



Residual efficacy of synergized pyrethrin + methoprene aerosol against larvae of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae)

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ABSTRACT

Wheat flour and different packaging surfaces (cardboard, flour bag, muslin bag, paper bag, pallet wrap, plastic overwrap, polyethylene) were exposed to aerosol formulations of either 1% active ingredient (AI) pyrethrin (synergized with piperonyl butoxide) + 33.6% (AI) methoprene or 3% (AI) pyrethrin + 33.6% AI methoprene. The mixture was formulated as specified on the insecticide labels to give a 100 to 1 ratio of active ingredient pyrethrin to methoprene. Residual bioassays were conducted every two weeks for 16 weeks post-exposure to the aerosol by placing four-week-old larvae of the red flour beetle, *Tribolium castaneum* (Herbst), or the confused flour beetle, *Tribolium confusum* (Jacqueline duVal), on treated flour or a treated packaging surface with untreated flour added. *T. castaneum* was clearly the more susceptible of the two species. Less than 2% of *T. castaneum* larvae exposed to aerosol-treated flour or packaging surfaces emerged as normal adults, regardless of the pyrethrin concentration. Most of the *T. castaneum* larvae on treated flour did not advance to the pupal stage because they were either developmentally arrested or died as larvae. They were able to develop further on the treated packaging surfaces, but still could not emerge as adults. *T. confusum* larvae exposed to aerosol-treated flour or packaging surfaces were able to develop to the pupal or adult stage. Emergence of normal-appearing adults from *T. confusum* larvae exposed on the packaging surfaces treated with 1% pyrethrin + methoprene gradually increased (range of 29.7 ± 2.9 to $49.0 \pm 6.7\%$, depending on the surface), whereas adult emergence of larvae exposed to treated flour peaked at 10 weeks post-exposure. However, when *T. confusum* was exposed to 3% pyrethrin + methoprene treated flour or packaging surfaces, adult emergence was reduced. Overall there were few significant differences attributable to the individual packaging surfaces.

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1. Introduction

The red flour beetle, *Tribolium castaneum* (Herbst), and the confused flour beetle, *Tribolium confusum* (Jacqueline duVal) are cosmopolitan pests of stored grains, cereal products, and fruit and nut products (Fedina and Lewis, 2007). They also infest structures including mills, food warehouses, retail stores, and urban homes (Rees, 2004). Product contamination by whole insects, eggs, insect fragments, frass, and cast skins can occur in processing plants and warehouses (Baur, 1984). Infestations of *T. castaneum* and *T. confusum* inside flour mills could have economic consequences including direct contamination and costs associated with treatment and monitoring, rejection and return of contaminated products, and loss of consumer goodwill (Campbell and Arbogast, 2004).

Pest management programs for stored-product insects inside mills and warehouses may include applications with insecticides of different liquid formulations including aerosols (Toews et al., 2005, 2009; Arthur and Peckman, 2006; Peckman and Arthur, 2006; Arthur and Campbell, 2008). In the United States (US), pyrethrins synergized with piperonyl butoxide (PBO) are labeled for use as an aerosol to control insects in flour mills. The insect growth regulator (IGR) methoprene is labeled for direct application to stored grains, as a residual contact spray, and as an aerosol. Multiple studies show that IGRs incorporated into insect diets result in reduced adult emergence following larval exposure (Oberlander et al., 1997). However, there are few recent publications in scientific journals evaluating efficacy of aerosols in field settings. In field trials with pyrethrin aerosol, survival of adults increased when provided with a flour food source either during or after insecticidal exposure (Arthur, 2008; Arthur and Campbell, 2008). Methoprene has excellent residual toxicity (Daglish and Wallbank, 2005), but there

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are no assessments of residual persistence of methoprene applied as an aerosol for flour beetle control. Small-scale tests have been conducted with methoprene aerosol against the Indian meal moth, *Plodia interpunctella* (Hübner) (Jenson et al., 2010a,b). Results of these tests show excellent efficacy of methoprene toward late-instar larvae.

Synergized pyrethrins applied as an aerosol can give immediate direct control of adult *T. castaneum* and *T. confusum*, but *T. confusum* appears to be the more tolerant species (Arthur, 2008). Similarly, studies with the IGRs hydroprene and pyriproxyfen applied as surface treatments also indicate *T. confusum* was the more tolerant of the two species (Arthur and Hoernemann, 2004; Arthur et al., 2009). The objective of this study was to evaluate the residual efficacy of aerosol applications of PBO-synergized pyrethrin combined with the IGR methoprene against *T. castaneum* and *T. confusum* larvae when applied to flour or different packaging materials commonly found in flour mills.

2. Materials and methods

2.1. Insecticides and treatment arenas

Field trials were conducted in a flour mill in the south-central USA equipped with an automatic aerosol application system (Entech Systems, Kenner, LA, USA). Each floor of the mill had an individual sprayer positioned about 4.5 m above an individual floor in the mill so that each floor of the flour mill could be treated separately. About 25–30% of the total surface area of the floor where the tests were conducted was occupied by milling equipment. Regular applications of a mixture of 1% or 3% active ingredient pyrethrin (Entech Fog-10 or Entech Fog-30, Entech Systems, Kenner LA, USA) and methoprene (Diacon II[®], 288 mg active ingredient (AI)/ml or 33.6%, Central Sciences International, Schaumburg, IL, USA) are done as part of the insect pest management program of the flour mill. The Entech Fog-10[®] formulation (EPA Reg. No. 73049-400-40391) is comprised of 1.0% active ingredient (AI) pyrethrins, 2.0% piperonyl butoxide (PBO) synergist, 3.3% N-octyl bicycloheptane dicarboximide and 93.7% refined petroleum solvent, and is applied at the rate of 29 mL/28 m³. The Entech Fog-30[®] formulation (EPA Reg. No. 73049-400-40391) contains 3.0% pyrethrins, 6.0% PBO synergist, 10% N-octyl bicycloheptane and 81% refined petroleum solvent, and is applied at the rate listed for the 1% AI pyrethrin formulation. The methoprene was added to either of these pyrethrin formulations at the label rate of 0.3 mL/28 m³ (hence the ratio of pyrethrin formulation to methoprene is about 100 to 1), and this mixture is applied through the aerosol system.

T. castaneum and *T. confusum* larvae used in experiments were obtained from colonies at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), Manhattan, KS, USA, maintained on a diet of 95% whole-wheat flour supplemented with 5% (w/w) brewer's yeast. These insecticide-susceptible cultures had been reared for more than twenty years at the CGAHR. All cultures were maintained in a low-light environmental chamber at 27 ± 3 °C and 70 ± 5% r.h. Concrete arenas used in the experiments were constructed using plastic 100 × 15 mm plastic Petri dishes with the bottom covered with concrete patching material (Rockite[®], Hartline Products, Cleveland, OH, USA). To prepare dishes (hereby termed arenas), a water-based slurry was prepared by mixing 2000 g Rockite[®] with 1.0 L of tap water and pouring approximately 20 mL into the bottom of a Petri dish and allowing it to dry uncovered overnight.

