<tr>
<td>3rd</td>
<td>96 (88)</td>
<td>—</td>
</tr>
<tr>
<td>4th</td>
<td>100 (90)</td>
<td>—</td>
</tr>
<tr>
<td>5th</td>
<td>99 (92)</td>
<td>99 (88)</td>
</tr>
</tbody>
</table>

N=80–120. * All larvae died within a few days after treatment. ** Treated larvae either became dauer larvac or died by the 20th day of the 5th instar. —: not tested.

RESULTS

Effects of fenoxycarb on the length of each larval instar, cocoon formation and pupation are presented in Fig. 1 and Table 1.

All larvae treated with 1 µg of fenoxycarb at the 1st instar died within a few days, but 0.1 µg caused prolongation of the 1st instar only. No significant effect was observed with 0.01 µg. A few (1%) precocious trilmolts, which made a cocoon and pupated in the 4th instar, appeared after application of 0.1 µg.

In the 2nd instar, larvae treated with 10 µg of fenoxycarb died within a few days. Application of 1 ng to 1 µg extended the length of the 2nd instar, but did not affect the length of the 5th instar. A few larvae (3%) treated with 1 µg became precocious trilmolts.

Application of 1 ng or more fenoxycarb to 3rd instar larvae was effective in terms of the period of the 3rd instar. Feeding periods of 5th instar larvae were also prolonged, and dauer larvae appeared with doses of more than 1 µg. The number of dauer larvae increased with increasing doses and no larvae began spinning with 100 µg. The ratio of normal pupation also decreased dose-dependently.

In the 4th instar, 0.1 and 1 ng was the lowest dose for prolongation of the period of the 4th and 5th instar, respectively. Dauer larvae appeared by 0.1 µg or more. Normal pupation was significantly inhibited with more than 0.01 µg.

Extra (5th) larval molts were observed in the 3rd and 4th instar treatments. The lowest doses which induced an extra molt were 0.1 µg and 0.1 ng at the 3rd and 4th instar, respectively. The duration of the 5th instar of these larvae was shortened to 4–6 d, and feeding periods of the 6th instar ranged from 7 to more than 20 d depending on the dose. More than half of the pentamolts which were treated with

Fig. 1. Relationship between dose of fenoxycarb and period of each instar. B. mori larvae were topically treated with fenoxycarb at the beginning of the 1st (A), 2nd (B), 3rd (C), 4th (D) and 5th (E) instar. The length of the 5th instar represents the feeding period of larvae that initiated spinning. Asterisks (*, **) in bars indicate significant difference from the control at p<0.05 and p<0.01, respectively, by a MANN-WHITNEY U-test. “All dead” means that all larvae died within a few days of the treatment. D means that no larva initiated spinning. Numbers in parentheses represent percentages of dauer larvae.
0.1 ng to 0.01 µg in the 4th instar spun cocoons and pupated normally.

In the 5th instar, the feeding period was prolonged dose-dependently by 0.01 ng or more fenoxycarb. Ninety percent of larvae treated with 0.01 µg became dauer larvae and no larvae could spin cocoons with 0.1 µg or more. The ratio of normal pupation was drastically decreased by application of more than 0.01 ng.

DISCUSSION

In this study, a response of B. mori larvae to various amounts of fenoxycarb were clearly shown for each instar. In the 1st and 2nd instar, sensitivity of larvae to fenoxycarb was rather low; topical application of 1 µg at the 1st instar or 10 µg at the 2nd instar killed all larvae within a few days, but lower doses had little effect on subsequent instars.

On the other hand, application after the 3rd instar prolonged the feeding period of the 5th instar and prevented spinning and normal pupation. The maximal non-effective doses for prolongation of the feeding period of the 5th instar, abnormal pupation and appearance of dauer larvae were lower with treatment at later instars; thus, the 5th instar was the most sensitive stage at which 0.01 ng inhibited normal pupation by about 50%. The maximal non-effective doses in terms of the periods of instar treated showed a similar tendency.

Cappellozza et al. (1990) reported that dauer larvae were induced by feeding with 59.6 µg (10^{-7} g) of fenoxycarb per larva at the 5th instar. This value is extraordinarily small (about 10^6 times smaller) compared with the results presented here, and may be a result of differences in experimental design. In this study, silkworms were topically treated only once at the beginning of the instar. On the other hand, in Cappellozza et al. (1990), the dosage was estimated from fenoxycarb-treated mulberry leaves fed for 6 to 8 d in the 5th instar. Continuous administration by feeding may be the cause of such a strong effect. Plantevin et al. (1991) induced dauer larvae by both topical and dietary application at the 2nd, 3rd or 4th instar in the silkworm. Dauer larvae were not induced by treatment at the 2nd instar in this study.

The application of fenoxycarb affected molting. Treatment at the 1st or 2nd instar caused a small number of precocious trimolters, whereas treatment at the 3rd or 4th instar caused supernumeral pentamolters. Trimolters or pentamolters were reported to be induced by another JHA, methoprene, and it was proposed that application of JHA influenced activities or timing of endocrine organs to change molting (Komori, 1978; Oshiki and Tamura, 1985; Kadono-Okuda et al., 1986). Since the incidence of trimolters or pentamolters was low in this study, more detailed experiments are necessary to analyze these phenomena.

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