Residual efficacy of aerosols to control *Tribolium castaneum* and *Tribolium confusum*

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Abstract

Aerosol insecticides can be important components of insect management plans for mills, food warehouses, and processing plants. In the United States, synergized pyrethrin is used alone or combined with an insect growth regulator (IGR), either methoprene or hydroprene. The presence of food material can result in increased survival of adult *Tribolium castaneum* (Herbst) or *Tribolium confusum* Jacquelin du Val exposed to synergized pyrethrins, but larvae appear to be more susceptible than adults. Results of field trials involving methoprene and pyriproxyfen indicate residual persistence of the IGRs. *Tribolium castaneum* is more susceptible than *T. confusum* to IGRs, but combination of pyrethrin with the IGR may produce an additive effect on *T. confusum.*

Keywords: Aerosol, Insecticide, Insect growth regulator, *Tribolium castaneum*, *Tribolium confusum*

1. Introduction

Aerosol insecticides [also known as ultra low volume (ULV), fogging, and space sprays], can be used as part of management programs for flour mills, processing facilities, and food warehouses in the United States (Peckman and Arthur, 2006). Several recent field studies have examined distribution and efficacy of pyreithrin or a pyrethroid applied alone or combined with the insect growth regulator (IGR) methoprene (Arthur et al., 2009; Jenson et al., 2010a, b), but there is little research regarding the residual activity and toxicity of an aerosol application. Methoprene and also the IGR pyriproxifen have residual efficacy when applied to different surfaces (Arthur et al., 2009; Jenson et al., 2009), therefore it is assumed that an IGR applied as an aerosol would also provide some level of residual control.

Insect populations inside a mill or food warehouse could be directly exposed to an aerosol or be to the residual deposits resulting from an application. Efficacy can therefore be measured by direct and indirect methods of exposure. However, it is often difficult to bring live insects of any life stage inside a commercial facility, and indirect methods of evaluation may be required to evaluate residual efficacy. The objectives of this study were to determine residual efficacy of pyrethrin combined with either methoprene or pyriproxyfen aerosol, through direct and indirect methods of exposure.

2. Materials and methods

2.1. Aerosol Field Trial 1

A field trial was conducted in a flour mill with an installed aerosol/ultra low volume (ULV) system that dispensed either a 1% pyrethrin (Entech Fog 10®)+ methoprene (Diacon II®) mixture, or a 3% pyrethrin (Entech Fog 30® + methoprene mixture, as specified on the product label for these formulations. Treatment arenas were constructed by filling the bottom portion of a Petri dish, which measured about 62cm^2, with slurry of a driveway patching material (Rockkite®) to create a smooth concrete surface. After these arenas were prepared in the laboratory, they were sent to the mill for direct exposure studies.

Trials were first done with the 1% pyrethrin + methoprene mixture. For each of 5 replicates, 20 arenas were sent to the mill, and exposed to the aerosol/ULV application, which was generally done on a Saturday. These arenas were placed on the fourth floor of the mill, directly on the floor so that there were no obstructions to the movement or drifting of the aerosol/ULV particles. Upon completion of the application, the area was vented for several hours according to label instructions, and the arenas were collected, boxed, and shipped to the Center for Grain and Animal Health Research (CGHAR) in Manhattan, KS. Upon arrival at the CGHAR, which was considered to be time 0, four arenas were randomly selected. Approximately 300 mg of flour media was placed on each arena. In one arena 10 eggs of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, were placed on

the flour, on a second arena 10 3-week old *T. castaneum* larvae were placed on the flour, on a third arena 10 4-week-old *T. castaneum* larvae placed on the flour, and on the fourth arena 10 *T. castaneum* pupae were placed on the flour. In another set of four arenas that were not exposed to the aerosol/ULV, 300 mg of flour was put in each one, and they were set up as described above with each of the four life stages as untreated controls. All arenas containing the life stages were placed in an incubator at 27°C and 60% r.h., and held until adult emergence was completed in the untreated controls. This required approximately 2, 3, 4, and 7 weeks for the pupae, 4-week-old larvae, 3-week-old larvae, and eggs, respectively. The emergence of adults that appeared to be normal with no morphological deformities was the criterion used for assessment, with main effects of concentration, residual time, and life stage. Data were analyzed using the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS, 2007).