2.2. Aerosol exposures and bioassays

Four separate replicates (different spraying times) were conducted for each of the two aerosol concentrations. Each four-

replicate experiment was performed throughout different two-month periods, with the 3% pyrethrin treatments done between August and September, and the 1% pyrethrin treatments performed between November and January. The times for these applications were determined by mill management and could not be altered for the experiment. Prior to the aerosol exposures at each of the insecticide concentrations, treatment arenas were prepared by one of two methods. In the first method, arenas were prepared for direct exposure of wheat flour to the aerosol. In the second method, different packaging materials were exposed to the aerosols. Seven different packaging materials, all of which are commonly found within flour mills, were tested in this portion of the study. The treatment arenas with the packaging surfaces were prepared by cutting a circular piece of the packaging the same diameter as the Petri dish and attaching this piece to the concrete using caulking applied around the circumference. The seven materials were a paper bag material, a 2 mm thick commercial cardboard, heavy commercial plastic sheets, pallet wrap material, commercial flour bags, polyester woven bag material, and, a cotton muslin bag material. These tests were conducted at the same time but flour data and packaging data were analyzed separately because of the different methodologies.

In studies where direct exposure in flour was assessed, at each replicate for each concentration, 6 g of the wheat flour mixture described above was added to each of 18 concrete arenas. For the test exposing the packaging surfaces, 54 arenas of each of the seven surfaces (378 total) were included along with the flour arenas. Immediately prior to aerosol treatment, the arenas containing flour and the arenas containing the packaging surfaces were placed directly on the floor, within a 6 m × 6 m unobstructed area of the floor, such that no treatment arena was placed within 0.6 m of a potential overhead barrier (i.e., milling equipment, walls, doorways, etc.). These arenas were not placed in this area in any particular order or grouping, and were considered to be “haphazardly arranged”. For each replicate of each aerosol concentration, two control arenas containing only flour and two control arenas containing each of the seven packaging surfaces were held in another on-site building that was not exposed to the aerosol treatment. During the aerosol treatment, the lids of each arena were placed open side up underneath each treatment arena. The aerosol was applied through the application system, and after 2.5 h the arenas were retrieved from the mill. After treatment, arenas were covered with lids, bundled, secured with masking tape, and placed in dark, UV-light minimizing storage containers. These storage containers were returned to Manhattan and held on a counter in a laboratory in the Department of Entomology at Kansas State University.

A series of nine residual bioassays were done over a period of 16 weeks. For each bi-weekly post-exposure bioassay of the exposed flour, four treated arenas were randomly chosen from the larger sample of 18 exposed arenas for each replicate and concentration. The 6 g of exposed flour from each of the four arenas was divided into two sub-samples of 3 g; one was used for residue analysis (data not shown) and the other 3 g subsample was divided into three samples of 0.5 g each transferred individually to new unexposed concrete arenas created as described above. This gave a total of 12 arenas each containing 0.5 g of the flour that was exposed to aerosol during each replicate of the field trial. Ten 4-week-old *T. castaneum* larvae were placed on the flour in six of the arenas and ten 4-week-old *T. confusum* larvae were placed on the flour in each of the other six arenas. This addition of larvae was denoted as day 0 and the arenas were monitored every two days for 30 days, as will be described below in more detail. The two control arenas that contained 6 g of flour each and held on-site in an untreated building were prepared in the same

manner as described for the treated arenas and used in the bioassays at time 0. At each subsequent bi-weekly bioassay, new control arenas were prepared to accompany the tests with the treated arenas.

In packaging screening experiments, at each two-week bioassay period six arenas of each type were selected from the respective boxes containing the treated arenas. Approximately 0.5 g of flour media was added to each arena. Three arenas were used for the bioassays with *T. castaneum* and three were used for bioassays with *T. confusum*. Ten four-week-old *T. castaneum* or *T. confusum* larvae were put in each of the three treatment arenas for packaging surface and untreated control arena. Dishes were sealed with parafilm and held in an environmental chamber set at $27 \pm 1^\circ\text{C}$. A $0.32 \times 0.26 \times 0.07$ m plastic pan was filled with tap water to provide some level of humidity inside the chamber. At the 0-week bioassay, the six untreated controls of each of the seven packaging materials were prepared as described above. The controls for each of the subsequent bi-weekly bioassays were prepared separately to accompany the tests with the treatment arenas. The controls for both the flour tests and packaging tests were done thusly because of the sheer number of arenas required for the treatments at each replication and concentration for the nine bioassays (0–16 weeks every two weeks).

Arenas containing larvae placed on the exposed flour and the arenas containing larvae placed on the exposed packaging surfaces with flour added were sealed with parafilm. Every two days for 30 days, the treated arenas and control arenas for both studies were examined and individuals classified as follows: dead larvae, arrested larvae, larvae-pupae intermediates, dead pupae, arrested pupae, pupal-adult intermediates, adults-unable to shed cuticle, adults with unfolded wings, adults with twisted wings (nine categories for morphological deformities), and visually normal adults (10th category). Dead larvae and dead pupae were visibly discolored and withered, arrested larvae and pupae were still alive but had not advanced to the next stage, hence were considered “arrested”. The intermediate categories occurred during the molting process. Once an individual's status had been determined as described above it was removed from the given arena. After each observation, the arenas were re-sealed and returned to the environmental chamber. At the conclusion of the 30-day observation period, all individuals that were still alive but in the larval or pupal stage were classified as having been arrested in that stage, because by this time virtually all larvae in untreated controls had emerged as normal adults. All observations of normal adults or adults or immatures with morphogenic deformities were totaled. Hence, there were a total of nine categories for morphological defects or arrested larvae and pupae, with normally-appearing adults as a separate category (visual normal as described above).

2.3. Statistical analysis

For bioassay data from the flour study, the six sub-samples were totaled to give 60 observations for each species at each concentration and replicate. Similarly, for the packaging surface study, the 30 observations for each species at each concentration, replicate and packaging surface were totaled. For the flour study, treatments were initially analyzed as a 2 (species) by 2 (concentration) by 9 (bioassay week) factorial, but then subsequently analyzed by species due to the higher susceptibility of *T. castaneum*. For the packaging surface study, treatments were analyzed as a 2 (species) by 2 (concentration) by 7 (packaging surface) by 9 (bioassay week) factorial but were also subsequently analyzed by species.

