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ScienceDirect
Journal of Stored Products Research 44 (2008) 58–64
Journal of STORED PRODUCTS RESEARCH
www.elsevier.com/locate/jspr

Distribution and efficacy of pyrethrin aerosol to control *Tribolium confusum* (Coleoptera: Tenebrionidae) in food storage facilities ☆

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Accepted 4 April 2007

Abstract

A field test was conducted whereby adult *Tribolium confusum* (du Val), the confused flour beetle, were exposed in Petri dishes lined with filter paper and containing 0–2000 mg of flour, to a pyrethrin–CO_2 aerosol inside an empty warehouse. Applications were made according to label directions, and beetles were exposed to the aerosol for 2 h. After exposure, knockdown was assessed, and beetles were held for 2 weeks either in the same Petri dishes in which they were exposed or transferred with the flour to new Petri dishes lined with clean filter paper. Resident insect populations inside the test warehouse and outside were assessed through the use of pheromone traps. Virtually all beetles were knocked down at the time of removal from the exposure environment. Recovery from knockdown, or survival, increased with the presence of flour, and varied depending on the position of the exposure dishes inside the warehouse. Survival was also greater for beetles transferred along with the exposed flour to new Petri dishes lined with unexposed filter paper, compared to beetles that were held with the flour and the Petri dish that was exposed to the aerosol. Inside the warehouse, *Plodia interpunctella* (Hübner), the Indian meal moth, and *Trogoderma variabile* Ballion, the warehouse beetle, were the major species collected in pheromone traps. Large numbers of *T. variabile* were captured outside the warehouse, but their numbers declined during the study, while captures of *P. interpunctella* outside remained relatively constant. The intermittent aerosol treatments did not seem to affect these resident populations.
Published by Elsevier Ltd.

Keywords: Aerosols; Tribolium; Control; Storage

1. Introduction

Aerosol space treatments are often used to control insects inside flour mills, food warehouses, and other indoor storage facilities. Space treatments can be defined as "the dispersal of insecticides into the air by foggers, misters, aerosol devices, or vapor dispensers to kill exposed adults or crawling insects" (Peckman and Arthur, 2006). Although there are several commercial aerosols that can be used inside food warehouses to control stored-product beetles, there is often little published information regarding efficacy of these aerosols, or how they can be distributed inside a given facility. Synergized pyrethrins and the organophosphate dichlorvos are two insecticides that have historically been used for aerosol applications inside processing facilities and food warehouses. More recently, new pyrethroid and insect growth regulators have been registered as aerosols for use against stored-product insects, but most documented reports of efficacy describe tests done under laboratory conditions (Arthur and Gillenwater, 1990; Arthur, 1993). Most of the available field data on aerosols are from older studies with the organophosphate dichlorvos (Childs, 1967; Gillenwater et al., 1971; Cogburn and Simonaitis, 1975; Kirkpatrick and Gillenwater, 1979, 1981), not with the newer reduced-risk formulations and application systems that are currently on the market.

A diverse community of insect pests is associated with food facilities and is the target of an aerosol treatment. Two of the major beetle pests of stored and processed food

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0022-474X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.jspr.2007.04.001

are *Tribolium castaneum* (Herbst), the red flour beetle, and, *Tribolium confusum* (du Val), the confused flour beetle. Both of these species are generally harder to kill with aerosols and contact insecticides than other stored-product beetles, and have been used as indicator species in different laboratory research trials (Arthur, 1988, 2000a; Arthur and Puterka, 2002). In addition, laboratory tests have shown that the presence of food material, either during or after exposure to an insecticide, often leads to an increase in survival of *T. castaneum* and *T. confusum* (Arthur, 2000b, c).

There are few published results in scientific journals regarding field-scale trials of aerosol insecticides, especially within the last couple of decades. Part of the difficulty is finding available research sites to conduct replicated tests. We were able to secure an empty warehouse in a non-operational commercial food facility as a test location for the work reported here. The objectives of our study were to determine: (1) the susceptibility of adult *T. confusum* to a commercial pyrethrin aerosol, (2) the effects of food material on aerosol efficacy, and (3) the spatial pattern of efficacy within the warehouse. As an ancillary objective, the impact of the treatments on resident pest populations within the warehouse was also assessed using pheromone traps.

2. Materials and methods

This study was conducted in an empty warehouse in Kansas City, MO. The warehouse was approximately 21.0 m along the south and north walls, 45.5 m along the east and west walls, and 12.3 m high, with a volume of about 11,753 m^3. The warehouse was largely empty except for some small piles of debris scattered on the floor. At the south end were several bay doors that were used for loading and unloading trucks, and a pedestrian entry door at the southwest corner. At the north end there was a pedestrian door to the outside, and along the west side there were two doorways to an adjacent warehouse. These openings were sealed with plastic prior to application of the aerosol. An aerosol application system with two dispensing nozzles, approximately 12.3 and 27.7 m from the south wall, was installed in the center below the roofline, approximately 12 m off the ground.