2.2. Aerosol Field Trial 2

This field trial was conducted during summer and autumn of 2007 by first creating concrete exposure as described for the first aerosol field trial. For each replicate trial in this test, twenty four of these arenas exposed to an aerosol applications of pyrethrin + the IGR pyriproxyfen (Nygard®), at the label rate for both products, by placing six concrete arenas at each of the following positions: low and open on the floor, low and obstructed on the floor (underneath a pallet), and at similar positions called "high", which was about 4 m from the ground.

The aerosol was usually applied late Friday afternoon, the dishes were picked up on Saturday morning, and the next week shipped back to the CGHAR. At the time the dishes were received (week "0"), one dish from each position was removed and about 300 milligrams of flour was placed in each dish. Ten 4-week-old *T. castaneum* larvae were placed in each dish. Companion dishes of untreated controls were set up as well. The remaining dishes were held and then assessed at residual intervals of 2, 4, 6, 8, and 10 weeks after they were originally exposed to the aerosol. This process was repeated for three separate spray replicates. In another series of three replications, the tests were repeated using the procedures described above, and conducting residual bioassays with larvae of *Tribolium confusum* Jacqueline du Val (Coleoptera: Tenebrionidae), the confused flour beetle. Data were analyzed using the GLM Procedure of SAS with residual time, exposure position, and species as main effects.

2.3. Aerosol Field Trial 3

This trial was conducted during spring and summer of 2008 using different methods of efficacy assessment. Treatment arenas were constructed and sent to the field site, where they were placed at the same positions as described for the 2007 study. In this trial, only *T. castaneum* was used as the test species. When the arenas exposed to the aerosol arrived in at the CGHAR, one arena from each of the four exposure positions was selected. Three hundred mg of flour was placed in each of the arenas, along with ten mixed-sex 1 to 2-week old adult *T. castaneum*. Untreated controls are also set up as well following the same procedure. The adults are allowed to remain on the arenas for one week at 27°C and 60% r.h. inside a Percival® incubator, and then removed. The arenas containing the flour were then returned to the incubator and held for an additional period of six weeks to record progeny from the exposed parental adults. At this time all of the adults in the untreated controls had emerged, so they were counted and the arenas discarded. In the treatment dishes, the beetles had advanced only to the larval stage. Therefore, an additional 300 mg of flour was added to each of the arenas and the arenas returned to the incubator for an additional period of four weeks. After that time, the arenas were removed from the arenas, the emerged adults were tabulated, and the arenas discarded. Data were analyzed using the GLM Procedure of SAS with residual time and exposure position as main effects.

3. Results

3.1. Aerosol Field Trial 1

Main effects treatment, concentration, and life stage were all significant at $P < 0.01$ ($F = 787.5$, df = 1, 228; $F = 16.7$, df = 1, 228; $F = 48.7$, df = 3, 228), but week post-treatment was not significant ($F = 0.3$, df = 4, 228, $P = 0.85$), indicating equal effectiveness of the aerosol residues at all weekly exposure intervals. The only interactions that were significant ($P < 0.05$) were concentration by treatment, life stage by treatment, and concentration by life stage by treatment (all others $P \geq 0.05$). Data were further analyzed by combining the data for post-exposure week.

In all comparisons, the percentage of emerged adults was less in the treatments than in the controls ($P < 0.01$), and for life stages except pupae, adult emergence was lower in the arenas exposed to the 3% pyrethrin + methoprene ULV than in the 1% pyrethrin + methoprene ULV ($P < 0.01$) (Table 1). Except for pupae, adult emergence was zero for all life states in the treatment arenas exposed to the 3% pyrethrin + methoprene ULV. Adult emergence from pupae in the treatment arenas was the same at both insecticide concentrations.

Table 1 Percentage of adult emergence (mean ± SE) of eggs, 3-week-old larvae, 4-week-old larvae, and pupae of *Tribolium castaneum* in concrete arenas exposed to 1% pyrethrin + methoprene and 3% pyrethrin + methoprene, at 0-4 weeks post-treatment (data combined for the post-treatment bioassays). Means within columns for each concentration followed by different letters indicate significant differences in adult emergence among exposed life stages ($P < 0.05$, Waller-Duncan *k*-ratio *t*-test)[a].

