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# Assessing effects of esfenvalerate aerosol applications on resident populations of *Tribolium castaneum* (Herbst), the red flour beetle, through direct and indirect sampling

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ABSTRACT

Small-scale field sheds were infested to establish resident populations of the red flour beetle, *Tribolium castaneum* (Herbst), and either left untreated (control) or treated every two or four weeks with an aerosol spray of esfenvalerate (Conquer®). Prior to treatments, sheds were infested by placing flour (food) patches underneath shelves in the shed, and two trials were done in separate blocks. Aerosol efficacy was assessed using pheromone traps to estimate live adults (indirect sampling) and by collecting dead adults and estimation of eggs, larvae, pupae, and adults in the food patches (direct sampling). Beetle populations readily colonized the food patches, and overall populations of each life stage in the food patches were similar in the controls and in the 2- and 4-week aerosol treatments. However, the proportion of individuals in the egg and larval stages was greater in the control versus the aerosol treatments. There were more live adults trapped in the controls than in the aerosol treatments, with lower adult numbers in the two-week aerosol spray than in the four-week sprays, and more dead adults in the food patches in the control and 4-week spray than in the 2-week spray. Indirect sampling using pheromone traps gave consistent indications of aerosol efficacy, regardless of the extent of food patch colonization; however; the presence of the food patches allowed continued population development, and as a result the frequency of aerosol application had little impact on *T. castaneum* populations in the food patches.

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## 1. Introduction

The red flour beetle, *Tribolium castaneum* (Herbst), is a cosmopolitan stored-product insect that can infest raw grains, mills and processing plants, and finished food products (Rees, 2004). It is a major pest of flour mills in the United States, which have relied on fumigation of structures with methyl bromide as the primary management strategy (Campbell et al., 2010a,b). The worldwide phase-out of methyl bromide has led to increased research to examine and evaluate non-fumigant options for controlling *T. castaneum*, which include spray and aerosol applied insecticides. There are a number of laboratory studies that show pyrethroid insecticides such as cyfluthrin (Tempo®) will give residual control of adult *T. castaneum* (Arthur, 1998, 2000). Similarly, insect growth regulators such as hydroprene (Gentrol®) and pyriproxyfen (NyGard®) will give residual control through inhibition of the molting and developmental processes of immature stages of *T. castaneum* (Arthur, 2001; Arthur et al., 2009).

The presence of food sources can lead to increased survival of adult *T. castaneum* after they have been exposed to contact insecticides (Arthur, 2000). Food facilities also contain refugial areas where adult *T. castaneum* can escape exposure to a residual insecticide (Toews et al., 2005a,b). Insects may escape direct exposure because populations exist in inaccessible areas where insecticide applications do not reach or because only a small relative portion of the area inside a structure is treated (e.g., spot or crack and crevice applications). Because much of the insect population is in cryptic habitats, which may or may not be exposed to insecticides and which cannot be readily sampled, it is difficult to determine the efficacy of insecticide applications applied to structures (Arthur and Campbell, 2008). Recent studies with contact insecticides applied as surface treatments to limited areas document increased adult mortality and subsequent reduction in capture in traps, but limited control of resident populations of *T. castaneum* (Toews et al., 2005a,b, 2009).

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Insecticides applied as an aerosol can disperse throughout an area and may give more complete coverage than can be achieved with residual surface treatments. However, the ability of aerosol insecticides to impact populations or the cumulative impacts of multiple applications on populations has not previously been evaluated under controlled conditions. Different aerosol formulations of synergized pyrethrins have been shown to provide effective control of adult *T. castaneum*, but also show that the related species *Tribolium confusum* (Jacqueline DuVal), the confused flour beetle, is the more susceptible of the two species (Arthur, 2008). Toews et al. (2010) evaluated aerosol formulations of either synergized pyrethrins or the pyrethroid esfenvalerate (Conquer®) for direct mortality of different life stages of *T. castaneum*. In commercial food facilities, the presence of equipment, packages and structural features may hamper aerosol distribution, and thus provide refugial areas where insects can escape exposure (Arthur and Campbell, 2008). In addition, aerosol insecticides are often applied at regular intervals, so that the cumulative effect of repeated removal of a percentage of the population or the build up of insecticide residues needs to also be taken into consideration. The objective of the current study was to evaluate how the frequency of aerosol pyrethroid insecticide applications impacts *T. castaneum* population growth under simulated field conditions.

