PUBLICATIONS:


Growth inhibition and total loss of reproductive potential in *Tribolium castaneum* by *Artocarpus hirsuta* lectin

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Summary

The α-galactoside binding lectin from *Artocarpus hirsuta* inhibits growth and has anti-fertility activity on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (red flour beetle). Postembryonic development was affected to a significant level when freshly hatched first instar larvae of *T. castaneum* were fed on a lectin-treated diet at 0.5% (w/w) concentration. Although larval survival till the tenth day did not differ significantly, larval weight did. Similarly, time taken for pupation, percentage pupation, time taken for adult emergence and percentage adult emergence were adversely affected. Larval–pupal intermediates comprised 11% and pupal–adult intermediates comprised around 50% of the treated population after 30–35 days. Finally, the F1 breeding pairs completely failed to reproduce successfully since the female adults from the breeding pairs were unable to lay a single egg.

Key words: Lectin, *Artocarpus hirsuta*, growth inhibition, *Tribolium castaneum*

Lectins are carbohydrate binding proteins occurring ubiquitously in nature which have diverse roles in plants, animals and microbes. They are widely used as tools in the study of different biological processes (Sharon, 1993). One of the important biological activities of plant lectins is the insecticidal activity, vital as a plant defense mechanism. The GlcNAc (N-acetylglucosamine) specific lectins from wheatgerm (*Triticum aestivum*, WGA) and rice (*Oryza sativa*L.) and GalNAc (N-acetylgalactosamine) specific lectins from osage orange (*Maclura pomifera*) and peanut (*Arachis hypogea*) at the levels of 0.2 and 1.0 % (w/v) have been implicated in the insecticidal activity against the insect, cowpea weevil (*Callosobruchus maculatus*) (Huesing et al., 1991; Murdock et al., 1990). The mannose binding snowdrop lectin (*Galanthus nivalis*, GNA) is toxic (at 5.3 mM) against the rice brown plant hopper (*Nilaparvata lugens*), at 2% (w/v of dietary protein) against tomato moth (*Lacanobia oleracea*) larvae and at 0.1% (w/v) against potato aphid

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(Aulacorthum solani) (Powell et al., 1998; Fitches et al., 1997; Down et al., 1996). Several mannose-specific lectins (at <0.1% w/w of the diet) from the families Amaryllidaceae and Alliaceae are found to be toxic to insects (especially Coleoptera) (Gatehouse et al., 1992). The significance of studying insecticidal lectins extensively lies in the possibility of cloning and transferring the lectin genes into plants susceptible to insects thereby conferring resistance to them (Cavalieri et al., 1991; Rao et al., 1998; Zhu et al., 1996). Artocarpus hirsuta, a variety of jackfruit, has galactose-specific lectin present in the seeds (Gurjar et al., 1998; Gaikwad et al., 1998). In the present paper, we report the insecticidal activity expressed by this lectin against the red flour beetle.

The A. hirsuta lectin was purified by ion-exchange chromatography as described by Gurjar et al. (1998). Eggs of Tribolium castaneum were obtained by infesting freshly sieved flour (40 apertures/mm²) with adults obtained from a laboratory culture maintained on wheat flour at 28±2°C and 60% rh. After 24 h the adults were removed and the flour sieved again to obtain the eggs. These eggs were maintained at 28±2°C to obtain freshly emerged first instar larvae.

The purified lectin was dissolved in 20 mM phosphate buffer, pH 7.2, to give a 40 mg/ml stock solution. From this stock, 0.25 ml (10 mg) was thoroughly incorporated into 2 g of sieved wheat flour giving a concentration of 0.5% (w/w) of the pure protein. The control diet received only 0.25 ml of the phosphate buffer alone in 2 g of diet (wheat flour). Both the treated and the control diets were allowed to dry at 25±2°C. After complete drying, these diets were used for the first experiment wherein 15 newly hatched first instar larvae of T. castaneum were released to monitor the growth and development. Ten days after the start of the experiment, larvae were weighed five at a time and their survival was recorded. The larval and pupal counts were taken every week by sieving the flour. Once pupation had begun in any treatment, observations were made every day for adult emergence. Adults emerging from the treated diet as well as control diet were kept separately as breeding pairs for the second experiment, to monitor egg laying and hatchability. All the experiments were replicated three times. Data obtained were compared by the Student's t-test (Snedecor and Cochrane, 1967).

