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Effect of methoprene on the progeny production of *Tribolium castaneum* (Coleoptera: Tenebrionidae)[†]

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Abstract

BACKGROUND: *Tribolium castaneum* (red flour beetle) is a serious insect pest of stored products around the world. Current control measures for this species have several limitations: loss of registration of insecticides, insecticide resistance and consumer concerns about chemical residues in food. The objective of this study was to determine whether methoprene affects progeny production of *T. castaneum*. Late-instar larvae or young adults were exposed to methoprene-treated wheat, and progeny production was determined. The pairing of male and female adults was performed as untreated × untreated, treated × untreated or treated × treated, to study sex-based effects.

RESULTS: There were three outcomes to late-instar larvae held on methoprene-treated wheat kernels (0.001 and 0.0165 ppm): (1) failure to emerge as an adult; (2) emergence as an adult, and almost no offspring produced; (3) emergence as an adult and normal production of offspring. Male larvae were more susceptible to methoprene than female larvae. In contrast, young adults exposed to methoprene (1.67–66.6 ppm) showed no reduction in offspring production.

CONCLUSION: Methoprene concentrations will decline with time following its application. However, this research indicates that methoprene can still reduce populations of *T. castaneum* by reducing their progeny production, even if adults emerge. (© 2011 Society of Chemical Industry

Keywords: methoprene; larvae; progeny; all-or-nothing; bimodal

1 INTRODUCTION

Synthetic insecticides, both as contact insecticides and fumigants, have been used extensively to control stored-product insects since the 1960s.^{1–3} However, at present there is an emphasis on the use of insecticides that have biorational properties,⁴ owing to many concerns about the use of currently available insecticides. This concept also includes the use of reduced-risk insecticides, such as insect growth regulators (IGRs).^{5,6} There are three types of IGRs: juvenile hormone agonists, ecdysteroid agonists and chitin synthesis inhibitors.⁷

Methoprene,⁸ hydroprene,⁹ fenoxycarb¹⁰ and pyriproxyfen^{7,11} act as juvenile hormone analogues (JHAs), and they have been used commercially to control insect pests.⁵ Juvenile hormone analogues are lethal to embryos when applied during blastokinesis,^{12,13} or to larvae, which then produce malformed pupae.^{5,14} Much of the research on JHAs has focused on mortality due to the disruption of development during metamorphosis.^{15–21}

However, the effects of juvenile hormones and JHAs can also be sublethal, as JH is involved in many physiological systems.^{22–33} For example, JHAs affect diapause status,^{34,35} pheromone production,³⁶ mating,³⁷ heat tolerance³⁸ and behaviour.³⁹ Juvenoids often affect reproduction. Juvenile hormone analogues applied at the immature stages reduce the offspring production by affecting females during their adulthood both in stored-product insects^{40,41} and in non-stored-product insects.^{42–46} Furthermore, JH in the male larva affects spermatogenesis during the adult stage.⁴⁷ Juvenile hormone or its analogues applied to female adults can affect reproduction by affecting vitellogenesis, growth of follicles or protein synthesis in ovaries,⁴⁷ or causing abnormalities in ovaries.⁴⁸ JH or JHAs, when applied to adult males, affect sexual communication³¹ or mating³⁷ respectively.

Red flour beetle, *Tribolium castaneum* (Herbst), is a serious insect pest of both raw grains and processed grain products^{49,50} and is found in different habitats in grain storage and processing facilities: warehouses,⁵¹ flour mills,⁵² food processing plants⁵³ and retail stores.⁵⁴ Current control measures for *T. castaneum* include the use of contact insecticides,⁵⁵ application of diatomaceous earth,⁵⁶ use of fumigants such as phosphine⁵⁷ or sulfuryl fluoride⁵⁸ and application of low temperature.^{60,61} *Tribolium*

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castaneum populations originating in different geographical locations have become resistance to certain neurotoxic insecticides; some of these insecticides are still in use.⁵²

Grain storage managers are moving to alternate and safer control methods owing to concerns about neurotoxic insecticides. Methoprene, isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate,⁸ is one of the most widely used JHAs.^{7,41} Although there is information on the toxicity of methoprene to *T. castaneum*,⁶² little information is available on how JHAs affect the male and female reproductive systems and the reproductive capacity of this species. The objective of the present study was to determine whether methoprene affects progeny production by *T. castaneum* when applied to larvae or adults.