Exposure dishes were prepared using standard 100×15 mm plastic Petri dishes, with the bottoms lined with 9 cm filter paper positioned in the dish and creased along the edge. Dishes were filled with 0, 250, 500, 1000, or 2000 mg of whole-wheat flour, and 10 one- to two-week-old adult *T. confusum* were placed in each dish. There were 36 dishes per flour quantity or 210 dishes in total. These beetles were obtained from pesticide-susceptible colonies maintained at the Grain Marketing and Production Research Center, Manhattan, KS. All dishes were held overnight in a laboratory at approximately 27 °C, 50% relative humidity, and were transported by car the next morning to the field site.

Upon arrival at the field site, the dishes containing the various flour treatments were placed on the floor of the warehouse in the following manner. Eighteen locations were selected, a row of six along each sidewall and one row down the center. Each row started in the corners, with one dish in the center, and the remaining locations spaced approximately 9.2 m apart in between the two end points. At each location, a piece of cardboard measuring 0.61×0.46 m was placed on the floor, and two complete sets of dishes containing the five concentrations of flour (0–2000 mg) were randomly placed on the cardboard. The lid of each dish was removed and placed on the cardboard open side up, and the bottom of the dish that contained the flour and insects was put inside the lid. Control locations not exposed to aerosol treatment were setup in an adjacent warehouse. Three locations were used: one on each wall and one in the center of the room, and the dishes were setup in the same way as in the treatment warehouse (10 dishes at each point, 2 dishes with each concentration of flour, 30 in total). A separate control group of 21 dishes with beetles and no flour, which corresponded with each of the 18 points in the warehouse and the three points in the adjoining room, was placed outside the warehouse, underneath a small flight of stairs.

The aerosol applied in this study was cylinderized Aerotech®75, 0.7% pyrethrins, 5.0% piperonyl butoxide synergist, and 94.3% inert ingredients, with CO_2 propellant to facilitate dispersion. The warehouse doors were shut and the opening between the warehouse and the adjoining room was sealed with polyethylene plastic. The actual amount by weight of aerosol that was needed for the total volume of the room (11,753 m^3) was approximately 3178 g, or 270 mg/m^3. The aerosol cylinder was put on a scale, and once the required 3178 g was dispensed the system was turned off. The insects were exposed to the aerosol for 2 h, at which time a person equipped with a respirator entered the room, collected the Petri dishes, and took them into an adjoining room where there was no exposure to the aerosol. This process was done in a series of collections, i.e. the Petri dishes were collected onto a cart, brought into the room, laid on a table, and then the person went back into the room to collect more dishes. This entire process took about 15 min.

As the beetles were brought into this adjoining room, all individuals in each dish from the two sets of five dishes were visually classified as either knocked down (on their backs and incapable of extended movement) or running (upright and mobile). Beetles and flour from one set of the exposed dishes were then transferred to new Petri dishes lined with clean filter paper. After the beetles were transferred, the dishes in which they were originally exposed were discarded. The other set of beetles were held in the same dishes in which they were exposed. Knockdown was also assessed for the beetles in each of the 21 dishes placed outside the testing room (untreated controls) in the adjacent room and outside the building using the same procedure.

All individual dishes were put in cardboard boxes, covered, and returned to the laboratory in Manhattan, and held on an open counter in the same room in which they were prepared. The percentage of active beetles was assessed again at 1, 2, 3, 7, and 14 d after the beetles had been removed from the warehouse. For all of these assessments, those adults that were mobile and running were considered to have "survived" exposure.

Four separate trials were conducted on 23 and 30 September, and 7 and 14 October 2003, and all procedures for each replicate were as described above. Statistical analyses were conducted with the General Linear Models (GLM) procedures of the Statistical Analysis System (SAS Institute, 2001) to determine significance for main effects, i.e. quantity of flour, beetles transferred to new dishes or not, and the percentage of active beetles at 1, 2, 3, 7 and 14 d post-treatment. The percentage of active beetles at the 18 positions within the test warehouse were averaged into weekly values for the statistical analysis since they are not independent replicates, and the time assessments were treated as a repeated measure because all observations were made on the same set of dishes. Data for percentage of active beetles for the flour treatments were plotted using equations generated from Table Curve 2-D software (SYSTAT Software, Richmond, CA, USA). Data for the treatment no flour, beetles not transferred to new dishes,