A. hirsuta lectin at 0.5% (w/w) concentration reduced the larval weight of T. castaneum by 55% after 10 days feeding as compared to the control (Table 1). Time taken for pupation as well as adult emergence of the larvae fed on the treated diet was greater by 23% and 16%, respectively, when compared with the larvae fed on the control diet (Table 1). Abnormal intermediates were also found during the course of development. Pupal-adult intermediates (50%) were more common than larval-pupal intermediates (11%) (Fig. 1). When the adults of T. castaneum emerging following lectin treatment were kept as breeding pairs, the females laid no eggs, whereas in control batches, the females laid on an average 7.33±1.23 eggs.

Similar effects have been observed with snowdrop lectin (GNA) at 2% (w/w) concentration causing reduction in biomass of the tomato moth by 32% in 21 days and significantly slower larval development as assessed by instar duration and reduced fecundity for the rice brown plant hopper (Fitches et al., 1997; Down et al., 1996). Percentage pupation and percentage adult emergence are both reduced. Con A reduces the size and fecundity of the peach-potato aphid at 1–9 mM concentration in a liquid diet (Gatehouse et al., 1999). Snowdrop lectin expressed in transgenic rice decreases the survival and overall fecundity of the rice brown plant hopper, retards insect development and has a deterrent effect on insect feeding (Rao et al., 1998).

Retarded development and decreased biomass are the major consequences of most lectins in insects. Since the lectin does not disturb the larval feeding pattern, their role in disrupting the growth may be due to other effects, as shown by Rembold et al. (1982) and Mukherjee and Ramachandran (1989) with azadirachtin. Since lectins are known to bind to carbohydrate receptors, it is possible that this hampers the supply of glucose which is needed for chitin build up, thus resulting in growth inhibition. The fact that lectins are involved in plant defense against insect and fungal pathogens has tremendous potential in crop protection.

<table>
<thead>
<tr>
<th>Table 1. Effect of A. hirsuta lectin at 0.5% concentration on the growth and development of T. castaneum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Larval weight after 10 days (mg) X ± SD/5 larvae</td>
</tr>
<tr>
<td>Time taken for pupation (days) X ± SD</td>
</tr>
<tr>
<td>Time taken for adult emergence (days) X ± SD</td>
</tr>
</tbody>
</table>

*p<0.05.
Fig. 1. Effect of *A. hirsuta* lectin on *T. castaneum* (red flour beetle). a, b, c: Normal larva, pupa and adult, respectively. d: Larval–pupal intermediate; e, f: pupal–adult intermediates. Intermediates were observed in the lectin treated group.

Lectin genes from *Griffonia simplicifolia*, *Galanthus nivalis*, *Artocarpus integrifolia*, *Bauhinia purpurea* and *Codium fragile* have been expressed in transgenic plants such as cowpea, wheat, rice and sorghum by genetic engineering making them resistant to the insect pests (Cavaliere et al., 1991; Rao et al., 1998; Zhu et al., 1996).

References


Effect of sublethal concentrations of flufenoxuron on growth, development and reproductive performance of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Summary