2 MATERIALS AND METHODS

2.1 Methoprene

The commercial product Diacon II[®] (288 g of S-methoprene L⁻¹) (Central Life Sciences, Schaumburg, IL) was used as the source of methoprene. Wheat was treated with a series of concentrations of methoprene in distilled water (w/w based on the active ingredient of the commercial product). Tribolium castaneum larvae or adults were exposed to a series of concentrations. Larvae were exposed to methoprene concentrations 0.001, 0.0165 or 0.033 ppm on wheat. In previous experiments, these concentrations caused approximately 25, 50 and 75% mortality respectively.³⁸ The survivors at the adult stage were used to determine the sublethal effects on their progeny production. Adults were exposed to methoprene concentrations 1.67, 16.65 or 66.6 ppm on wheat. For controls, a formulation that contained all the adjuvants but no methoprene, provided by manufacturers of Diacon II, was used at equivalent active ingredient concentrations (0.033 ppm for larvae and 66.6 ppm for adults). In earlier experiments, the response of T. castaneum to these concentrations of adjuvant was not different from the response to water.³⁸ Hence, only the adjuvant mixture was used as a control in the present experiment. All the solutions were prepared immediately before spraying.

2.2 Spraying and exposure of insects to methoprene

Adjuvant and methoprene solutions were sprayed onto hard red spring wheat, *Triticum aestivum* L. (moisture content 14.1–14.5%), medium containing 80% whole wheat, and 20% cracked wheat. Wheat medium was laid onto a single grain thickness on a wax sheet. Each spray treatment (adjuvants or methoprene) was 3 mL, which was sprayed onto 300 g of the wheat medium (moisture content 14.1–14.5%). Spraying was carried out under a fume hood using an artist's brush (Paasche Airbrush Company, Chicago, IL). Spraying onto wheat was carried out in the manner described in previous work.³⁸

Tribolium castaneum from a colony that had been in the laboratory since 1989 were used in the experiments. Insects were reared and experiments conducted at 30 °C, 60% RH, in the dark, on a medium containing 95% unbleached white wheat flour and 5% brewers' yeast (flour medium) (ICN Biomedicals, Inc., Aurora, OH). Insects for the experiments were produced by introducing 200 adults into the above medium (250 g) to lay eggs for 24 h. The experiments were conducted under the same environmental conditions as for the rearing of insects, as mentioned above. Late-instar larvae (14 days old) were separated from the rearing medium using a sieve (425 μ m mesh). This age determination was based on allowing 3 days for incubation as previously observed

under the temperature, humidity and darkness used in this experiment. In the larval exposure experiment, the larvae were introduced into the wheat medium treated with a particular concentration of methoprene (300 larvae 300 g^{-1} medium). Between 4 and 8 days following the introduction, the pupae were separated from the treated wheat medium using a sieve (2 mm mesh) and sexed.⁶³ Each pupa was held in a separate vial with flour medium (approximately 1.5 g) until adult emergence. Two weeks after adult emergence, one male and one female were introduced into a vial containing flour medium (7 g). After 7 days, adults were sieved out (850 μ m mesh); the flour medium containing offspring was held for 5 weeks, and the progeny adults were counted.

In the adult exposure experiment, the untreated pupae were separated from the rearing wheat flour medium with a sieve (600 µm mesh) and sexed, and each pupa was held in an individual vial containing rearing flour medium (approximately 1.5 g) until adult emergence. When the adults were 2-4 days old, each adult was placed in an individual vial containing the wheat medium (4 g of 80% whole wheat and 20% of cracked wheat) treated with a given concentration of methoprene and held for 7 days. Following this, the adults were sifted from that medium using a sieve (2 mm mesh), paired in 7 g of flour medium (each pair in a separate vial) and held for 7 days. These adults were sifted out (using 600 mm mesh), and the offspring production in the vials was assessed 5 weeks later, as described above. Only the vials in which both adults were alive at the time of sifting out were included in the experiment. To avoid contamination, all the procedures with spraying, introducing larvae, sexing pupae and handling adults were performed from the lowest concentration to the highest, and the new concentration was passed through the artist's brush before spraying onto wheat. In both experiments, each pair of adults sifted out of the flour medium after the oviposition period were frozen at -10 °C for verification of sex in the cases where no progeny was produced.

