Horizontal transfer of methoprene in *Tribolium castaneum*

Tucker, A.M.*[1], Campbell, J.#[2], Arthur, F.H.[2], Zhu, K.Y.[1]
[1] Kansas State University, Department of Entomology, 123 West Waters Hall, Manhattan, KS 66506 USA.
Email address nos7147@ksu.edu
[2] United States Department of Agriculture, Agricultural Research Service, Center for Grain and Animal Health Research, 1515 College Ave., Manhattan, KS 66502, USA.

* Corresponding author
\# Presenting author

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Abstract

Aerosol applications of reduced risk insecticides such as pyrethrins, pyrethroids, and insect growth regulators are becoming more commonly used to manage stored-product insects in food facilities. However, these applications have a limited ability to penetrate into hidden refugia, where the majority of the pest population is located. Horizontal transfer of insecticides could occur as individuals directly treated or exposed to treated surfaces move into hidden refugia and encounter untreated individuals. In this series of studies, the potential for horizontal transfer of methoprene from treated *Tribolium castaneum* , the red flour beetle, to untreated individuals was evaluated. Adding larvae, pupae, or adults treated with methoprene to flour patches with untreated *T. castaneum* larvae, resulted in increased pupa and adult deformities and higher numbers of dead focal individuals, which suggests the potential for this mechanism. The transfer mechanism might be flour substrate contamination, transfer during contact of individuals, and/or cannibalism of individuals exposed to insecticides. Experiments focused on isolating the impact of contact and cannibalism on horizontal transfer did not detect a significant increase in mortality. Experiments focused on flour substrate contamination resulted in decreased adult emergence as well as lower survival, and higher rates of deformities. These findings suggest that substrate contamination is the more likely mechanism for horizontal transfer, and although horizontal transfer can occur, the impact of this process on populations needs further evaluation.

Keywords: Red flour beetle, *Tribolium castaneum*, Methoprene, Horizontal transfer

1. Introduction

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, is a serious pest of stored-products in food facilities such as flour mills, rice mills, warehouses, and retail stores. They are difficult to manage because they can exploit both the stored commodity and locations within equipment and the building structure where food material accumulates. To control these hidden pest populations, the food industry has often relied on periodic structural treatments such as fumigation or heating, that have the ability to penetrate into these hidden refugia to eliminate or reduce infestations (Bell, 2000; Fields and White, 2002). Fumigation using methyl bromide has been widely used to manage *T. castaneum* populations in flour mills, but its use is being phased out under the Montreal protocol agreement (UNEP, 2000). A potential alternative to the use of methyl bromide is the use of aerosol insecticides, although these applications do not have the ability to penetrate into commodities or the building structure. A widely used aerosol insecticide formulation involves the combination of an insect growth regulator (IGR) such as methoprene with synergized pyrethrins (Arthur, 2008). Direct application of these compounds or exposure to previously treated surfaces has been shown to cause high mortality of *T. castaneum* (Arthur, 2008). However, since only a small percentage of the total *T. castaneum* population in a facility is in exposed areas where they may come in contact with the aerosol insecticide (Toews et. al., 2005), aerosol treatments at first appear to be unlikely to have a dramatic impact on *T. castaneum* populations. Although assigning a cause and effect relationship is difficult, there is evidence suggesting regular applications of aerosol formulations of methoprene and synergized pyrethrins as part of an IPM program can suppress *T. castaneum* populations (Campbell et. al., 2010).

There are several possible mechanisms for how aerosol applications might have a cumulative impact on the hidden pest population within a structure. Insecticides may move into hidden refugia exploited by insects through drift of aerosol, movement of treated flour, and/or movement of insects that have been exposed to insecticide. Horizontal transfer of insecticides occurs when active members of the population ingest or come into contact with an insecticide and then return to a location where other members of the population come in contact with and/or consume the translocated insecticide (Buczkowsi and Schal, 2001). Horizontal transfer of insecticides has been demonstrated in several insect systems. Contact, cannibalism, and ingestion of fipronil residues are possible horizontal transfer mechanisms of fipronil in the German cockroach, *Blattella germanica* (L.) (Buczkowski and Schal, 2001). Red imported fire ants, *Solenopsis invicta* (Buren), transferred methoprene from S-methoprene baits by food sharing with adjacent colonies (Aubuchon et al., 2006).