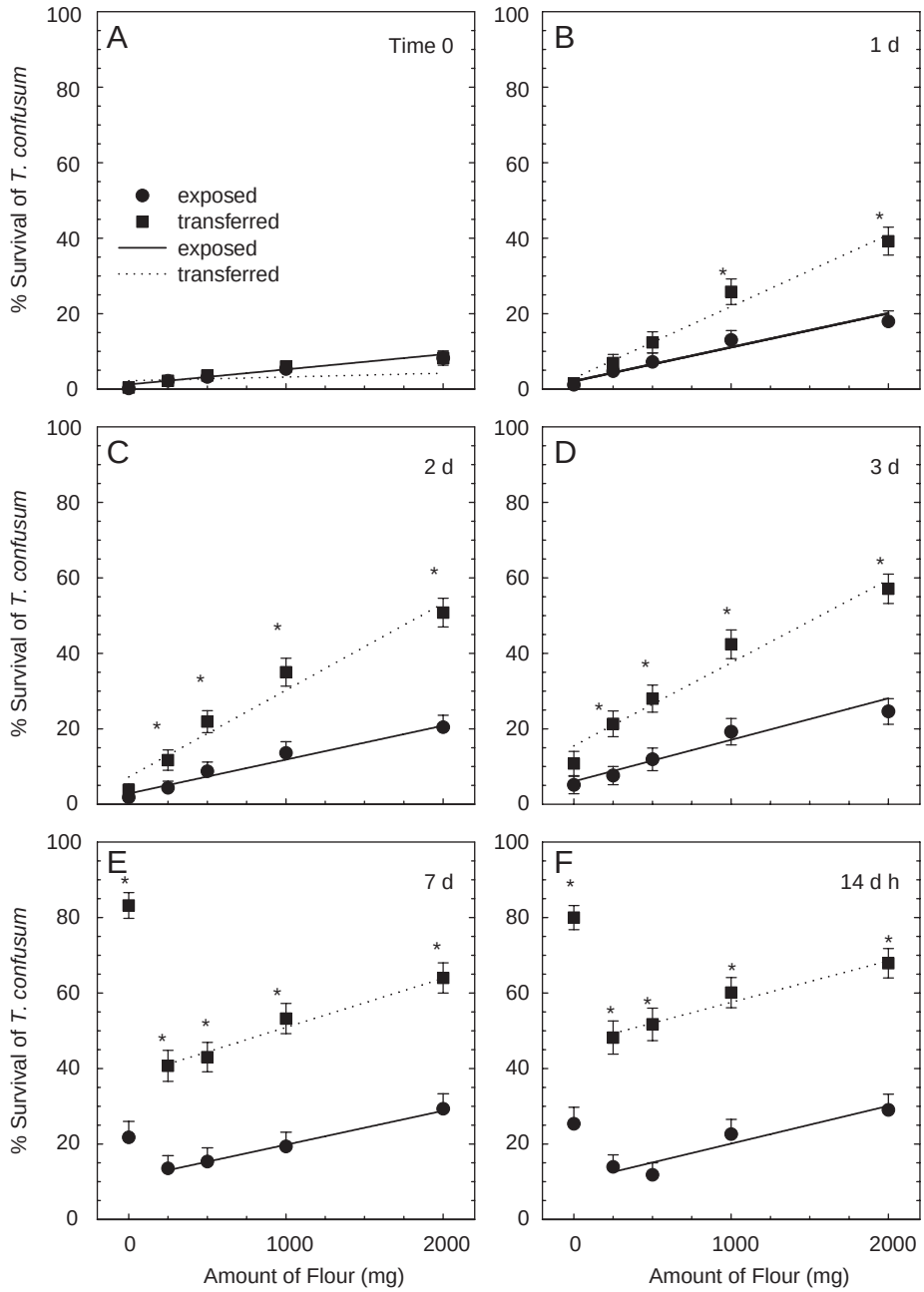

Fig. 1. Percentage survival (mean ± SEM) of adult *T. confusum*, exposed with 0–2000 mg of flour, and either held in the original dishes in which they were exposed to pyrethrin aerosol or transferred to new dishes. Survival assessments made at 0, 1, 2, 3, 7, and 14 d post-exposure.

were used to assess efficacy patterns among the 18 positions in the warehouse. Surfer software (Golden Software, Golden, CO, USA) was used to make contour plots at each post-treatment interval.