2.3 Pairing

One adult female, either emerged from a treated larva or treated as adult, was paired with one adult male. There were four pairings: both female and male untreated, only female treated, only male treated or both female and male treated. When both sexes were treated, the same concentration of methoprene was used for the two sexes.

2.4 Statistical analysis

This experiment had two stages. The first stage was a completely randomized design (CRD) with one of four methoprene treatment concentrations (0, 0.001, 0.0165 or 0.033 ppm) applied to larvae. The count of adults emerging was analyzed using a log-linear model.^{64–66} Treatment values for adult emergence were compared using a chi-square test and were considered significantly different using a type-1 error of 0.05 (Table 1).

The second stage of the experiment was the production of progeny from the parents in a factorial set of treatments. The developing larvae were sexed as pupae and then, as adults, were combined in a CRD, in a factorial experiment with two factors. The two factors were the methoprene concentration applied to male larvae (0, 0.001, 0.0165 or 0.033 ppm) and the same methoprene concentration applied to the females as larvae. Of 16 possible combinations, only ten were used. One restriction was that the same concentration was used in males and females. For example, males treated with 0.001 ppm were paired only

Table 1. Tribolium castaneum larvae treated with different methoprene concentrations, sexed as pupae and held until emergence to adult

Methoprene concentration (ppm)	Emergence to adult ^a (%)	Number of pupae sexed			
0	94.1 a	253			
0.001	62.8 b	441			
0.0165	27.9 с	283			
0.033	4.6 d	787			
^a Percentage emergence followed by the same letter are not significantly different (likelihood ratio (I.B) $\chi^2 = 969.10$ df = 3					

P < 0.0001).

with females treated with 0.001 ppm. In the larval experiment, the offspring production was bimodal. The progeny size of 30 was an approximate demarcation for the two subsets of progeny produced by a pair of parent adults. Therefore, the frequency of pairs with equal or less than 30 offspring (low progeny production) was analysed using a log-linear model^{64–66} and employed specific contrasts to determine differences between treatments (type-l error 0.05). As small progeny production when both males and females were treated with adjuvants was zero, 1 + X was used in the analysis. For pairs with a progeny production greater than 30, analysis was done using ANOVA procedures (SAS)⁶⁶ to determine whether they differed from the untreated control.

The adult exposure experiment was also a CRD and used a two-factor factorial set of treatments (incomplete), as in the experiment with larvae. However, here the distribution of progeny was unimodal. Therefore, the raw counts (*X*) were transformed to log (1 + X) scale⁶⁷ and were analyzed using ANOVA.⁶⁶ The differences from the control were tested using Dunnett's test (*P* = 0.05).

3 RESULTS

3.1 Treated as larvae

There was an increase in mortality (failure to emerge as adult) with increased methoprene concentration. As the number of live adults produced at 0.033 ppm was not enough for pairing, that concentration was not used (Table 1). When both sexes were treated with the adjuvants (control) as larvae, there was 81.9 ± 2.4 progeny pair⁻¹ (mean \pm SEM), and the distribution was normal (Kolmogorov–Smirnov test; P = 0.122) and unimodal (Fig. 1A). When one or both of the sexes were treated with methoprene, the progeny distribution was not normal (Kolmogorov–Smirnov test; P < 0.010). Also, it was bimodal (Figs 1B to G), with two distinct responses to methoprene: either a pair had very low progeny production (most pairs had zero progeny) or a pair's progeny production was similar to that for controls.