For both the flour study and the packaging surface study, the nine categories for morphological deformities and the 10th category for adult emergence were analyzed separately as binomial data (% of individuals with and without a deformity category) using the GENMOD Procedure of the Statistical Analysis System (SAS, 2007). For the flour study, data were pooled over the 6 sub-samples and 9 bioassay weeks for each combination of deformity-species –concentration and replicate. Data were similarly pooled for the packaging study over the 3 sub-samples and 9 bioassay weeks. For the flour study, treatments were first analyzed as a 2 (species) by 2 (concentration) factorial. Because the species by concentration interaction was significant ($P < 0.05$) for six of the 10 categories, treatments were analyzed as a one-way treatment structure for the four species-concentration combinations, and the four treatments were compared using LSD-type pairwise comparisons in GENMOD. For the packaging surface study, treatments were analyzed as a 4 (species by concentration) by 7 (packaging surface) factorial. The interaction between the species-concentration treatment factor and the packaging surface was significant for all nine morphological deformity categories and for emerged normally-appearing adults. Again, *T. castaneum* showed a much higher susceptibility than *T. confusum*, therefore simple-effect pairwise comparisons were used to compare the two concentrations for each species within each packaging surface.

3. Results

3.1. Aerosol deposition on flour

Adult emergence in the untreated controls was almost 100% and no corrections were necessary for treatment mortality. The initial analysis on emergence of adults with no visible deformities showed that the main effects for species, concentration of pyrethrin, and bioassay week were all significant at $P < 0.01$ ($F = 427.6$, $df = 1, 108$; $F = 28.9$, $df = 1, 108$; $F = 3.0$, $df = 8, 108$, respectively). The species by concentration and the species by bioassay week interactions were also both significant at $P < 0.05$. However, significant interactions resulted because *T. castaneum* was far more susceptible to the pyrethrin-methoprene aerosol than was *T. confusum*. When analysis was run separately for each species, then concentration, bioassay week, and the interaction were not significant for *T. castaneum* ($F = 3.5$, $df = 1, 54$, $P = 0.07$; $F = 0.4$, $df = 8, 54$, $P = 0.90$; $F = 0.9$, $df = 1, 54$, $P = 0.49$, respectively). Average emergence of normal-appearing adults of *T. castaneum* from the larvae placed on flour exposed to the 1% and 3% pyrethrin + methoprene concentrations was $0.13 \pm 0.10\%$ and $1.2 \pm 0.51\%$, respectively (data averaged for bioassay week). The analysis for normal-appearing adults of *T. confusum* showed significance at $P < 0.01$ for concentration and bioassay week respectively ($F = 48.7$, $df = 1, 54$; $F = 3.2$, $df = 8, 54$), but not the interaction ($P = 0.63$, $F = 0.8$, $df = 8, 54$). In the 1% pyrethrin + methoprene aerosol treatment, adult emergence gradually increased during the 16-week residual testing period, with the maximum emergence of $46.2 \pm 2.7\%$ occurring at week 16 (Fig. 1). In the 3% pyrethrin + methoprene aerosol treatment, adult emergence was comparatively less than in the 1% pyrethrin + methoprene aerosol treatment on five of the nine bioassay weeks; with maximum emergence of $26.2 \pm 1.9\%$ occurring at week 10 (Fig. 1).

An initial analysis for differences over the 16-week residual testing period was done for each of the nine morphological categories. For all species and insecticide concentration combinations there were significant differences in each of the nine categories with respect to week ($P < 0.05$, Waller-Duncan k -ratio- t -test). Regression analysis, with week as the independent variable, was used to determine if there were significant trends over time, but

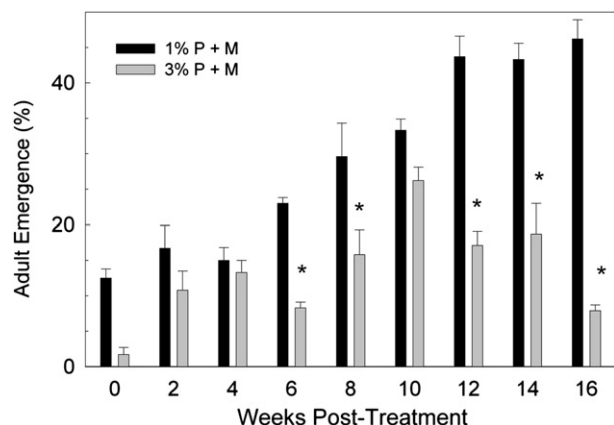


Fig. 1. Percentage of adult emergence (mean $\pm$ SEM) from 4-week-old larvae of *Tribolium confusum* on flour treated with 1% pyrethrin + methoprene or 3% pyrethrin + methoprene (P + M) at 0–16 weeks after treatment, significant differences in emergence ($P < 0.05$, t -test) at each bi-weekly bioassay are denoted with an asterisk.

there was no significant regression for any of the nine categories ($P \geq 0.05$). Because of this lack of a trend with respect to week, the data were combined for further analysis.

Results for the combined analysis for each of the morphological defects showed *T. castaneum* larvae exposed to 1% and 3% pyrethrin + methoprene aerosol applications exhibited morphological defects sooner (that is, with higher percentages in earlier morphological categories), than *T. confusum* exposed to 1% pyrethrin + methoprene (Table 1). When *T. castaneum* larvae were exposed, about 67% either died or were arrested in the larval stage. About 30% were able to develop to the pupal stage, where they died, were arrested as pupae, or became malformed pupal-adult intermediates. Hence, most of the morphological effects occurred almost immediately after the 4-week-old larvae encountered the residues in the flour. There were few differences between the 1% and 3% pyrethrin formulations. In contrast, more of the *T. confusum* larvae were able to develop beyond the larval stage when exposed on the flour treated with the 1% pyrethrin, and a greater percentage of the deformities occurred in the pupal and adult stages compared to *T. castaneum*. Increasing the concentration to 3% pyrethrin resulted in an increase in the percentages of dead pupae and pupal-adult intermediates, as well as a decrease in emergence of normal adults.

Table 1

Percentage (mean $\pm$ SEM) of individual beetles that were in each of the nine morphological deformity categories, averaged over the 16-week residual bioassays, by species and aerosol insecticide formulation (1% pyrethrin + methoprene or 3% pyrethrin + methoprene (P + M)^a. Flour was exposed directly to the aerosols and bioassays were done by placing 4-week-old larvae of each species on the treated flour.