The effect of sublethal concentrations 0.00141% (LC<sub>20</sub>), 0.00251% (LC<sub>50</sub>) and 0.00336% (LC<sub>50</sub>) of a dispersible concentrated formulation of the insect growth regulator flufenoxuron (Cascade®), on larval growth and development, adult reproductive potential and egg hatchability of the red flour beetle, *Tribolium castaneum*, was investigated. When neonates were subjected to sublethal concentrations of flufenoxuron in artificial diet for 24 h, there were dose-dependent effects on larval weight, percent pupation and percent adult emergence, as well as time taken for adult emergence. A small proportion of larval-pupal as well as pupal-adult intermediates were observed at all concentrations. Adults emerging from the LC<sub>20</sub> and LC<sub>50</sub> concentrations laid mostly non-viable eggs, and the few larvae which emerged from viable eggs died at the first instar stage. At the LC<sub>50</sub> concentration, all the adults that emerged were deformed and subsequently died. Flufenoxuron exhibited transovarial ovicidal activity resulting in the production of non-viable eggs upon exposure of adults of different ages (2 days old, 3 days old and 4 days old) to treated diet. It was observed that in 2-day-old adults, fecundity decreased with an increased concentration. In the case of 3-day-old adults, there was no difference in fecundity with respect to the concentrations tested, although it was significantly less than the control. In the case of 4-day-old adults there was a drastic reduction in fecundity at LC<sub>50</sub> and the eggs laid were abnormal at all concentrations. Topical application of sublethal concentrations of flufenoxuron to adults of either sex reduced the fecundity in a dose-dependent manner. Furthermore, the fecundity was reduced drastically in pairs where both the sexes were treated as compared to the pairs where only one sex was treated. Eggs showed a decrease in hatching percentage with increasing concentrations of flufenoxuron mixed with diet to which the eggs were exposed.

*Key words: Tribolium castaneum, flufenoxuron, sublethal effects*

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Introduction

Insect growth regulators (IGRs) include compounds that affect molting and metamorphosis by mimicking juvenile hormone (JH agonists) or antagonizing JH activity (ecdysteroid agonists) or by interfering with cuticle formation (chitin synthesis inhibitors) (Smet et al., 1990; Oberlander et al., 1991; Oberlander et al., 1997). The potential of IGRs to suppress insect pests in stored commodities was first suggested by Thomas and Bhatnagar-Thomas (1968). Several studies have advanced the possibility that IGRs can be used in the management of stored product pests (Strong and Diekman, 1973; Williams and Amos, 1974; Hoppe and Suchy, 1975; McGregor and Kramer, 1975; Oberlander et al., 1975; Silhacek and Oberlander, 1975; Loschiavo, 1976; Nickle, 1979).

*Triobolium castaneum* (Herbst) is an economically important pest of stored products worldwide feeding on a wide range of commodities (Arboagast, 1991). Although efficacy and toxicological effects of chitin synthesis inhibitors have been extensively investigated (Oberlander et al., 1997), few studies have dealt with their sublethal effects. Radwan et al. (1978) studied the effect of sublethal doses of *Dimilin* on the reproductive performance of *Spodoptera littoralis* Boisduval for three consecutive generations by treating the fourth instars of each generation. The only other study reported so far is by Biddinger and Hull (1999) wherein they reported the sublethal effects of several classes of IGRs on the tufted apple bud moth, *Platynta ideaeusalis*. Studies on such effects are also important for the assessment of overall ecological impact since non-target species in the periphery of the treated area often receive sublethal doses.

The study reported here was undertaken to investigate the effect of sublethal doses (*LC*$_{20}$, *LC*$_{30}$, and *LC*$_{40}$) of a dispersible concentrated formulation of the chitin synthesis inhibitor flufenoxuron (Cascade®) on *Triobolium castaneum* by exposing various developmental stages to treated diet or topical application and observing the effect on larval growth and development, adult reproductive potential and egg hatchability.

Materials and Methods

A stock culture of *T. castaneum* was maintained on a diet containing wheat flour and 5% Brewers yeast at 29±1°C and 60% relative humidity. Eggs were collected by sieving (sieve number 40) diet infested with adults. Newly emerged adults were obtained by collecting pupae and monitoring them for adult emergence. Flufenoxuron was thoroughly incorporated into the diet using acetone as the carrier solvent. The treated flour was kept at room temperature for 4 h for complete evaporation of the solvent before use in the experiments.

Determination of *LC*$_{20}$ through diet was carried out by releasing newly hatched first instar larvae of *T. castaneum* in a diet treated with various concentrations (by percentage, i.e., grams of active ingredient per gram of the diet) of flufenoxuron for 24 h. An acetone-mixed diet was used as control. The mortality count was taken after 7 days. Subsequently, the sub-lethal doses used in the experiments (*LC*$_{20}$, *LC*$_{30}$ and *LC*$_{40}$) were deduced by extrapolation of the probit mortality analysis.