The percentage of pairs with low progeny production (equal or less than 30 offspring) was calculated (Table 2). Compared with the progeny group size produced by males and females both untreated at the larval stage, the frequencies of production of the low progeny production group size were significantly higher when either sex or both sexes were treated with methoprene concentrations of 0.001 or 0.0165 ppm (likelihood ratio (LR) $\chi^2 = 55.10$, df = 6, P < 0.0001). In general, males were significantly more affected than females (LR $\chi^2 = 14.0$, df = 3, P = 0.0029). However, with individual concentrations, this

difference was significant at 0.0165 ppm (LR $\chi^2 = 10.34$, df = 1, P = 0.0013), but there was no difference at 0.001 ppm (LR $\chi^2 = 3.65$, df = 1, P = 0.0559). Concentration did not affect the frequency of low progeny production with only females treated (LR $\chi^2 = 0.51$, df = 1, P = 0.4749), with only males treated (LR $\chi^2 = 0.33$, df = 1, P = 0.4749), with both sexes treated (LR $\chi^2 = 0.69$, df = 1, P = 0.4078). Furthermore, means of the progeny subgroups with greater than 30 offspring in any of the treatments did not significantly differ from untreated controls (ANOVA, $F_{6.95} = 1.34$, P = 0.2480) (Table 2).

When both sexes were exposed to methoprene, the frequency of pairs with low progeny production was higher than for the female-only treatment either at 0.001 ppm (LR χ^2 = 10.24, df = 1, P = 0.0014) or at 0.0165 ppm (LR χ^2 = 10.16, df = 1, P = 0.0014). This was significant even when the datasets for the two concentrations were combined (LR $\chi^2 = 20.41$, df = 1, P < 0.0001). In contrast, low progeny production with maleonly treatment was not significantly different from that when both sexes treated either at 0.001 ppm (LR $\chi^2 = 1.73$, df = 1, P = 0.1880), at 0.0165 ppm (LR $\chi^2 = 0.0$, df = 1, P = 0.9454) or in the combined dataset (LR $\chi^2 = 0.79$, df = 1, P = 0.3746) (Table 2). The effect of methoprene on the percentage of pairs with low progeny production when both sexes of the pair were treated can be calculated by considering individual probability levels of at least one individual having low progeny production. It is assumed that having at least one adult of the pair with low progeny production will cause the pair to have low progeny production, as none of the untreated pairs had low progeny production. If a female were to be treated with 0.001 ppm methoprene, then the probability that it would have low progeny production would be 0.292, and for males it would be 0.560. Based on these individual probabilities, a predicted value for the expected probability can be calculated as

$$(f \times m) + (F \times m) + (f \times M)$$

where *f*, *m*, *F* and *M* represent the probabilities of having a low-progeny-production female, a low-progeny-production male, a normal female and a normal male respectively. Accordingly, for 0.001 ppm methoprene, the predicted value for the probability of low progeny production is 0.689, obtained as $(0.292 \times 0.560) + (0.780 \times 0.560) + (0.292 \times 0.440)$, which is similar to 0.733, the actual probability obtained in the experiment. For 0.0165 ppm methoprene, the predicted probability obtained from a calculation similar to that discussed above is 0.647, which is similar to the observed value of 0.633.

3.2 Treated as adults

When both the sexes were treated with the adjuvants (control) as adults, the frequency distribution of progeny production was unimodal, with 88.5 \pm 3.4 progeny pair⁻¹ (mean \pm SEM) (Fig. 2A). Unlike the larvae, the frequency distribution of progeny production of treated pairs was unimodal (Fig. 2B). In general, methoprene did not reduce the progeny production (Table 3). The only exception was the progeny production of pairs in which males were treated with 16.65 ppm, which was lower than when both the sexes were untreated.

4 DISCUSSION

There were three outcomes to methoprene treatment of larvae in this study: (1) larva failed to emerge as an adult; (2) larva





Figure 1. Frequency distribution of progeny production by pairs of parent adults exposed to different methoprene treatments as larvae: (A) both sexes untreated; (B) females treated at 0.001 ppm; (C) males treated at 0.001 ppm; (D) both sexes treated at 0.001 ppm; (E) females treated at 0.0165 ppm; (F) males treated at 0.0165 ppm; (G) both sexes treated at 0.0165 ppm.

