Aerosol efficacy and direct and indirect exposure of flour beetles

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DOI: 10.14455/DOA.res.2014.137

Abstract

Aerosol insecticides are being used with increased frequency in the United States to control insects in flour mills, particular *Tribolium castaneum* (Herbst), the red flour beetle, and *Tribolium confusum* Jacqueline DuVal, the confused flour beetle. We conducted a series of studies assessing direct and indirect exposures of different life stages of both species to pyrethrin applied alone or with the insect growth regulator (IGR) methoprene. In studies with pyrethrin, direct exposures gave the most effective control. *Tribolium castaneum* was the more susceptible of the two species to both pyrethrin and methoprene. When either untreated flour was mixed with treated insects, or treated flour was mixed with untreated insects (indirect exposure) efficacy was reduced. In addition, adults and larvae were less susceptible than eggs and pupae. There is also evidence that flour treated with the IGR can be transferred to untreated areas, thereby increasing efficacy of the IGR. Field studies were also conducted and showed differences in the distribution of pyrethrin combined with either methoprene or the IGR pyriproxyfen, depending on the obstructions present in the site. Results show that differences among life stages of a particular target species, the target species itself, and the structural composition of flour mills may all contribute to efficacy of aerosol insecticides.

Keywords: aerosols, insecticides, control, distribution, stored-product insects

1. Introduction

Aerosol insecticides are being evaluated in the United States (US) as an alternative to fumigation with methyl bromide (Arthur, 2012; Boina and Subramanyam, 2011). These insecticides are usually generated from liquid formulations, and a mechanical device or generator is used to apply insecticide particles in a range of 5 to 50 microns (Peckman and Arthur, 2006). Results from outdoor applications with adult flying mosquitoes generally state that a range of 15 to 30 microns is considered optimal, however, most of the literature describing results with aerosols reports studies conducted outdoors, often against mosquitoes, and research with aerosols inside structures is lacking in comparison to those outdoor studies (Campbell et al., 2014; Arthur et al., 2015). Most of the recent studies in the US have been conducted with *Tribolium castaneum* (Herbst), the red flour beetle, or *Tribolium confusum* Jacqueline DuVal, the confused flour beetle, as the target pests. These two species are major pests of milling facilities in the US.

Common aerosols used in the United States include natural pyrethrin used alone or combined with either of the insect growth regulators methoprene or pyriproxyfen. Pyrethrin has little residual activity, while residues of the IGR methoprene are persistent when applied as an aerosol (Sutton et al., 2011). Also, there is some evidence of an additive effect of pyrethrin and methoprene when they are combined (Sutton et al., 2011). Several recent studies have indicated that pyrethrin or pyrethroid aerosols will kill adults of either *T. castaneum* or *T. confusum* (Arthur and Campbell, 2008). In addition, there is evidence that immatures of

both *T. castaneum* and *T. confusum* are more susceptible to pyrethrin aerosols compared to adults when directly exposed (Arthur, 2008).

There is ample evidence to suggest that when adults of either *T. castaneum* or *T. confusum* are provided with a food source during exposure survival increases (Arthur, 2008). In addition, the food source will also enhance recovery from knockdown if the food source is provided after exposure (Arthur, 2008). Similarly, resident populations may not be completely controlled by aerosol applications because even though adults may be killed, immature can escape exposure to the aerosols and the populations will persist. Toews et al. (2009, 2010) conducted studies in which experimental arenas containing flour food sources were inoculated with different life stages of *T. castaneum*. These arenas were then placed underneath artificial shelving inside small sheds that were regularly treated with aerosol resmethrin, a pyrethroid insecticide. Even though dead adults were constantly present in the sheds, the populations in the experimental arenas persisted throughout the treatment period. Similar results were obtained in a study with pyrethrin applied as an aerosol (Arthur et al., 2013).