The effects of sub-lethal concentrations (*LC*$_{20}$, *LC*$_{30}$ and *LC*$_{40}$) of flufenoxuron through diet on survival and metamorphosis of the larvae were examined by releasing 10 newly hatched larvae in the treated diet for 24 h. An acetone-mixed diet was used as control. After 24 h, the larvae were transferred to a normal diet. On the 7th and 10th days after the start of the experiment, the larvae were weighed (five at a time) and their survival was recorded. Once pupation had begun in any treatment, observations were made every day for signs of adult emergence. Percentage pupation, time taken for pupation, percent adult emergence and time taken for adult emergence were recorded. All experiments were replicated five times for each treatment. Regression analysis was performed to determine dose-dependent effects.

Flufenoxuron mixed at various sub-lethal concentrations in diet was also used to determine the effect on fecundity of adults of different ages. An acetonetreated diet was used as a control. All experiments were replicated five times and data obtained were compared by ANOVA, Students *t*-test and the Z-test.

The effects of sub-lethal concentrations of flufenoxuron on the hatchability of eggs were determined by placing 20 eggs in a treated diet and recording hatching of eggs every day till hatching in the control was completed. The data were confirmed 3 days later. All experiments were replicated five times. Regression analysis was performed to determine dose-dependent relationships.

To study the effects of sub-lethal concentrations on topical application to adults, the *LC*$_{20}$ was determined by applying various concentrations of flufenoxuron to the ventral surface of the adult between the mesothoracic and metathoracic legs using a Hamilton micro-syringe. Subsequently, the *LC*$_{20}$ (0.1 μg/μl), *LC*$_{30}$ (0.4 μg/μl) and *LC*$_{40}$ (0.8 μg/μl) values were deduced by extrapolation of the probit mortality analysis and
applied to the adults. The dispensing volume of solution at any concentration was always 1 μl. Adults treated similarly with acetone alone were used as controls. Two hours after treatment, the treated adults were transferred to a normal diet. Crosses were performed as follows: treated males × untreated females; treated females × untreated males; treated males × treated females. Five replicates of each cross were made. Fecundity was observed for 7 days and analyzed by a t-test.

Results

LC$_{50}$ of flufenoxuron for the first instar larvae of *T. castaneum* through dietary treatment was 0.0042% (Fig. 1).

**Effects on growth and development**

Flufenoxuron at various sub-lethal dietary concentrations significantly reduced larval weight on the 7th and 10th days of their growth period compared to the control (Table 1). Reduction in weight of the larvae on the 10th day was observed to be proportional to that of the larvae on the 7th day when compared with the control (Fig. 2). In addition, the time taken for pupation and adult emergence was significantly greater than that of the control (Fig. 3). A significant reduction in percent pupation and percent adult emergence were observed with increasing concentrations of flufenoxuron (Fig. 4).

Sub-lethal concentrations of flufenoxuron through diet also adversely affected moulting of larvae resulting in the development of larval–pupal and pupal–adult intermediates (Table 1, Figs. 5,6). Furthermore, adults emerging from the larvae fed on diet at LC$_{20}$ and LC$_{30}$ laid non-viable eggs and those larvae which emerged from viable eggs died at the first instar stage. With LC$_{40}$ treated larvae developed into deformed adults that failed to retain their dorso-ventral posture. They were unable to ingest the food as their mandibles were incompletely chitinised and did not survive for long.

![Fig. 1](image1.jpg)  
 **Fig. 1.** Regression graph of dose-response for flufenoxuron on mortality of first instar larvae of *T. castaneum*.

![Fig. 2](image2.jpg)  
 **Fig. 2.** Regression graph of dose-response for flufenoxuron on weight of the larvae of *T. castaneum*. A. Equation for trendline 1 (7th day). B. Equation for trendline 2 (10th day).