Concentration	Life Stage	Treatment	Control
1% pyrethrin+ methoprene	Pupae	62.5 ± 6.2a	96.5 ± 1.3a
	4-week-old larvae	30.0 ± 7.9b	96.0 ± 8.7a
	3-week-old larvae	20.0 ± 8.7b	91.3 ± 2.6a
	Eggs	22.0 ± 6.9b	70.5 ± 4.2b
3% pyrethrin+ methoprene	Pupae	63.6 ± 5.7a	95.5 ± 1.9a
	4-week-old larvae	0.0 ± 0.0b	93.5 ± 1.7a
	3-week-old larvae	0.0 ± 0.0b	86.5 ± 2.5b
	Eggs	0.0 ± 0.0b	81.0 ± 3.2b

[a] Adults emergence always lower in treatments versus controls, and for each life stage except pupae emergence was lower for 3% pyrethrin + methoprene versus 1% pyrethrin + methoprene.

3.2. Aerosol Field Trial 2

Main effects treatment and species were both significant ($F = 2{,}710.4$, df = 1, 176, $P < 0.01$; $F = 5.0$, df = 1, 176, $P < 0.02$) for adult emergence from larvae in the arenas exposed to the aerosol, but neither the position at which the arenas were originally exposed (open or obstructed, on the floor versus 4 m off the floor) or the residual bioassay week were significant ($F = .01$, df = 3, 176, $P = 0.93$; $F = .04$, df = 3, 176, $P = 0.81$). The week by species and the treatment by species interactions were significant ($P < 0.01$) but the rest of the interactions were not significant ($P \geq 0.05$). *T. castaneum* was the more susceptible species, with adult emergence of 4.1 ±1.1% compared to 10.1 ± 2.2 % for *T. confusum* in arenas exposed to the aerosol. Adult emergence of larvae in the untreated controls was 92.1 ± 1.2% and 93.4 ± 2.1% for *T. castaneum* and *T. confusum*, respectively. The residues from the aerosol application appeared to be effective for 10 weeks, assessed by the methodology employed in this experiment.

3.3. Aerosol Field Trial 3

In the arenas that were exposed to the aerosol, there were very few adults, and no difference regarding where the arenas were placed when exposed to the aerosol (on the floor, off the floor, open versus obstructed positions, $F = 0.6$, df =3, 168; $P = 0.64$) or the residual bioassay time in which the adults were first put on the arenas exposed to the aerosol ($F = 0.4$, df =5, 168; $P = 0.84$). For the entire test, the average number of adults in the control arenas was 26.0 ± 1.2 compared to 0.7 ± 0.3 in the arenas exposed to the aerosol. In all of the arenas exposed to the aerosol, only 8 adults were found during the entire experiment, compared to more than 2,700 in the equal number of untreated control arenas.

4. Discussion

Results of these field trials show that the pyrethrin+IGR mixtures seem to have residual activity against larvae of *T. castaneum* and *T. confusum*. Recent field studies have also shown that regular applications of a pyrethrin+ methoprene mixture can suppress resident populations of *T. castaneum* (Jenson et al., 2010a). However, more detailed tests should be conducted to make an accurate assessment of the level of efficacy, especially because earlier studies have shown that *T. castaneum* is the more susceptible of the two species to pyrethrin aerosol (Arthur, 2008) and the IGRs hydroprene and pyriproxifen (Arthur and Hoernemann, 2004; Arthur et al., 2009). Although each individual mill or warehouse has its own characteristics that will affect insect control, certain common themes are present in all locations.

In the field trial with pyrethrin + methoprene, increasing the application rate of pyrethrin from 1% AI to 3% AI produced an apparent corresponding increase in residual efficacy even though the concentration of methoprene remained the same. When adult beetles were placed on these arenas at the same time as the immature stages there was no adult mortality, hence the assumption that it was the methoprene component that was providing the residual control. However, immature stages of *T. castaneum* and also *T. confusum* may be more susceptible to pyrethirn than the adults (Arthur, 2008), therefore any residual efficacy relating to the pyrethrin component would be reflected in the reduced adult emergence from exposure of eggs and larvae. Perhaps there was also an additive effect for the pyrethirn and the IGR.

The indirect methods of exposure employed for the field trials with pyrethrin and pyriproxifen, whereby the concrete arenas themselves were exposed to the aerosol, could be used for expanded field trials with other insecticides or application system. The flour apparently absorbed some of the residues from the exposed surface, and larvae encountered these residues through contact toxicity and feeding on the flour. Utilizing this procedure for evaluation of residual activity of aerosols would alleviate risks associated with bringing live insects inside an active commercial facility.

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