## 2. Materials and methods

This study was conducted in five wooden sheds that are part of the USDA-ARS Center for Grain and Animal Health Research (CGAHR), Manhattan, KS. Three of the sheds are 5.9 m long by 2.8 m wide by 2.0 m high (sheds 1–3) and two have slightly higher ceilings at 2.2 m high (sheds 4–5). The sheds were aligned east-west with the entry door at the west end, and each shed has a heat/air conditioning unit at the east end. The thermostat on the units was set at 25 °C, but actual air temperatures inside the shed generally ranged from 22 to 27 °C. The sheds have been used for a number of studies, and their construction and method of lining the interior has been previously described (Toews et al., 2005a,b, 2009). The exterior of the sheds consisted of framed white pine and plywood, had masonite exterior siding, and an asphalt shingled roof. Floors, walls, and ceilings of each shed were insulated and covered with interior plywood. The walls were also caulked and sealed and coated with food production grade paint primer and epoxy to seal cracks and crevices. Prior to the experiment, all interior surfaces of the sheds were covered with plastic sheeting (6 ml thick) and then the floor was covered with sheetrock. The panels on the floors were replaced, primed and painted to provide a finished floor surface, using commercial products as specified in Toews et al. (2009). Between each replicate of the experiment, plastic sheeting and floors were replaced to alleviate any residual contamination. Each shed contained three metal shelves (106.7 cm long by 44.3 cm wide by 11.6 cm tall) placed one each along the north and south sides about 2 m from the east end and 0.5 m from the walls, and one shelf placed midway along the east end about 0.5 m from the wall. A complete description of these shelves, along with figures showing placement of the shelves are provided in Toews et al. (2005a,b). Temperatures were monitored by placing a HOBO temperature-RH logger (Onset Computer Corporation, Bourne, MA, USA) in the approximate center of the floor of each shed. Twenty-four hours lights-on conditions were maintained using two 100-Watt incandescent bulbs mounted in the ceiling. These bulbs give 42.0 ± 2.6 lux ($n = 20$) at ground level (Toews et al., 2009).

The experiment was conducted using a *T. castaneum* strain originating from a flour mill in the central United States and established in laboratory cultures in 2001. The strain was reared on a diet of 95% unbleached whole-wheat flour and 5% Brewer's yeast, and reared under a 14–10 (L:D) photoperiod inside an incubator that maintained 27 ± 1 °C and 60 ± 2% relative humidity. Four food patches were placed in a row underneath each shelf and positioned about one meter from the wall. Each patch consisted of the bottom half of a plastic Petri dish (62 $cm^2$ in total area) containing 50 g flour and 17 each of 1-week old larvae, 3-week-old larvae, pupae, and adults. A strip of filter paper ca. 7.6 cm long and 1.9 cm wide, and folded at ca. 2.5 cm, was placed with the short end inside an individual dish and the long end touching the floor. The filter paper created a ramp to facilitate movement into and out of the food patches.

The insecticide used in the trials was the pyrethroid esfenvalerate (Conquer®, 3.5% active ingredient [AI], Paragon Professional Products, Memphis, TN, USA). Label directions for aerosol application of the product used in this study were to mix 296 ml of formulation in 3.8 L of oil solvent to give a 0.25% dilute spray, and apply this dilute spray at the rate of 29.6 ml/28.3 $m^3$. The oil solvent used for this test was Isopar M (Exxon Mobil Chemical Company, Houston, TX, USA). Given that the label is based on the volume of the space, and slightly different amounts of spray were applied to the different sized sheds. The applications were done by using a hand-held ultra low volume (ULV) mist sprayer (E2 MLDR Chemical Dispersal Unit, MicroGen Equipment Corporation, San Antonio, TX, USA). The sprayer flow rate was calibrated so that the smaller sheds were sprayed for 72 s and the larger sheds were sprayed for 85 s, which gave a target range of 30–35 g of insecticide dispensed for a single application. The person who applied the spray stood ~2 m inside the west end of the shed, held the sprayer at ~1 m off the ground, and operated the unit for the time intervals specified above.

The experiment was performed using two blocks. The first block was initiated on 14 November 2006 and the first spray treatment was done on 16 November 2006. In the first block, shed 1 was the untreated control, sheds 2 and 5 were sprayed every two weeks, and sheds 3 and 4 were sprayed every four weeks. The final spray for this first block was done on 29 March 2007. The final samples were taken on 26 April 2007, approximately 21 weeks after the start of experiment. Before the second block, the sheds were de-constructed and re-constructed as previously described. The second block was initiated on 23 June 2008 and the first spray performed on 25 June 2008. In this block, shed 2 was the untreated control, sheds 1 and 5 were sprayed every two weeks, and sheds 3 and 4 were sprayed every four weeks. The final spraying for this block was done on 15 October, and the final sampling was done on 29 October 2008, approximately 18 weeks after the start of experiment.

The sampling procedure was the same for both blocks, and consisted of weekly enumeration of the dead adults observed outside of food patches, sub-sampling of the beetle populations in the food patches, and indirectly monitoring adult activity using pheromone and kairomone baited traps. All dead adults on the floor of each shed and underneath the shelves were collected, counted, and discarded. Live adults in the sheds were monitored using plastic pitfall traps (Dome traps) baited with a commercial oil-based food attractant and an aggregation pheromone (Trécé Corporation, Adair, OK, USA) that were placed on floor in each corner of the sheds. The traps were sampled weekly, and captured adults were removed, counted, and discarded. To determine population levels in the flour food patches, a 1 g sample was taken from each of the four food patches underneath each shelf, and replaced with an equivalent amount of new flour. The four samples for each shelf were combined for analysis. The combined samples were weighed and sieved through a standard #60 mesh brass sieve (250 micron openings) to collect immatures and adults. The number of each developmental