<table>
<thead>
<tr>
<th>Dose</th>
<th>% larval survival $X \pm SE$</th>
<th>Larval weight (mg) $X \pm SE$</th>
<th>% pupation $X \pm SE$</th>
<th>Time taken for pupation (days) $X \pm SE$</th>
<th>% adult emergence $X \pm SE$</th>
<th>Time taken for adult emergence (days) $X \pm SE$</th>
<th>% LPI$^a$</th>
<th>% PA$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>100</td>
<td>1.66 $\pm 0.3$</td>
<td>5.15 $\pm 1$</td>
<td>100 $\pm 1$</td>
<td>22 $\pm 2^*$</td>
<td>93.18 $\pm 6$</td>
<td>27.4 $\pm 0.8^*$</td>
<td>2.27</td>
</tr>
<tr>
<td>LC$_{20}$</td>
<td>88</td>
<td>1</td>
<td>3.74 $\pm 0.2$</td>
<td>93.5</td>
<td>22 $\pm 2^*$</td>
<td>93.18 $\pm 6$</td>
<td>27.4 $\pm 0.8^*$</td>
<td>2.27</td>
</tr>
<tr>
<td>(0.00014%)</td>
<td>4.4</td>
<td>$\pm 0.8$</td>
<td>$\pm 6$</td>
<td>93.18 $\pm 6$</td>
<td>27.4 $\pm 0.8^*$</td>
<td>2.27</td>
<td>4.54</td>
<td></td>
</tr>
<tr>
<td>LC$_{30}$</td>
<td>70</td>
<td>3.3 $\pm 0.4^*$</td>
<td>2.96 $\pm 0.6^*$</td>
<td>88.86</td>
<td>32 $\pm 1.7^*$</td>
<td>77.75 $\pm 9.6^*$</td>
<td>38 $\pm 2.6^*$</td>
<td>5.2</td>
</tr>
<tr>
<td>(0.00025%)</td>
<td>-0.9$^*$</td>
<td>$\pm 1.6^*$</td>
<td>$\pm 9.6^*$</td>
<td>77.75 $\pm 9.6^*$</td>
<td>38 $\pm 2.6^*$</td>
<td>5.2</td>
<td>10.5</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Larval–pupal intermediates.

$^b$Pupal–adult intermediates.
Effects on fecundity

Through diet

Fecundity of adults of different ages was adversely affected when fed on the diet mixed with sub-lethal concentrations of flufenoxuron (Fig. 7). When the number of eggs laid by treated adults was compared with controls, it was observed that susceptibility to the insecticide was age dependent, and the average effect of different concentrations was not uniform. The older the adult, the greater the effect of flufenoxuron with reference to fecundity and percentage of abnormal
Fig. 6. A–C: Showing effect of \( \text{LC}_{25} \) on the development of first instar \emph{T. castaneum} larvae. A, larval–pupal intermediate with anterior part of the body covered with pupal case, middle part of the body showing sclerotization more than that of the posterior part; B, pupal–adult intermediate, ventral view; C, adult with un sclerotized area between head and prothorax and between prothoracic and mesothoracic sclerites. D–F: Effect of \( \text{LC}_{40} \) on the development of first instar \emph{T. castaneum} larvae. D, larval–pupal intermediate, ventral view; E, larval–pupal intermediate, dorsal view; F, pupal–adult intermediate, lateral view.

Fig. 7. Effect of sub-lethal concentrations of flufenoxuron on fecundity of \emph{T. castaneum}.

Eggs. Furthermore, the various sublethal concentrations of flufenoxuron had age-specific effects on fecundity. Fecundity of 2-day-old adults was significantly reduced \((p<0.05, \ t_{18,0.05} = 1.74)\) with increasing concentration of flufenoxuron. For 3-day-old adults it was found that all concentrations were equally effective in reducing fecundity, while for 4-day-old adults \( \text{LC}_{40} \) was found to be the most effective (Tables 2a, b). Abnormal eggs laid by the treated adults were observed to have no flour particles sticking to their surface, in contrast to normal eggs. Twin eggs were also observed in which the chorion was continuous with a small constriction between the pair (Fig. 8). These eggs did not develop further but shrank gradually and turned brown. The effects of \( \text{LC}_{20} \), \( \text{LC}_{30} \) and \( \text{LC}_{40} \) on adults of different ages were compared using the Z-test (Tables 3, 4). In case of 2- and 3-day-old adults, the percentage of abnormal eggs laid increased with increasing concentration of flufenoxuron. However, for 4-day-old adults the percentage of abnormal eggs laid was higher at \( \text{LC}_{30} \) and \( \text{LC}_{40} \) than at \( \text{LC}_{20} \) (Table 5). At \( \text{LC}_{20} \), the percentage of abnormal eggs laid was greater in 4-day-old adults than in 2- and 3-day-old adults; at \( \text{LC}_{30} \) and \( \text{LC}_{40} \), the percentage of abnormal eggs laid increased with increasing age of the treated adults.
Table 2
Effect of sublethal concentrations of flu fenoxuron on fecundity in adult T. castaneum of different ages
a) t-tests values at fixed age of adults for sub-lethal concentrations of flu fenoxuron (t_{0.05} = 1.74)