Morphological deformity	<i>T. castaneum</i>		<i>T. confusum</i>	
	1% P + M	3% P + M	1% P + M	3% P + M
Dead Larvae	30.4 $\pm$ 1.0a	23.3 $\pm$ 1.9b	18.7 $\pm$ 0.8c	18.2 $\pm$ 1.0bc
Arrested Larvae	36.5 $\pm$ 1.0b	39.8 $\pm$ 1.1a	7.1 $\pm$ 0.6c	6.8 $\pm$ 0.5c
Larvae-Pupae	1.5 $\pm$ 0.2a	1.4 $\pm$ 0.2a	0.2 $\pm$ 0.1b	0.5 $\pm$ 0.1b
Intermediates				
Dead Pupae	16.3 $\pm$ 0.8a	17.1 $\pm$ 0.8a	8.8 $\pm$ 0.6b	15.1 $\pm$ 0.8a
Arrested Pupae	4.9 $\pm$ 0.5b	6.3 $\pm$ 0.5a	3.4 $\pm$ 0.4c	4.6 $\pm$ 0.4c
Pupal-Adult Intermediates	8.1 $\pm$ 0.6d	10.5 $\pm$ 0.6c	19.0 $\pm$ 0.8b	27.4 $\pm$ 1.0a
Adults-Unable to Shed cuticle	0.8 $\pm$ 0.2b	0.2 $\pm$ 0.1b	4.8 $\pm$ 0.4a	5.3 $\pm$ 0.5a
Adults-Unfolded Wings	0.9 $\pm$ 0.2b	0.1 $\pm$ 0.1c	6.2 $\pm$ 0.5a	5.8 $\pm$ 0.5a
Adults-Twisted Wings	0.6 $\pm$ 0.2b	0.1 $\pm$ 0.1c	2.5 $\pm$ 0.3a	2.9 $\pm$ 0.4a
Adults-Normal	0.1 $\pm$ 0.1d	1.3 $\pm$ 0.2c	29.3 $\pm$ 1.0a	13.3 $\pm$ 0.7b

^a Means followed by the same letter within the same morphological deformity category are not significantly different at $P \geq 0.05$ (LS-MEANS).

3.2. Aerosol deposition on packaging materials

Adult emergence in the untreated controls of all packaging surfaces was again almost 100% and no corrections were necessary for mortality. Initial analysis of emergence of normal-appearing adults showed that main effects (packaging surface, species, concentration of pyrethrin, and bioassay week) were all significant at $P < 0.01$ ($F = 8.7$, $df = 6$, 756; $F = 4995.7$, $df = 1$, 108; $F = 1340.6$, $df = 1$, 756; $F = 118.9$, $df = 8$, 756, respectively). All associated interactions were significant at $P < 0.05$, except surface by week, surface by species by week, and surface by species by concentration by week. The significance of main effects was primarily due to *T. castaneum* being far more susceptible to the pyrethrin-methoprene aerosol compared to *T. confusum*. When the analysis for normally-appearing adults was run separately for each species only bioassay week was significant for *T. castaneum* ($F = 4.2$, $df = 8$, 378, $P < 0.01$; all other main effects and interactions were not significant ($P \geq 0.05$), but emergence at any bioassay week did not exceed 1%. Average emergence of normally-appearing adults of *T. castaneum* from the larvae placed on flour exposed to the 1% and 3% pyrethrin + methoprene concentrations was $0.13 \pm 0.06\%$ and $0.19 \pm 0.06\%$, respectively (data averaged for all surfaces and all bioassay weeks).

Main effects for packaging surface, concentration, and bioassay week were all significant at $P < 0.01$ ($F = 9.6$, $df = 6$, 378; $F = 1526.4$, $df = 1$, 378; $F = 124.3$, $df = 8$, 378; respectively), for emergence of normally-appearing *T. confusum* adults after larval exposure to aerosols. Only the surface by concentration and concentration by week interactions were significant ($P < 0.01$). At the 1% pyrethrin + methoprene concentration, adult emergence ranged from 29.7 ± 2.5 to $49.0 \pm 4.9\%$, with the greatest emergence on the paper bag surface (Fig. 2), which would seem to indicate less retention of the aerosol on this packaging surface compared to the others. At the 3% pyrethrin + methoprene concentration, the greatest adult emergence of $14.4 \pm 1.7\%$ occurred on the muslin bag surface, which had the lowest emergence at the 1% pyrethrin + methoprene concentration. Hence, results were inconsistent regarding the relative order of adult emergence on the seven surfaces at the two concentrations. However, it was clear that

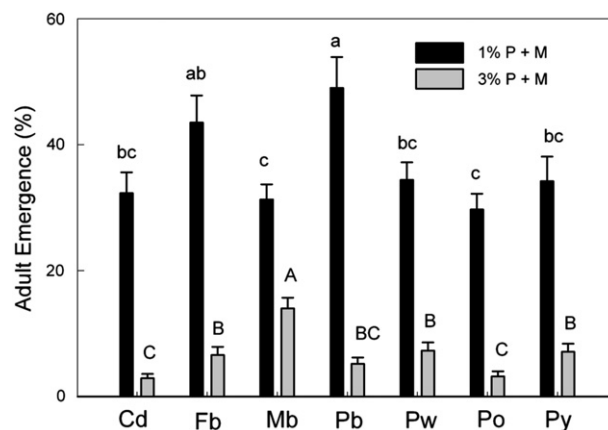


Fig. 2. Percentage of adult emergence (mean $\pm$ SEM) from 4-week-old larvae of *Tribolium confusum* on surfaces made of cardboard, flour bag, muslin bag, paper bag, pallet wrap, plastic overwrap, and polyethylene exposed to 1% pyrethrin + methoprene or 3% pyrethrin + methoprene (P + M). Data combined over the 16-week bioassays, significant differences in emergence ($P < 0.05$) on each surface exposed to 1% P + M are denoted by different upper-case letters, significant differences in emergence ($P < 0.05$) on each surface exposed to 3% P + M are denoted by different lower-case letters ($P < 0.05$, LS-Means).

adult emergence was greatly reduced on all surfaces at the higher aerosol concentration.

Mean percentages for the surface by concentration by week interaction for *T. confusum* are given in Fig. 3 (A–G). At the 1% pyrethrin + methoprene concentration, adult emergence on each of the surfaces followed the same general pattern of a gradual increase in emergence as the weeks progressed, indicating a time-dependent reduced residual toxicity of the aerosol application. By week 16 adult emergence on the seven surfaces ranged from $43.3 \pm 2.7\%$ for muslin bag to $77.5 \pm 2.5\%$ for paper bag surfaces (Fig. 3, C and D). At the 3% pyrethrin + methoprene concentration, there was greatly reduced adult emergence with a less distinct pattern of increasing emergence with time, especially for the muslin bag surface (Fig. 3C). Except for week 0 on the polyethylene surface (Fig. 3G), adult emergence was significantly lower at the 3% pyrethrin + methoprene concentration than at the 1% pyrethrin + methoprene concentration (PROC *t*-test in SAS, $P < 0.01$) on each surface at each bioassay week.