Because of its good persistence, lack of activity against adult insects, and activity against immature stages at very low concentrations, methoprene may be the most likely insecticide for horizontal transfer in food facilities. Several behaviors and chemical ecology characteristics of *T. castaneum* may also facilitate horizontal transfer of methoprene. Firstly, *T. castaneum* is a cannibalistic species, with both adults and larvae consuming other life stages (Park et al., 1965; Godfrey and Hassell, 1997). Secondly, *T. castaneum* have a male produced aggregation pheromone, 4,8-dimethyldecanal, which attracts both sexes and can increase encounters among treated and untreated individuals (Suzuki, 1980). Lastly, *T. castaneum* adults readily move among flour patches (Campbell and Runnion, 2003), which means that they could be exposed to settling aerosol insecticide(s) or surfaces with residual insecticide(s) and carry it into refugia. The combination of these behaviors allows for the possibility of horizontal transfer of methoprene. In this paper, we summarize a series of experiments designed to determine if horizontal transfer of methoprene from treated individuals to untreated individuals can occur with *T. castaneum* and if so what mechanism(s) are likely involved in the process (e.g., physical contact with a treated individual, cannibalism of a treated individual, and transfer of insecticide to a substrate such as flour which is then contacted and/or eaten by an unexposed individual (substrate contamination)). Horizontal transfer was evaluated using a biological assay method where levels of mortality during the observation period (mortality), development to the adult stage (adult emergence), and presence of any type of physical abnormality (deformities) were used as measures of the amount of methoprene transfer.

2. Does horizontal transfer of methoprene occur with T. castaneum?

2.1. Materials and methods

To generate methoprene-treated developmental stages, larvae, pupae, and adults were collected from a *T. castaneum* laboratory culture. Selected individuals were placed in concrete exposure arenas consisting of a plastic Petri dish (62 cm^2 surface area) containing ~0.1 cm of concrete patch material (Rockkite®, Hartline Products, Co., Inc., Cleveland, OH, USA) in the bottom (Arthur, 2008). Methoprene (Diacon® II, Central Sciences International, West Schaumberg, IL, USA) or distilled water was sprayed evenly onto the arenas using an airbrush (Badger Air-Brush Company, Franklin Park, IL, USA) in order to evenly coat the surface and the insects. The labeled rate of methoprene for a surface treatment (3 mg/m^2) was applied to treatment dishes (0.25 ml/62 cm^2) and both treatments and controls received 0.132 ml of liquid. After application, the exposure arenas and insects were allowed to dry for ~15 min. Insects were transferred to new Petri dishes and placed in a freezer for 24 hrs. Insects were frozen to prevent degradation of methoprene (Henrick, 2007) and prevent movement in order to standardize amount of contact with treated individual in experiments.

Single late stage *T. castaneum* larvae (focal individuals), not treated with insecticide, were added to 18 g plastic vials with ~ 5 g of flour and then a dead larva, pupa or adult methoprene or water treated individual was placed in the vial. An additional control group, consisting of just flour and focal larvae in the vial, was also used. Vials were held at 32°C. Focal individuals were observed daily for 30 days and developmental stage, mortality, and presence of external deformities was recorded. The level of cannibalization of the dead individual in each vial was quantified using an index: 0 – no feeding observed; 1 – bites and/or scratches 2 – missing appendages; and 3 – complete consumption of the dead insect. Seven blocks with 3-11 replicates of each treatment group in each block were performed.

2.2. Results and discussion

For focal individuals, emergence as adults, mortality, and deformities were compared among treatments using Fisher's exact probability pair-wise comparisons. Comparisons of adult emergence and mortality were not significantly different between methoprene treatments and controls, with a couple exceptions.

There was a significant difference in adult emergence between larvae exposed to flour with methoprene-treated pupae (82%) and their respective water treated control (97%) (P=0.04). Larvae exposed to flour and methoprene treated larvae had significantly higher mortality (37%) compared to larvae exposed to water treated larvae (14%) (P=0.05). In general, deformities were more prevalent when larvae were exposed to methoprene treated individuals. More individuals displayed deformities when exposed to methoprene treated larvae and flour (46%) or methoprene treated pupae and flour (26%) compared to those exposed to flour alone (6%) (P<0.05). There was a similar pattern of deformities for comparisons between focal larvae exposed to water treated larvae (11%) and methoprene treated larvae (46%) (P<0.01) and water treated pupae (3%) and methoprene treated pupae (26%) (P<0.01). There were no significant effects on larval development when exposed to methoprene treated adults compared to controls. Some level of physical deformities in the control groups was not unexpected since these can occur normally during development.