<table>
<thead>
<tr>
<th>Pair</th>
<th>2-day-old adult</th>
<th>3-day-old adult</th>
<th>4-day-old adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_1 - \mu_2$</td>
<td>3.50*</td>
<td>18.26*</td>
<td>21.47*</td>
</tr>
<tr>
<td>$\mu_1 - \mu_3$</td>
<td>6.11*</td>
<td>19.80*</td>
<td>21.81*</td>
</tr>
<tr>
<td>$\mu_1 - \mu_4$</td>
<td>8.31*</td>
<td>19.80*</td>
<td>28.48*</td>
</tr>
<tr>
<td>$\mu_2 - \mu_3$</td>
<td>2.61*</td>
<td>1.53 n.s.</td>
<td>0.3391 n.s.</td>
</tr>
<tr>
<td>$\mu_2 - \mu_4$</td>
<td>4.80*</td>
<td>0.92 n.s.</td>
<td>7.008*</td>
</tr>
<tr>
<td>$\mu_3 - \mu_4$</td>
<td>2.19*</td>
<td>0.61 n.s.</td>
<td>6.6697*</td>
</tr>
</tbody>
</table>

Conclusion: $\mu_1 > \mu_2 > \mu_4 > \mu_3 = \mu_4 = \mu_3 > \mu_4$

*Significant value. n.s. not significant.

$\mu_1 =$ average fecundity in control, $\mu_2 =$ average fecundity in LC_{20} treated adults, $\mu_3 =$ average fecundity in LC_{30} treated adults, $\mu_4 =$ average fecundity in LC_{40} treated adults.

b) t-tests values at fixed concentration for different age of adults (t_{0.05} = 1.74)

<table>
<thead>
<tr>
<th>Pairs</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC_{20}</td>
</tr>
<tr>
<td>$\mu_1 - \mu_2$</td>
<td>4.699*</td>
</tr>
<tr>
<td>$\mu_1 - \mu_3$</td>
<td>12.48*</td>
</tr>
<tr>
<td>$\mu_1 - \mu_4$</td>
<td>13.32*</td>
</tr>
<tr>
<td>$\mu_2 - \mu_3$</td>
<td>7.78*</td>
</tr>
<tr>
<td>$\mu_2 - \mu_4$</td>
<td>8.62*</td>
</tr>
<tr>
<td>$\mu_3 - \mu_4$</td>
<td>0.841 n.s.</td>
</tr>
</tbody>
</table>

Conclusion: $\mu_1 > \mu_2 > \mu_3 > \mu_4 = \mu_2 > \mu_3 > \mu_4$

*Significant value. n.s. not significant.

$\mu_1 =$ average fecundity in control, $\mu_2 =$ average fecundity of 2-day-old adult, $\mu_3 =$ average fecundity of 3-day-old adult, $\mu_4 =$ average fecundity of 4-day-old adult.

Table 3
2-day-old adult: 0.67
3-day-old adult: 2.2
4-day-old adult: 20

Topical application

When adults of either sex were topically treated with sub-lethal concentrations of flu fenoxuron, there was reduction in fecundity (Table 6a). Egg laying was significantly reduced (p<0.05, t_{0.05} = 1.86) at 0.4 $\mu$g/1 (LC_{20}) and 0.8 $\mu$g/1 (LC_{40}) when both sexes were treated and also when only member of a pair was treated. At both concentrations of flu fenoxuron, fewer eggs were laid by treated females crossed with normal males than by normal females when crossed with treated males, although the fecundity of such females was less than that of the controls. At 1 $\mu$g/1 (LC_{20}) of flu fenoxuron, pairs where both sexes were treated produced fewer eggs than pairs where only males were treated; there was no significant difference in fecundity between pairs where only females were treated and pairs where both sexes were treated (Table 6b).
Fig. 8. A–D: Abnormal eggs laid by adults of different ages treated with sub-lethal concentrations of flufenoxuron. A, abnormal egg; B, twin eggs with lateral fusion of chorion; C, twin eggs with vertical fusion of chorion; D, shrunken twin eggs.