The initial analysis for each of the nine morphological categories, by species, insecticide concentration, and packaging surface showed significant differences with respect to week for each of the nine categories ($P < 0.05$). However, as with the flour exposure portion of the experiment, regression analysis showed no significance with respect to week ($P \geq 0.05$). Data were then combined for each surface so as to analyze for differences between species and concentration with respect to these morphological categories (Table 2), similar to the analysis shown in Table 1. As the aerosol concentration increased there was an increase in arrested larvae of *T. castaneum*, and a decrease in pupal-adult intermediates, which was an indication that at the higher aerosol concentration the effects were occurring earlier in *T. castaneum*. There were more dead larvae and dead pupae of *T. confusum* as the aerosol concentration increased. Overall the results of the exposure appeared to be manifested later in *T. confusum* than in *T. castaneum*, similar to the results for the flour experiment. However, in the surface exposure portion of the study, the effects

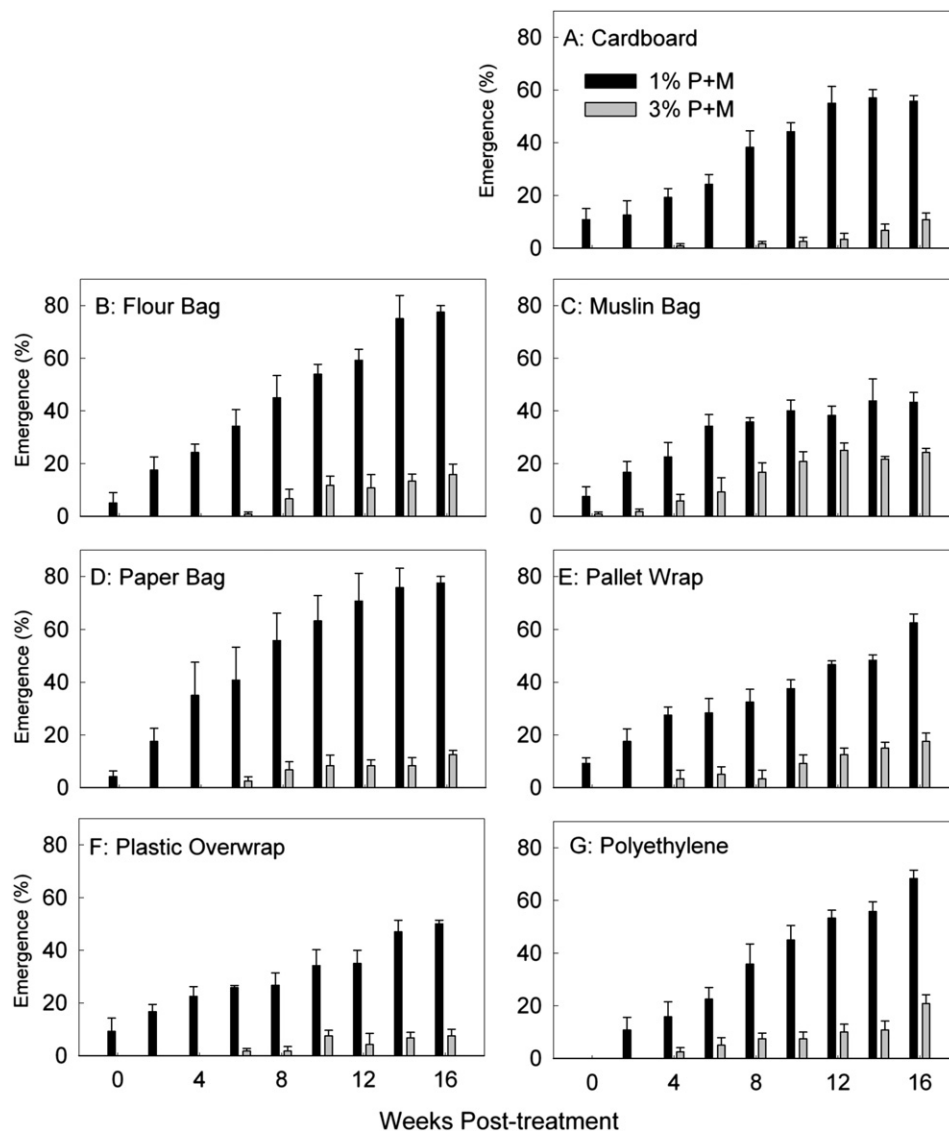


Fig. 3. A–G. Percentage of adult emergence (mean $\pm$ SEM) from 4-week-old larvae of *Tribolium confusum* on surfaces made of cardboard, flour bag, muslin bag, paper bag, pallet wrap, plastic overwrap, and polyethylene exposed to 1% pyrethrin + methoprene or 3% pyrethrin + methoprene (P + M). Approximately 0.5 g of a wheat flour mixture was added to the surfaces for bioassays. Adult emergence was significantly less at 3% P + M compared to 1% P + M on all surfaces after the 0-week bioassays ($P < 0.05$, *t*-test).

Table 2
Percentage (mean $\pm$ SEM) of individual beetles that were in each of the nine morphological deformity categories, averaged over the 16-week residual bioassays, by species and aerosol insecticide formulation (1% pyrethrin + methoprene or 3% pyrethrin + methoprene (P + M), averaged over the seven packaging surfaces^a. The surfaces were exposed directly to the aerosol concentrations, and bioassays were done by placing 4-week-old larvae of each species on the treated surfaces (abbreviations used for Table 3).

Morphological deformity	<i>T. castaneum</i>		<i>T. confusum</i>	
	1% P + M	3% P + M	1% P + M	3% P + M
Dead Larvae (DL)	29.2 $\pm$ 0.5a	30.3 $\pm$ 0.5a	9.7 $\pm$ 0.3c	19.6 $\pm$ 0.5b
Arrested Larvae (AL)	6.1 $\pm$ 0.3bc	19.1 $\pm$ 0.5a	3.4 $\pm$ 0.2dc	9.2 $\pm$ 0.3bc
Larvae-Pupae Intermediates (LPI)	2.0 $\pm$ 0.2a	1.9 $\pm$ 0.2a	0.0 $\pm$ 0.0b	0.3 $\pm$ 0.1b
Dead Pupae (DP)	24.8 $\pm$ 0.5a	24.4 $\pm$ 0.5a	10.4 $\pm$ 0.4c	23.0 $\pm$ 0.5b
Arrested Pupae (AP)	3.1 $\pm$ 0.2c	6.6 $\pm$ 0.3a	1.6 $\pm$ 1.1d	4.1 $\pm$ 0.2b
Pupal-Adult Intermediates (PAI)	31.3 $\pm$ 0.5a	15.8 $\pm$ 0.4c	23.3 $\pm$ 0.5a	30.0 $\pm$ 0.5a
Adults-Unable to Shed Cuticle (SC)	1.3 $\pm$ 1.4c	0.2 $\pm$ 0.1d	5.2 $\pm$ 0.3a	2.2 $\pm$ 0.2b
Adults-Unfolded Wings (UW)	0.4 $\pm$ 0.1c	0.2 $\pm$ 0.1c	5.3 $\pm$ 0.3a	2.5 $\pm$ 0.2b
Adults-Twisted Wings (TW)	0.2 $\pm$ 0.1c	^b	2.6 $\pm$ 0.3a	0.8 $\pm$ 0.1b
Adults-Normal (AN)	0.1 $\pm$ 0.1c	0.1 $\pm$ 0.1c	36.6 $\pm$ 0.6a	5.3 $\pm$ 0.3b

^a Means followed by the same letter within the same morphological deformity category are not significantly different at $P \geq 0.05$, LS-MEANS).