Milling facilities present barriers to aerosol efficacy from equipment, structural barriers, or methods of aerosol application. This report summarizes selected data from previous studies to describe effects of barriers on aerosol dispersion and efficacy toward *T. confusum*.

2. Materials and Methods

2.1. Laboratory study

Kharel et al. (2015) conducted studies using boxes of different heights (5, 10, or 20 cm high, all 15 cm wide, and 1 m long) to create an artificial barrier for aerosol dispersal. These tests were conducted inside small sheds at the USDA-ARS Center for Grain and Animal Health Research (CGAHR) in Manhattan, Kansas, USA. These sheds had an approximate volume of 42 m^3. These boxes were then placed inside the 42 m^3 sheds. In brief, 5 adult *T. confusum* were placed in each of four 30 mm dia. plastic Petri dishes, along with about 50 mg of flour, in a row of four dishes at depths of 7.5, 36, 64, and 91 cm inside the boxes from the front opening.

The insecticide used for the study was a commercial formulation of 1% pyrethrin aerosol (Entech Systems, Kenner, LA, USA). The insecticide label for the pyrethrin formulation specifies application at 30 ml of formulation per 28 m^3, hence 45 ml of the aerosol was applied to these sheds with a volume of 42 m^3, using a hand-held applicator to dispense the insecticide. These adults were then exposed for two hours, then removed from the shed and placed inside an incubator set at 27°C and 60% r.h. Tests were done at three target exposure temperatures, 22, 27, and 32°C but only the data for 27° C will be reported here. Adults were assessed at 2, 7, and 15 days post-exposure classifying beetles as active, affected (on their backs but still exhibiting movement), or dead. Final mortality data for the 15-day post-exposure assessment will be reported here.

2.2. Field study

This study was conducted at the Hal Ross Flour Mill at Kansas State University in Manhattan, Kansas, USA. A complete description of the methodology is given in Campbell et al. (2014). Three floors of the mill were used for these tests, which involved three different insecticides: pyrethrin plus either of the IGRs pyriproxyfen (Nygard®) or methoprene (Diacon II®), and also the organophosphate dichlorvos (Vap20®). All insecticides were applied by a commercial applicator using the label rates for each product.

The boxes described above were placed in an open area on each of the three floors, with 5 adult five adult *T. castaneum* exposed in a row of four individual 30 mm Petri dishes

underneath the three different boxes at the positions described for the laboratory study. All test insects were obtained from colonies maintained at CGAHR. In addition, a grid of 65 Petri dishes were placed throughout each of the three floors in positions considered to be open to the aerosols, adjacent to an obstruction, or underneath a piece of milling equipment (covered). The beetles were exposed for different time periods, depending on the particular aerosol. Untreated controls were placed on the first floor of the mill. After the exposures were completed, the beetles were removed from the mill and transported back to the lab at CGAHR. Assessments were done at 24 and 48 hours and at 1 and 2 weeks by classifying beetles as active, affected, or dead. An efficacy index was developed ranging from 1, the weakest response with all 5 beetles active, to 21, all 5 beetles dead. This index is described in detail in Campbell et al. (2014). Data reported here are only for the 2-week final assessments for the treatments, as control mortality was negligible.

3. Results and Discussion

3.1. Laboratory study

Mortality (y-axis) of adult *T. confusum* at the open position in front of the box was at least 80%, and general mortality decreased with increasing distance from the front of the box and with decreasing height of the box (Fig. 1). It was clear that the aerosol did not disperse throughout most of the area defined by the different box heights. Previous studies inside these same sheds showed excellent overall efficacy of the same pyrethrin applied in the same manner against different life stages of *T. castaneum* and *T. confusum* (Kharel et al., 2014ab). Different life stages of *T. castaneum* and *T. confusum* were exposed to the same aerosol formulation using the same application method. The insects were exposed for 2 hours, then removed from the sheds and placed on flour untreated flour (direct exposure), versus treating the flour and then exposing the adults (indirect exposure). Adult mortality was greatly reduced in the indirect exposure compared to the direct exposure, and more immatures successfully developed to the adult stage in the indirect compared to the direct exposures. When flour and insects were both exposed to the aerosol, the flour provided a means where mobile larvae and adults apparently escaped exposure to the aerosol. In general, there is also more evidence of positive effects of pyrethrin through direct exposure (Kharel et al., 2014ab).