Table 6. Effect of topical application of sub-lethal concentrations of flufenoxuron on the fecundity of T. castaneum

<table>
<thead>
<tr>
<th>Dose of flufenoxuron (µg/µl)</th>
<th>Treated male × normal female (µ₁)</th>
<th>Treated female × normal male (µ₂)</th>
<th>Treated male × treated female (µ₃)</th>
<th>Control (µ₄)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>56.75 ± 1.66</td>
<td>46.42 ± 0.16</td>
<td>19.97 ± 1.16</td>
<td>73.8 ± 2.46</td>
</tr>
<tr>
<td>0.8</td>
<td>27 ± 2.3</td>
<td>11.8 ± 0.8</td>
<td>7.1 ± 0.33</td>
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<tr>
<td>1</td>
<td>10.65 ± 0.6</td>
<td>9.1 ± 2.4</td>
<td>4.25 ± 2</td>
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</tr>
</tbody>
</table>

b) t-test values for different concentrations of flufenoxuron (t₀.₀₀５ = 1.86)

<table>
<thead>
<tr>
<th>Pairs</th>
<th>Dose of flufenoxuron (µg/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>µ₁–µ₂</td>
<td>5.54*</td>
</tr>
<tr>
<td>µ₁–µ₃</td>
<td>16.24*</td>
</tr>
<tr>
<td>µ₁–µ₄</td>
<td>5.138*</td>
</tr>
<tr>
<td>µ₂–µ₃</td>
<td>20.203*</td>
</tr>
<tr>
<td>µ₂–µ₄</td>
<td>9.934*</td>
</tr>
<tr>
<td>µ₃–µ₄</td>
<td>17.7*</td>
</tr>
<tr>
<td>Conclusion</td>
<td>µ₁&gt;µ₂&gt;µ₃</td>
</tr>
</tbody>
</table>

*Significant value. n.s. not significant.

Effect on hatching

Flufenoxuron at various sub-lethal concentrations significantly reduces the hatching of eggs as compared to controls. The percentage hatching decreased with an increase in concentration of flufenoxuron (Fig. 9) in a dose-dependent manner.

Discussion

Sublethal concentrations (LC₂₀, LC₅₀, and LC₄₀) of flufenoxuron incorporated in the diet and fed for 24 h to newly hatched first instar larvae of T. castaneum were shown to affect growth, development and fecundity. Subsequent feeding on a flufenoxuron-free diet
Fig. 9. Regression graph of dose-response for flufenoxuron on % hatching of eggs of *T. castaneum*.

did allow the larvae to gain sufficient weight to achieve molting. However, significant growth retardation was reflected by lower larval weight and a delay in the time taken for both pupation and adult emergence. Significant reductions in pupation as well as adult emergence were also observed. At lower concentrations (LC$_{20}$ and LC$_{50}$) more larval–pupal and pupal–adult intermediates were generated, although no abnormal adults were observed. At the highest concentration tested (LC$_{90}$), deformed adults were encountered. One type had deformed wings only whereas the other had undeveloped and deformed body parts and unsclerotized patches on the exoskeleton of the thorax, in addition to wing deformities. Similar observations were reported by Arthur (2001) on both *T. castaneum* and *Tribolium confusum* on exposure to hydroprene. Developmental abnormalities such as larval–pupal and pupal–adult intermediates as well as deformed adults, similar to those found with the use of JH analogues, suggest that flufenoxuron may influence reproduction by causing hormonal imbalance (Bull, 1986; Deecker et al., 1989; Deecker et al., 1990a, 1990b). The severity of adult deformity was so great that such adults could not retain their dorso-ventral posture. Furthermore, due to incomplete chitinisation of the mandible, they could not feed and, as a result, died within a week of emergence. Our findings support those of Neumann and Guyer (1987) and Clarke and Jewess (1990) who observed such effects on *Heliotis virescens* and *Spodoptera littoralis*, respectively.