^b Data actually 0.

of exposure were manifested later for *T. castaneum* compared to the flour exposures, but the end result was that less than 1% of the *T. castaneum* larvae exposed on the flour or on the surfaces were able to emerge as normal adults.

Because the interaction between the species by concentration treatment factor and the packaging surface was significant ($P < 0.01$) for all morphological categories and *T. castaneum* again showed a much higher susceptibility than *T. confusum*, simple-effect pairwise comparisons were used to compare the two concentrations for each species within each packaging surface. On all seven surfaces, there were more arrested larvae of *T. castaneum* at the higher pyrethrin concentration, and more arrested pupae on six of the seven surfaces at the higher pyrethrin concentration (Table 3). As mentioned previously, few adults of *T. castaneum* emerged on any surface. For *T. confusum*, there were more dead larvae, more arrested larvae, more dead pupae, and more arrested pupae on all surfaces at the higher pyrethrin concentration (Table 4). On all surfaces, there were fewer emerged adults at the higher pyrethrin concentration, indicating that deleterious effects from this higher concentration of pyrethrin combined with methoprene appeared earlier in the life cycle for *T. confusum*, resulting in less adult emergence (Table 4).

4. Discussion

This study was conducted in an active commercial facility to provide realistic data on residual efficacy of aerosols. Our results showed that the 1% pyrethrin + methoprene and the 3% pyrethrin + methoprene aerosols gave residual control of *T. castaneum* and *T. confusum*. However, there was a clear difference in the susceptibility of the two species. In previous comparative studies with the IGRs hydroprene and pyriproxyfen, *T. castaneum* was also the more susceptible of the two species, as assessed by adult emergence from exposed larvae (Arthur and Hoernemann, 2004; Arthur et al., 2009). In the current study, most of the effects of the insecticide exposure occurred in the 4-week-old larval stage of *T. castaneum*, whereas in *T. confusum* the effects from the exposure occurred in either the pupal or adult stages as the post-treatment weeks progressed and the residues began breaking down in the flour. Although outside and inside temperatures were not measured in this study, the individual replications were conducted at different times throughout the year, with little difference between replicates, hence temperature effects were limited.

There were also indications of a possible additive effect of the pyrethrin and the efficacy of the methoprene IGR, as shown in the results for *T. confusum*, the more tolerant species. Even though the

concentration of methoprene remained the same, the morphological effects that occurred in the pupal and adult stages increased with the increasing pyrethrin concentration. The amount of PBO synergist was 2% in the 1% pyrethrin formulation and 6% in the 3% pyrethrin formulation, but this is the same ratio of synergist to pyrethrin in the two formulations. Although additive and synergistic effects have been noted before in the response of stored-product insects to contact insecticides with different modes of action (Akbar et al., 2004; Athanassiou, 2006; Nayak and Daglish, 2007; Chintzoglou et al., 2008), this is the first report of an additive effect between pyrethrin and methoprene applied as an aerosol. The immature stages of *T. castaneum* and *T. confusum* are more sensitive to pyrethrin than the adult stages (Arthur, 2008), which could also contribute to the additive effects of the aerosol mixture which is directed toward the immature stages. Since these same formulations did not show adult toxicity in a previous study (Arthur, 2010), it was assumed that the pyrethrin would rapidly dissipate, and most of the residual effects would be due to the methoprene component of the aerosol. Both in this study and a previous study that utilized the same aerosol application system (Arthur, 2010), adult emergence was reduced when different life stages of *T. castaneum* were exposed on concrete surfaces treated with 1% pyrethrin + methoprene compared to 3% pyrethrin + methoprene.

The life stage used in our test was the 4-week-old larval stage, and as this stage prepares to molt, the introduction of a juvenile hormone analog apparently produces more of the intermediate forms associated with molting between the larval and pupal stage, and the pupal and adult stage (Sehnal and Meyer, 1968; Tunaz and Uygun, 2004). This has occurred in other studies with methoprene and *T. castaneum* and *T. confusum* (Amos et al., 1974), with methoprene and *T. castaneum* (Hoppe, 1981), and in studies with hydroprene for both species (Arthur, 2001; Arthur and Hoernemann, 2004). In the results reported in this paper, most of the *T. castaneum* exposed as 4-week-old larvae on the treated flour either died or were arrested as larvae, and would most likely eventually die in that stage or as incomplete larval-pupal molts. Larval *T. confusum* exposed on the treated flour molted to the pupal stage but were arrested, died, were intermediate pupal-adult molts, or were adults with morphological deformities.

In studies with IGRs applied as contact insecticides to a concrete surface, the flour food source appeared to absorb the residues from the surface, as evidenced by reduced adult emergence after exposure of larvae of either *T. castaneum* or *T. confusum* (Arthur, 2001; Arthur and Hoernemann, 2004; Arthur et al., 2009). Evidence of residual persistence on a concrete surface has also been demonstrated in field studies in which IGRs have been applied as aerosols

Table 3

Percentage (mean $\pm$ SEM) of *T. castaneum* that were in each of the nine morphological deformity categories, averaged over the 16-week residual bioassays, by species and aerosol insecticide formulation (1% pyrethrin + methoprene or 3% pyrethrin + methoprene (P + M), for each of the seven packaging surfaces^{a,b}. The surfaces were exposed directly to the aerosol concentrations, and bioassays were done by placing 4-week-old larvae of each species on the treated surfaces.