These results indicate that some horizontal transfer of methoprene occurred, but typically not enough to cause a high rate of mortality and the degree of effect was influenced by the developmental stage initially exposed to methoprene. The limited mortality and adult emergence effects might be due to only a single individual being added to the flour. Adding methoprene treated adults did not have any detectable impact on larvae, which could be due to differences in the cuticle between adults and immatures and/or that there is less cannibalization of adults. Since the adults are the stage most likely to move between flour patches (Campbell and Runnion, 2003) and therefore most likely to be exposed to insecticide treatments, this could reduce the potential for horizontal transfer of methoprene in food facilities. Since this experimental design did not allow us to determine the relative contribution of the three mechanisms of horizontal transfer (contact, substrate contamination, and cannibalization), additional experiments were performed.

3. Does physical contact and/or cannibalization result in horizontal transfer of methoprene?

3.1. Materials and methods

The impact on *T. castaneum* development due to larvae direct contact with, and/or cannibalization of, methoprene treated individuals, without the potential for contamination of the flour substrate, was evaluated in this experiment. *T. castaneum* development stages were treated with methoprene or water, and frozen as described in the previous experiment. Five previously methoprene or water treated individuals were placed in an 18 g plastic vial with no flour and then one late-stage larva (focal individual) was added to each vial and its fate was observed for 30 days as described in the previous experiment. Two additional controls were also prepared: flour added to vial (flour control) and no food (no flour or dead insects) added (starvation control). Three blocks with 5 replicates of each treatment in each block were performed.

3.2. Results and discussion

There was 100% focal individual mortality and 0% adult emergence for larvae exposed to methoprene treated individuals and this was a significantly greater response than that in the controls (Fisher's exact probability pair-wise comparisons, P<0.05). Focal individual mortality when exposed to flour (20%) was lower compared to the starvation controls (79%) (P<0.01) or water treated adult controls (67%) (P=0.01). Observed deformities were also significantly higher in the larvae exposed to methoprene treatments as compared to their flour and water exposed counterparts. For example, 72% of larvae exposed to methoprene treated pupae had deformities compared to 13% of larvae exposed to just flour (P=0.01). Similarly, larvae exposed to methoprene treated pupae (72%) had significantly higher deformities as compared to larvae exposed to pupae treated with water (0%) (P<0.01). The high rate of mortality in the no food treatment group shows the effects of starvation, and these individuals typically died as adults and had very few deformities. The individuals exposed to water treated adults also had a high mortality rate and this is also likely from starvation due to the potential difficulties for larvae to cannibalize the dead adult.

Cannibalization rates were highly variable among individuals, as was found by Stevens (1989), but 100% mortality was obtained in all methoprene treatments. This suggests that physical contact is sufficient to generate these effects, with cannibalism being less important, but with this experimental design it is not possible to isolate the contact and cannibalization factors. Since larvae exposed to treated adults had high mortality, and this is the stage most likely to be exposed and than to move into an untreated area, this suggests that horizontal transfer might occur in a food facility.

4. Does level of cannibalism affect horizontal transfer of methoprene?

4.1. Materials and methods

Because cannibalization rates varied so much among replicates in the previous experiment, in this experiment separate treatment categories were created based on the level of cannibalization. Methoprene and water treated *T. castaneum* pupae were generated as previously described. One dead methoprene or water treated pupa was placed in a 90 mm plastic Petri dish with one late-stage larva (focal individual). After 48 h, the dead pupa was observed to determine its level of cannibalization as previously described and the associated focal larvae were then sorted into one of four cannibalization rank groups. These larvae were then transferred individually to new Petri dishes with ~1 g of flour and their fate recorded as previously described. Larvae given flour or no flour for that initial 48 h period were used as controls. Two blocks, with 22 and 25 replicates per treatment group, respectively, were performed.

4.2. Results and discussion

There were no significant differences in adult emergence, mortality, or deformities among larvae exhibiting different levels of cannibalization of methoprene treated or water treated pupae (contingency table and χ^2 test, $P>0.05$). The only pair-wise comparisons that were significant ($P\leq 0.05$) were between larvae exposed to flour and larvae exposed to no food, the latter having high mortality and low adult emergence. There was 79% mortality of the larvae that were exposed to no food compared to larvae that were exposed to flour only (0%) ($P<0.01$). These results suggest that cannibalization is not likely to be a primary mechanism for horizontal transfer of methoprene in this system. We are not aware of studies evaluating differences in methoprene efficacy resulting from consumption versus cuticle contact alone, but it could be that *per os* efficacy of methoprene is less than that due to contact with the cuticle.