3.2. Field study

The results in the field trials for pyrethrin + pyriproxyfen (NyGard) and pyrethrin + methoprene (Diacon II) in the box exposure portion of the study followed same pattern as those obtained for the laboratory. Results for pyrthein plus NyGard and pyrethrin plus Diacon were similar so only the data for pyrethrin plus Diacon are reported here. The efficacy of the aerosol, as measured by the index, decreased with decreasing box height and increased distance from the front open end of the box (Fig. 2). The IGRs do not kill adults, so the direct efficacy on the adults was attributable to the pyrethrin component of the mixture. Efficacy for diclorvos was the highest value of 21 for all but the exposure positions farthest from the open end of the 5-cm high box. Dichlorvos has excellent vapor toxicity and disperses well but gives no residual control (Subramanyam et al., 2014). The summarized data for the positions in the floor of the mill also show effects of structural composition on efficacy, particularly for pyrethrin plus pyriproxyfen (Fig. 3).

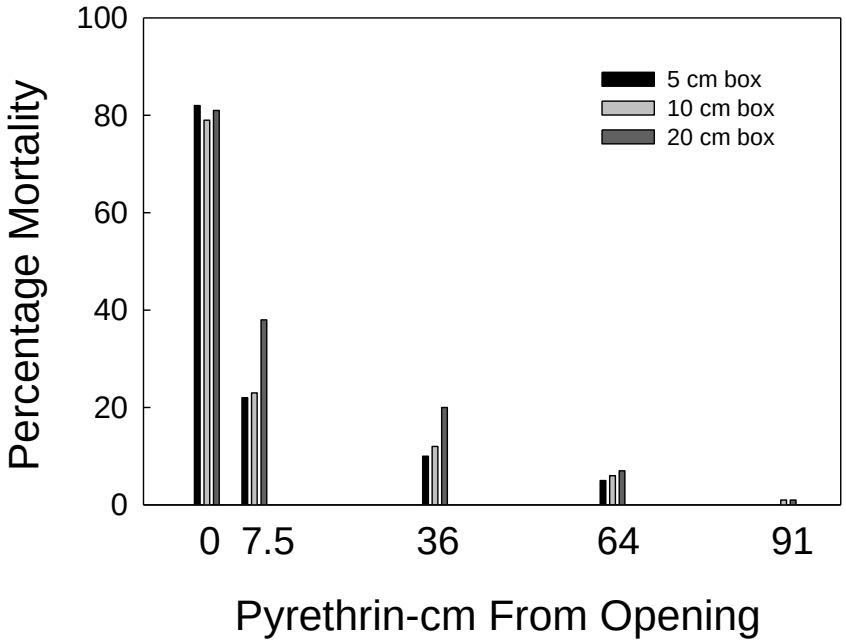

Figure 1 Final 15-day mortality (% mean ±SE) of adult *T. castaneum* exposed to 1% pyrethrin aerosol in front of experimental boxes that were either 5, 10, or 20 cm in height, 15 cm wide, and 1 m long. Insects were exposed in front of the box and at different distances from the front opening inside the box. Complete details in Kharel et al. (2015).