	Cardboard		Flour bag		Muslin bag		Paper bag	
	1% P + M	3% P + M	1% P + M	3% P + M	1% P + M	3% P + M	1% P + M	3% P + M
DL	28.9 $\pm$ 1.1a	25.7 $\pm$ 1.3a	27.7 $\pm$ 1.4a	29.3 $\pm$ 1.4a	35.8 $\pm$ 1.5a	32.7 $\pm$ 1.4a	19.7 $\pm$ 1.2b	26.8 $\pm$ 1.3a
AL	6.1 $\pm$ 0.7b	30.6 $\pm$ 1.4a	4.6 $\pm$ 1.2b	12.2 $\pm$ 1.0a	11.0 $\pm$ 1.1b	15.3 $\pm$ 1.1a	7.7 $\pm$ 0.8b	20.4 $\pm$ 1.2a
API	2.5 $\pm$ 0.5a	1.7 $\pm$.04a	1.2 $\pm$ 0.3a	1.2 $\pm$ 0.3a	2.8 $\pm$ 0.5b	1.5 $\pm$ 1.4a	1.7 $\pm$ 0.4a	2.7 $\pm$ 0.5a
DP	26.3 $\pm$ 1.3a	22.5 $\pm$ 1.3b	26.7 $\pm$ 1.3a	28.5 $\pm$ 1.4a	24.9 $\pm$ 1.3a	23.4 $\pm$ 1.4a	23.1 $\pm$ 1.3a	24.6 $\pm$ 1.3a
AP	2.7 $\pm$ 0.5b	5.7 $\pm$ 0.7a	3.2 $\pm$ 0.5b	7.6 $\pm$ 0.8a	2.5 $\pm$ 0.5b	5.8 $\pm$ 0.7a	4.9 $\pm$ 0.7a	6.6 $\pm$ 0.8a
PAI	32.7 $\pm$ 1.4a	13.1 $\pm$ 1.0b	33.7 $\pm$ 1.4a	19.9 $\pm$ 1.2b	21.8 $\pm$ 1.3a	20.7 $\pm$ 1.2a	39.9 $\pm$ 1.5a	18.3 $\pm$ 1.2b
AC	0.7 $\pm$ 0.3a	0.2 $\pm$ 0.2a	2.5 $\pm$ 0.5a	0.5 $\pm$ 1.2b	0.7 $\pm$ 0.3a	0.1 $\pm$ 0.1b	1.7 $\pm$ 0.4a	0.3 $\pm$ 0.2b
UW	0.1 $\pm$ 0.1a	0.2 $\pm$ 0.2a	0.5 $\pm$ 0.2a	0.6 $\pm$ 0.2a	0.2 $\pm$ 0.1a	0.4 $\pm$ 0.2a	1.0 $\pm$ 0.3a	0.1 $\pm$ 0.1b
TW	0.0 $\pm$ 0.0a ^c	— ^d	0.0 $\pm$ 0.0a ^c	— ^d	0.1 $\pm$ 0.1a	— ^d	0.3 $\pm$ 0.2a	— ^d
AN	0.0 $\pm$ 0.0a ^c	0.2 $\pm$ 0.0a	0.0 $\pm$ 0.0a ^c	0.2 $\pm$ 0.1a	0.2 $\pm$ 0.1a	0.0 $\pm$ 0.0a ^c	0.1 $\pm$ 0.1a	0.2 $\pm$ 0.2a

	Pallet Wrap		Plastic Overwrap		Polyethylene	
	1% P + M	3% P + M	1% P + M	3% P + M	1% P + M	3% P + M
DL	25.9 $\pm$ 1.3a	27.9 $\pm$ 1.4a	30.4 $\pm$ 1.4a	33.7 $\pm$ 1.4a	38.7 $\pm$ 1.5a	32.7 $\pm$ 1.5a
AL	4.8 $\pm$ 0.7b	20.5 $\pm$ 1.2a	9.4 $\pm$ 0.9b	24.4 $\pm$ 1.3a	2.7 $\pm$ 0.5b	14.7 $\pm$ 1.1a
API	2.6 $\pm$ 0.5a	2.1 $\pm$ 1.4a	2.7 $\pm$ 0.5a	2.3 $\pm$ 0.5a	1.3 $\pm$ 0.3b	2.1 $\pm$ 0.4a
DP	24.4 $\pm$ 1.3a	24.4 $\pm$ 1.3a	25.0 $\pm$ 1.3a	21.7 $\pm$ 1.3a	23.3 $\pm$ 1.3a	25.7 $\pm$ 1.3a
AP	3.4 $\pm$ 1.6b	15.4 $\pm$ 1.1a	2.6 $\pm$ 0.5b	5.6 $\pm$ 0.7a	2.7 $\pm$ 0.5b	6.6 $\pm$ 0.87a
PAI	34.9 $\pm$ 1.5a	15.4 $\pm$ 1.1b	29.0 $\pm$ 1.4a	12.2 $\pm$ 1.0b	29.0 $\pm$ 1.4a	12.6 $\pm$ 1.0a
AC	2.17 $\pm$ 0.3a	0.6 $\pm$ 0.2b	0.7 $\pm$ 0.3a	0.1 $\pm$ 0.1b	1.6 $\pm$ 0.4a	0.4 $\pm$ 0.2b
UW	1.1 $\pm$ 0.3a	0.1 $\pm$ 0.1a	0.2 $\pm$ 0.1a	0.2 $\pm$ 0.1a	0.6 $\pm$ 0.2a	0.4 $\pm$ 0.2a
TW	0.3 $\pm$ 0.2a ^c	— ^d	0.0 $\pm$ 0.0a ^c	— ^d	0.2 $\pm$ 0.1a	— ^d
AN	0.6 $\pm$ 0.2a ^c	0.2 $\pm$ 0.2a	0.1 $\pm$ 0.1a ^c	0.2 $\pm$ 0.1a	0.0 $\pm$ 0.0a ^c	0.0 $\pm$ 0.0a ^c

^a Abbreviations from Table 2.

^b Means between concentration for each category for each morphological category followed by the same letter are not significantly different ($P \geq 0.05$, LS-MEANS).

^c Averages rounded to 0.

^d Data actually 0.

in combination with pyrethrins (Arthur, 2010). In the current test, the aerosols showed residual persistence on the seven packaging surfaces, and possible absorption of the residues by the flour food source, similar to results with IGRs and contact insecticides.

The increased effects on *T. confusum* with the 3% pyrethrin + methoprene aerosol compared to the 1% pyrethrin + methoprene aerosol would again seem to indicate an additive effect of the two insecticides.

Table 4

Percentage (mean $\pm$ SEM) of *T. confusum* that were in each of the nine morphological deformity categories, averaged over the 16-week residual bioassays, by species and aerosol insecticide formulation (1% pyrethrin + methoprene or 3% pyrethrin + methoprene (P + M), for each of the seven packaging surfaces^{a,b}. The surfaces were exposed directly to the aerosol concentrations, and bioassays were done by placing 4-week-old larvae of each species on the treated surfaces.