5. Does physical contact alone result in horizontal transfer of methoprene?

5.1. Materials and methods

The impact on *T. castaneum* larvae of direct contact with methoprene treated adults, without the potential for contamination of the flour substrate or cannibalism was evaluated in this experiment. Methoprene or water treated adults were generated in the same way as previously described, however, these individuals were not killed. One treated adult and one late-stage larva (focal individual) was placed in a 90 mm plastic Petri dish containing a paper tent (2x1 cm), which served as a refuge area. After 24 hrs, the larva was moved into a new Petri dish with ~1g of flour and a new paper tent. The focal individuals were observed for 30 days as previously described. Larvae treated as above, but not exposed to an adult *T. castaneum*, and larvae added directly to flour were used as additional controls. There were three blocks of 10 replicates for each treatment per block.

5.2. Results and discussion

There were no significant differences in adult emergence, mortality, and deformities between larvae exposed to methoprene treated adults and control larvae exposed to just flour (contingency table and Fisher's exact probability pair-wise comparisons, all comparisons $P\geq 0.05$). The same was true when comparing larvae between the methoprene and water treatments, except larvae exposed to methoprene treated adults had more deformities (26%) than larvae exposed to water treated adults (3%)($P=0.01$). These findings suggest that short term contact between individuals may not be an important mechanism for horizontal transfer of methoprene, although previous experiments suggested that transfer of methoprene from adults to larvae may be less than with other developmental stages. In these experiments the degree of contact was not quantified, and more physical contact between the individuals might further increase our ability to detect a response in adult emergence and mortality.

6. Does contamination of flour alone result in horizontal transfer of methoprene?

6.1. Materials and methods

Methoprene treated *T. castaneum* life-stages were generated as previously described. Five, 15, or 30 dead individuals of each life stage, respectively, were placed in 18 g plastic vials with ~5 g flour, and then agitated on a shaker table for 30 min to simulate individuals moving in flour. Individuals were sieved from the flour and one late-stage focal larva was placed in the vial and observed as previously described. The experiment was performed in six blocks with a total of 12-15 replicates per a treatment.

6.2. Results and discussion

Focal individual adult emergence was less and mortality and deformities were greater for all comparisons between larvae exposed to flour conditioned with methoprene treated individuals and unconditioned flour, regardless of the number of treated individuals or developmental stage in the flour ($P \leq 0.05$) (contingency table and Fisher's exact probability pair-wise comparisons). The same pattern was observed for larvae exposed to flour conditioned with methoprene treated individuals and flour conditioned with water treated individuals ($P \leq 0.05$), except in five pair-wise comparisons. For example, 77% of larvae had deformities after exposure to flour conditioned with 15 methoprene treated larvae as compared with 0% of larvae having deformities after exposure to unconditioned ($P<0.01$) or 23% of larvae having deformities after exposure to flour conditioned with 15 water treated larvae ($P<0.01$). Deformities seen in the water treatments were generally minor, compared to those in the methoprene treatment, which frequently resulted in death of the focal individual. All comparisons of larvae exposed to unconditioned flour only were not significantly different from flour conditioned with water treated individuals, regardless of number or development stage ($P \geq 0.05$). These findings indicate that methoprene can be readily transferred from cuticle of treated individuals to the flour substrate and that contact and/or ingestion of this flour by larvae can have detrimental effects. In food storage facilities, this may be the more likely way methoprene can be transferred from treated adults to untreated developmental life-stages and therefore impact the population dynamics.

7. Conclusions

Our results indicate that horizontal transfer of methoprene between treated and untreated *T. castaneum* can occur under laboratory conditions, although the strength of the response varied considerably among experiments. Transfer of methoprene through contact with flour or direct contact appears to be a primary mechanism in our experiments. Strongest responses occurred when larvae were exposed to more than one treated individual. The fact that treated adults were able to transfer methoprene to the flour substrate indicates that horizontal treatment of methoprene could occur in flour mills since adults are primarily the individuals moving between hidden refugia. There is still a need to look at horizontal transfer under more realistic conditions and determine the potential population effects of horizontal transfer of methoprene.

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