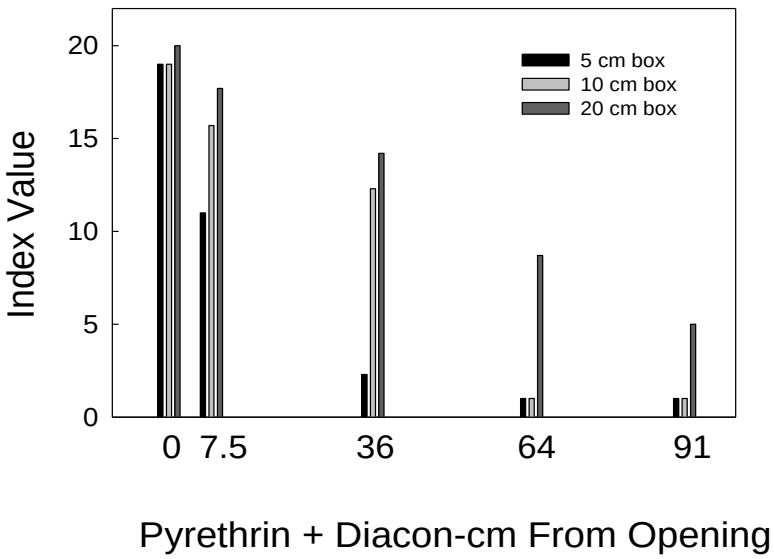

Figure 2 Index values ranging from complete survival (1) to complete mortality (21) of twenty adult *T. castaneum* exposed to three aerosols, pyrethrin + pyriproxyfen (NyGard®, Py + NyGard ; pyrethrin + methoprene (Diacon II®, P + Diacon), or the organophosphate dichlorvos. The complete index is explained in Campbell et al. (2014, see Reference). The beetles were exposed inside the boxes and positions described above.

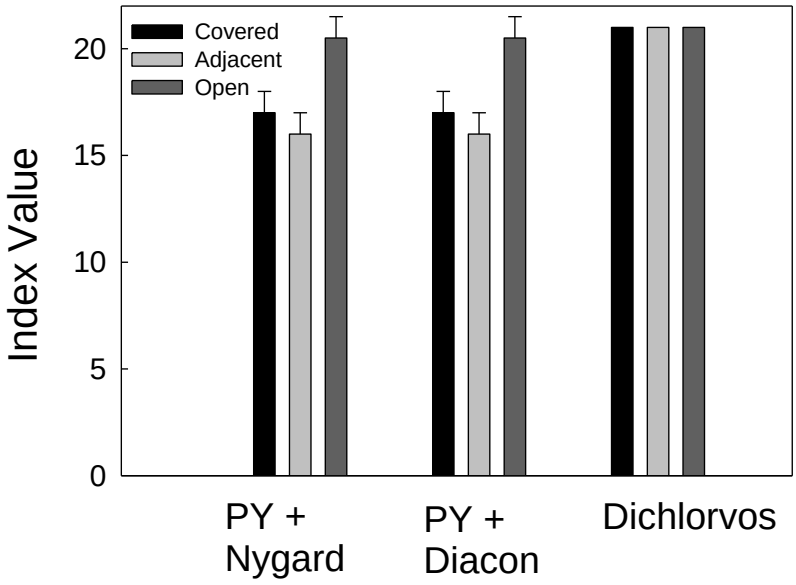

Figure 3 Index values ranging from complete survival (1) to complete mortality (21) of adult *T. castaneum* exposed to three aerosols, pyrethrin + pyriproxyfen (NyGard®, Py + NyGard ; pyrethrin + methoprene (Diacon II®, P + Diacon), or the organophosphate dichlorvos. The same index described above was used to assess efficacy. Beetles were exposed inside a flour mill in either open, adjacent to obstructions, or covered by milling equipment.

4. Conclusions

Flour mills, production plants, and food warehouses present different challenges for stored product insect pest management. Laboratory and field studies show that aerosols will give control of *T. castaneum* and *T. confusum*, but the level of control is dependent upon several factors, including the specific aerosol, the insect species, and the life stage of that species. The presence of food material either during or after adult flour beetles are exposed to aerosol can lead to increased recovery from exposure. The structural complexity of a mill or warehouse can also affect efficacy, especially when barriers to aerosol dispersion are present.

Acknowledgments

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