	Cardboard		Flour Bag		Muslin Bag		Paper Bag	
	1% P + M	3% P + M	1% P + M	3% P + M	1% P + M	3% P + M	1% P + M	3% P + M
DL	8.2 $\pm$ 0.8ba	17.5 $\pm$ 1.3a	9.9 $\pm$ 1.4b	19.9 $\pm$ 1.2a	11.8 $\pm$ 1.0a	19.4 $\pm$ 1.2a	6.9 $\pm$ 0.8b	19.8 $\pm$ 1.2a
AL	3.1 $\pm$ 0.5b	9.4 $\pm$ 0.9b	3.9 $\pm$ 1.2b	12.3 $\pm$ 1.0a	4.8 $\pm$ 0.7b	7.4 $\pm$ 0.8a	1.87 $\pm$ 0.4b	8.9 $\pm$ 0.9a
API	0.0 $\pm$ 0.0a ^c	0.5 $\pm$ 0.2a	0.6 $\pm$ 0.2a	0.1 $\pm$ 0.1a	0.3 $\pm$ 0.2b	0.3 $\pm$ 0.2a	0.3 $\pm$ 0.2a	0.8 $\pm$ 0.3a
DP	10.9 $\pm$ 0.9b	27.5 $\pm$ 1.4a	8.6 $\pm$ 0.9b	20.5 $\pm$ 1.2a	10.6 $\pm$ 0.9b	20.5 $\pm$ 1.2a	8.0 $\pm$ 0.8b	25.6 $\pm$ 1.3a
AP	1.6 $\pm$ 0.4b	3.7 $\pm$ 0.6a	1.4 $\pm$ 0.4b	4.3 $\pm$ 0.6a	2.4 $\pm$ 0.5b	4.1 $\pm$ 0.6a	1.0 $\pm$ 0.3a	6.9 $\pm$ 0.8a
PAI	25.9 $\pm$ 1.3b	33.7 $\pm$ 1.0a	18.4 $\pm$ 1.2b	30.0 $\pm$ 1.4a	28.6 $\pm$ 1.4a	28.9 $\pm$ 1.4a	20.6 $\pm$ 1.2b	25.9 $\pm$ 1.3b
AC	6.7 $\pm$ 0.8a	1.8 $\pm$ 0.4b	4.4 $\pm$ 0.6a	2.3 $\pm$ 0.5b	4.8 $\pm$ 0.7a	1.8 $\pm$ 0.4b	3.8 $\pm$ 0.6a	3.4 $\pm$ 0.6a
UW	5.6 $\pm$ 0.7a	2.3 $\pm$ 0.5b	5.6 $\pm$ 0.7a	2.5 $\pm$ 0.5b	3.3 $\pm$ 0.5a	1.9 $\pm$ 0.4a	5.4 $\pm$ 0.7a	2.5 $\pm$ 0.5b
TW	2.8 $\pm$ 0.5a	0.6 $\pm$ 0.2b	3.6 $\pm$ 0.6a	1.5 $\pm$ 0.5b	2.4 $\pm$ 0.4a	1.7 $\pm$ 0.4a	3.3 $\pm$ 0.5a	1.0 $\pm$ 0.3b
AN	35.3 $\pm$ 1.5a	2.9 $\pm$ 0.5b	43.5 $\pm$ 1.5a	6.6 $\pm$ 0.8b	31.3 $\pm$ 1.4a	14.0 $\pm$ 1.1a	49.0 $\pm$ 1.5a	5.2 $\pm$ 0.72b

	Pallet Wrap		Plastic Overwrap		Polyethylene	
	1% P + M	3% P + M	1% P + M	3% P + M	1% P + M	3% P + M
DL	8.9 $\pm$ 0.9b	14.4 $\pm$ 1.1a	10.0 $\pm$ 0.9a	23.4 $\pm$ 1.3a	13.2 $\pm$ 1.0a	24.4 $\pm$ 1.1b
AL	4.8 $\pm$ 0.6b	7.4 $\pm$ 0.8a	2.8 $\pm$ 0.5b	12.1 $\pm$ 1.0a	4.7 $\pm$ 0.6b	7.9 $\pm$ 0.8a
API	0.6 $\pm$ 0.2a	0.8 $\pm$ 0.3a	0.3 $\pm$ 0.2a	0.3 $\pm$ 0.2a	0.7 $\pm$ 0.2b	0.2 $\pm$ 0.1b
DP	10.2 $\pm$ 0.9b	16.5 $\pm$ 1.1a	11.8 $\pm$ 1.0b	28.8 $\pm$ 1.4a	13.6 $\pm$ 1.0b	23.5 $\pm$ 1.3a
AP	1.8 $\pm$ 0.4b	2.1 $\pm$ 0.4a	1.3 $\pm$ 0.3b	4.4 $\pm$ 0.6a	2.4 $\pm$ 0.4b	4.2 $\pm$ 0.6a
PAI	23.1 $\pm$ 1.3b	43.0 $\pm$ 1.5a	26.7 $\pm$ 1.3a	23.7 $\pm$ 1.3b	21.0 $\pm$ 1.2b	26.9 $\pm$ 1.4a
AC	6.2 $\pm$ 0.8a	2.1 $\pm$ 0.4b	7.6 $\pm$ 0.8a	2.0 $\pm$ 0.4b	3.7 $\pm$ 0.6a	2.1 $\pm$ 0.4b
UW	7.3 $\pm$ 0.8a	4.7 $\pm$ 0.6b	6.8 $\pm$ 0.8a	1.7 $\pm$ 0.48b	4.2 $\pm$ 0.6a	2.7 $\pm$ 0.5b
TW	2.7 $\pm$ 0.5a	1.6 $\pm$ 0.4b	3.1 $\pm$ 0.40a	0.4 $\pm$ 0.2b	2.6 $\pm$ 0.5a	0.8 $\pm$ 0.3b
AN	34.4 $\pm$ 1.4a	7.3 $\pm$ 0.8b	29.7 $\pm$ 1.4a	1.5 $\pm$ 0.4b	34.2 $\pm$ 0.4a	7.1 $\pm$ 0.8b

^a Abbreviations from Table 2.

^b Means between concentration for each morphological category followed by the same letter are not significantly different ($P \geq 0.05$, LS-MEANS).

^c Averages rounded to 0.

Although adult emergence of *T. castaneum* was similar on both the treated flour and the treated surfaces to which flour was added, more of the exposed larvae advanced to the pupal stage on the surfaces compared to the flour. Also, adult emergence of *T. confusum* at the 1% pyrethrin + methoprene concentration was generally greater on the treated surface than on the treated flour. This is not unusual because of the direct exposure of the flour to the aerosol. Food resources inside a flour mill could be considered as patchily distributed. *T. castaneum* moves among flour resource patches, can distribute eggs among multiple patches, and can complete the life cycle in a small resource patch (Campbell and Hagstrum, 2002; Campbell and Runnion, 2003). Spatial and temporal variation in these food patches inside a flour mill could result in multiple life stages of *T. castaneum* and *T. confusum* being present when an aerosol is applied, hence an aerosol that has residual activity and directed against the immature stages may have an overall impact at the population level. Some evidence is seen in the results from a multi-year trapping program at the mill utilized in this study, in which implementation of a sanitation program plus application of the pyrethrin + methoprene aerosols resulted in reduced populations of *T. castaneum* (Campbell et al., 2010a,b). The residual control provided by the aerosol could have contributed to this population reduction.

In conclusion, results of this study show *T. castaneum* and *T. confusum* varied in their response to the pyrethrin + methoprene aerosol. The 1% pyrethrin gave virtually complete control of *T. castaneum*; therefore if this is the only one of the two species found in a milling facility, increasing the concentration may not be necessary. In addition, the residual control from our study was such that the timing of applications and the distribution of aerosols inside a mill are areas warranting further research. Reducing the frequency of aerosol applications without compromising control could result in a cost savings for a milling facility.

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