Adults emerging from lower concentrations (LC$_{20}$ and LC$_{50}$) laid mostly non-viable eggs, and those eggs which hatched resulted in larval mortality during the first instar. This is possibly due to impairment of cuticle secretion in the affected embryos, as has been reported in case of *Leptinotarsa decemlineata* as a result of treatment with diflubenzuron (Grosscurt, 1978). This is the first report on the effects of varying sublethal concentrations of flufenoxuron on the reproduction of the surviving adults of *T. castaneum*.

There was considerable variability in the response of adults of *T. castaneum* of different ages with respect to fecundity when fed on a diet treated with sub-lethal concentrations of flufenoxuron. Two-day-old adults showed a dose-dependent effect, whereas in 3-day-old adults all the concentrations were equally effective. However, in the case of 4-day-old adults the highest sub-lethal concentration (LC$_{90}$) was the most effective. Furthermore, in the younger adults there was a dose-dependent effect on the number of abnormal eggs laid. In most insects the egg is covered by a sticky “shell” of protein secreted by the accessory glands. Abnormal eggs lacked the sticky layer as reflected by the lack of flour sticking to their surface, and this may be due to the absence of accessory gland secretion. The overall trend, however, revealed an age-dependent effect on fecundity in *T. castaneum* adults of sub-lethal concentrations of flufenoxuron.

The reduction in the fecundity of treated females and reproductive potential of males as a result of topical application of sub-lethal concentrations of flufenoxuron may be the result of egg sterilization in treated females through the disruption of oogenesis, as reported by Wing et al. (1988), and by interference with spermatogenesis in males, respectively. However, we found that disruption of oogenesis had more impact. Smaghe and Degheele (1992) found that RH-5849, a precursor of the ecdysone agonist tebufenozide, reduced fecundity by causing the resorption of oocytes in adult female *Spodoptera littoralis*. It is apparent from the present study that sub-lethal concentrations of flufenoxuron exhibit transovarial ovidical activity, as described previously in different flies (Wright and Harris, 1976; Wright and Spates, 1976; Irvie and Wright, 1978; Chang, 1979); the boll weevil, *Anthonomus grandis* (Moore and Taft, 1975); and the codling moth, *Cydia pomonella* (Moffitt et al., 1983). Chlorfluazuron and pyriproxyfen have a similar effect on *H. virescens* females (Neumann and Guyer, 1987) and the white flies, *Bemisia tabaci* and *Trialeurodes vaporariorum* (Ishaaya and Horowits, 1992; Ishaaya et al., 1994), respectively.

The present study reveals that flufenoxuron is more effective through ingestion than by topical application in the case of *T. castaneum*, in contrast to the findings of Clarke and Jewess (1990), according to which in *S. littoralis*, sub-lethal concentrations of flufenoxuron were 10 times more effective by topical application than by ingestion. This discrepancy may be due to
penetration differences (Biddinger and Hull, 1999) since *T. castaneum* and *S. littoralis* belong to different insect orders.

Eggs placed in a diet treated with sub-lethal concentrations of flufenoxuron exhibited a dose-dependent, inverse relationship with respect to hatchability. Similar findings have been reported for *S. littoralis* where the eggs were dipped in either PH-6040 (Ascher and Nemny, 1974) or diflubenzuron and BAY SIR 8514 (Ascher et al., 1979).

In addition to direct mortality, the sub-lethal effects of flufenoxuron on development and fertility of surviving individuals should help in suppressing subsequent generations. Thus information about the sub-lethal effects on pest species, as well as on beneficial arthropods, is needed to fully integrate compounds such as IGRs into future IPM programs. How these sub-lethal responses affect subsequent populations in the field has been poorly investigated and is extremely difficult to ascertain. Thus care should be taken when making assumptions based on laboratory data.

References


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PAPERS/POSTERS PRESENTED IN SYMPOSIA AND CONFERENCES.