Table 2.	able 2. Mean progeny production of one female and one male adult pair of Tribolium castaneum exposed to methoprene as larvae							
Methoprene concentration (ppm)		Progeny production (mean \pm SEM)						
Male	Female	Production by pairs producing less than 30 offspring	Production by pairs producing greater than 30 offspring	Percentage of pairs with low progeny production	Total number of pairs			
0	0	-	81.9 ± 2.4	0	28			
0	0.001	3.4 ± 3.4	80.1 ± 3.4	29.2	24			
0.001	0	0.1 ± 0.1	$\textbf{76.3} \pm \textbf{7.7}$	56.0	25			
0.001	0.001	1.8 ± 1.2	79.6 ± 5.6	73.3	30			
0	0.0165	0.2 ± 0.2	77.5 ± 3.8	20.0	25			
0.0165	0	3.4 ± 2.3	85.1 ± 7.7	64.0	25			
0.0165	0.0165	1.1 ± 0.9	67.0 ± 5.5	63.3	30			

Table 3. Mean progeny production by a pair of one female and one male adult of *Tribolium castaneum* exposed to methoprene as adults

Methoprene concentration (ppm)		Number of offspring			
Males	Females	(mean \pm SEM)	Number of pairs		
0	0	88.5 ± 3.4	39		
0	1.67	84.5 ± 3.0	30		
1.67	0	81.3 ± 3.4	30		
1.67	1.67	85.2 ± 2.4	30		
0	16.65	88.4 ± 3.2	30		
16.65	0	$71.4\pm3.0^{\text{a}}$	30		
16.65	16.65	84.7 ± 3.8	30		
0	66.6	98.4 ± 3.0	30		
66.6	0	84.8 ± 4.1	40		
66.6	66.6	81.7 ± 3.6	30		
^a Significantly different from control (neither female nor male treated					

" Significantly different from control (neither female nor male treated with methoprene); Dunnett's test, P = 0.05.

emerged as an adult, and produced almost no offspring; (3) larva emerged as an adult and produced offspring, the same as controls. Failure to emerge as an adult owing to methoprene treatment is well documented.^{7,16,40} In insects with complete metamorphosis, juvenile hormone titers generally remain low in the later part of the last larval instar and the pupal stage for normal development of insects.⁶⁸ High JHA levels during immature stages disrupt development of the insect,^{22,69} so much so that the insect dies.⁷⁰

In the second type of outcome, adults had normal morphology and movement but they produced very few offspring. Both male and female reproductive systems develop during the larval and the pupal stages.^{49,71} This study indicates that methoprene may disrupt this reproductive development in those *T. castaneum* that withstand the lethal effects and develop into adults. Other studies have shown similar effects of reducing the offspring when larvae are exposed to JHAs: *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae)^{43,72} and *Adoxophyes orana* (Fischer von Roslerstamm) (Lepidoptera: Tortricidae).⁴⁵

In the present study, progeny production of both males and females was adversely affected, with males being more sensitive to methoprene than females. There are several mechanisms by which methoprene could have suppressed progeny production. Exposure of immature stages of males to JHAs disrupts spermatogenesis⁷³ and functioning of accessory glands in some in-



Figure 2. Frequency distribution of progeny production by pairs of parent adults exposed to different methoprene treatments as adults: (A) both sexes untreated; (B) both sexes treated with 66.6 ppm.

sect species; the degree to which the target tissue is affected differs with the species. Supportive tissues and aedeagus might also be affected by JH, although no conclusive information is available.⁴⁷ In females, JH affects the development of oviducts,⁴⁷ follicular growth,⁷⁴ oocyte maturation⁴⁷ and functioning of accessory glands.^{47,75} Externally applied JHAs can affect the morphology of genitalia.⁷⁶ Males could be more sensitive to methoprene because one or more of the tissues of the male reproductive system are more sensitive than tissues in the female reproductive system.

An important finding in the present study was that only some of the exposed larvae were susceptible to methoprene, as evident from the bimodal distribution of progeny produced. There are several possible explanations for this effect. Individual insects may have received different doses of methoprene because of variation in methoprene distribution in the grain sample or different rates of movement, development or feeding. On account of genetic differences, individual insects could have different susceptibility to methoprene owing to differences in uptake, degradation and susceptibility of target tissues.^{2,14,52} Finally, it could be that there is a defined window of sensitivity during the development of T. castaneum larvae, during which methoprene prevents the normal development of reproductive systems. Although all larvae used in the experiment were from the eggs laid during the same 24 h period, there are always differences in the rate of development. Thus, larvae exposed to methoprene within the window of sensitivity would have non-functioning reproductive systems as adults. In contrast, those larvae exposed to methoprene outside the window of sensitivity would not be affected.

There are several examples of juvenile hormone or JHAs having a window of sensitivity. For example, larvae are sensitive and adults are not sensitive to JH and JHA.5,23,47 Within the egg and larval stages there are also examples of JH-sensitive phases. Acheta domesticus (L.) (Orthoptera: Gryllidae) embryo cuticle development is sensitive to JHA pyriproxifen for only a few days.⁷⁷ With Oncopeltus fasciatus (Dallas) (Hemiptera: Lygaeidae), JH must be present during the fourth-instar larvae for the normal development of accessory glands, and JH deficiency during that period cannot be recovered by having JH at a later stage.⁴⁷ In Cydia pomonella (L.) (Lepidoptera: Tortricidae), methoprene applied to two-day-old and six-day-old fifth-instar female larvae reduces fecundity, whereas methoprene applied to four-day-old female larvae does not.⁴⁶ Melipona scutellaris Illiger (Hymenoptera: Meliponini) has a JH-mediated control of female genes within a specific window during the late larval stage (L₃).⁷⁸ Therefore, it could be that males have a longer window of sensitivity than females, explaining why greater effects were seen in males. However, further research is required to determine the exact mechanism by which methoprene causes T. castaneum to become sterile.

In the present experiment with T. castaneum 2-4-day-old adults, methoprene did not alter their progeny production. Maturation of the reproductive systems in T. castaneum of both sexes takes approximately 5 days after eclosion.⁴⁹ The present data suggest that JH may not be involved in the final stages of development of the reproductive systems in T. castaneum, once the adult has emerged. Alternatively, JH levels may be high in the adult,^{14,28,71,79} and external application of methoprene would not be enough to disrupt the reproductive system. In general, there is no adult mortality due to methoprene application,^{5,38} but methoprene or other JHAs have adversely affected the reproductive systems of adults in several other species: Tribolium confusum Jacquelin du Val (Coleoptera: Tenebrionidae),¹⁵ Lasioderma serricorne (F.) (Coleoptera: Anobiidae),⁸⁰ Callosobruchus maculatus (F.) (Coleoptera: Bruchidae)⁸¹ and Rhyzopertha dominica (F.) (Coleoptera: Bostrychidae).⁸²

This study revealed that the progeny production of *T. castaneum* was reduced at 0.001 ppm, approximately 1600–5000 times lower than the label rate of 1–5 ppm methoprene on wheat (Central Life Sciences, Schaumburg, IL). The progeny production was reduced from approximately 82 offspring week⁻¹ female⁻¹ in untreated wheat to 24 offspring week⁻¹ female⁻¹ in treated wheat. This

has a number of implications for the use of methoprene in stored-grain insect pest management. The presence of adults in grain treated with methoprene may be less of a concern, as the majority of these adults may not be able to produce progeny. Methoprene degrades with time, dropping below the concentrations that prevent adult emergence. The present data show that methoprene should reduce populations for much longer periods of time by reducing the progeny production of the survivors. Grain should be treated at the label rate; however, there are a number of reasons for insects not being exposed to the label rate: incomplete coverage of grains by the sprayed concentration; movement of insects through the grain mass without adequate exposure; degradation of methoprene over time. Larvae exposed to sublethal concentrations of methoprene may emerge, but their progeny production as adults may be adversely affected. Previous studies state that methoprene applied on surfaces remains effective in controlling the development of insects for months.^{83,84} The present study reveals that methoprene will reduce progeny production even after its dose drops below the label rate. Thus, the study contributes to expanding the uses of methoprene in insect pest management.

Further work is required to determine whether the effects of methoprene on progeny production are seen at concentrations lower than were tested, and whether similar effects are observed in other stored-product insects. Methoprene reduces the heat tolerance in adult *T. castaneum*.³⁸ It would be interesting to determine whether larvae that survive methoprene exposure have reduced heat tolerance as adults. As described above, these findings not only provide insight into the mode of action of methoprene on reproductive physiology in insects but also pave the way for enhancing the use of methoprene as a reduced-risk insecticide.

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