To evaluate the resident pest populations, pheromone traps were placed inside the warehouse and outside around the perimeter of the building. Outside the facility, Delta traps (Scentry Biologicals Inc., Billings, MT; or Trécé Inc., Adair, OK) were placed approximately 1 m off the ground. This trap type is triangle shaped (10 cm wide per side) at the ends and 18 cm long with a sticky surface on the three interior walls (540 cm^2 trapping surface). Delta traps were baited with two lures: IMM + 4 pheromone lure (Trécé Inc., Adair, OK) that attracts four pyralid moth species, although *Plodia interpunctella* (Hübner), the Indian meal moth, was the only species captured at this site, and *Trogoderma* spp. (*Trogoderma variabile* Ballion and *Trogoderma granarium* Everts) pheromone lure. Eight traps were placed outside and were attached to the sides of the building at evenly spaced intervals to completely surround the structure.

Inside the warehouse, two types of pheromone traps were used: Dome traps and Pherocon II traps (Trécé Inc., Adair, OK). Dome traps are pitfall traps with a pheromone lure for *T. castaneum* and *T. confusum* suspended over the trap and the bottom of the pitfall contained a food oil attractant. Dome traps, which were placed on the floor, are designed to capture walking insects. Pherocon II traps have roughly diamond-shaped openings at both ends with the interior of the trap having a sticky surface (280 cm^2). This type of trap is designed to capture flying insects and traps were suspended between 1.5 and 2.1 m off the floor. In the Pherocon II traps, lures for *Trogoderma* spp. and *P. interpunctella* were used (Trécé Inc., Adair, OK). Twelve locations were used in the warehouse with both trap types at each location: three rows of four evenly spaced traps along the north and south walls and down the center of the warehouse.

3. Results

Survival of untreated controls during the time of exposure and the 2-week post-exposure intervals was virtually 100% and no corrections or adjustments were necessary for control mortality. These data were therefore eliminated from the statistical analysis. The two main effects "amount of flour" and "removing the *T. confusum* adults from the exposure dishes versus not transferring them to new dishes", plus the repeated measure "time", were all significant at $P<0.01$ ($F=81.1$, d.f. = 4,30; $F=654.2$, d.f. = 1,30; $F=217.9$, d.f. = 5,20; respectively). All associated interactions were also significant at $P<0.01$. Regression equations were used to fit straight lines to the data (Fig. 1(A–F); Table 1) for the percentage of beetles that had survived the aerosol exposure at each post-treatment assessment, with the amount of flour as the independent variable and the percentage of survivors either in the original dish in which they were exposed or in the untreated dishes to which they were transferred as the dependent variable. The regression equations explained between 88.8% and 99.6% of the variation that could be obtained from any model fitted to the data set.

Table 1
Equation parameters (mean ± SE) for linear equations $y=a+bx$, where y = percentage active *T. confusum* 0, 1, 2, 3, 7, and 14 d after exposure to pyrethrin aerosol in Petri dishes with 0–2000 mg of flour (x)

Days	a	b	Max R^2	R^2	% R^2
Not transferred					
0	–[a]	0.003 ± 0.001	0.294	0.287	97.6
1	–[a]	0.009 ± 0.002	0.524	0.498	95.0
2	–[a]	0.009 ± 0.002	0.552	0.535	96.9
3	6.01 ± 2.24	0.011 ± 0.002	0.601	0.575	95.7
7	10.8 ± 2.78	0.009 ± 0.002	0.507	0.505	99.6
14	10.1 ± 3.76[b]	0.010 ± 0.003	0.447	0.397	88.8
Transferred					
0	–[a]	0.003 ± 0.001	0.294	0.287	97.6
1	–[a]	0.019 ± 0.004	0.571	0.558	97.7
2	–[a]	0.023 ± 0.004	0.701	0.675	96.3
3	15.5 ± 4.74	0.022 ± 0.005	0.590	0.564	95.6
7	37.8 ± 3.66	0.013 ± 0.003	0.568	0.559	98.4
14	46.5 ± 4.01[b]	0.010 ± 0.003	0.429	0.415	96.7

Beetles were either held in the same dishes in which they were exposed (not transferred) or transferred to untreated dishes after they were exposed. Maximum R^2 (Max R^2) for any equation fitted to the data, R^2 values of the linear equations, and R^2 of each equation as a percentage of the maximum (% R^2).

[a]Regression through origin of 0,0.

[b]Regression through origin of 250 mg on the x-axis.

Initial knockdown was at least 99%, but *T. confusum* adults began to recover from knockdown at each post-exposure assessment until recovery leveled off at 7 and 14 d. As the amount of flour increased in both sets of exposure dishes, the percentage survivors generally increased as well, indicating that the presence of the flour food source could have compromised the effectiveness of the pyrethrin aerosol when the beetles were directly exposed to the aerosol (Fig. 1(A–F)). However, when the beetles along with the flour in which they were exposed were transferred to new dishes (Fig. 1(A–F), solid line and circles), survival in those dishes was less than in the dishes in which the beetles were exposed and then held along with the flour (Fig. 1(A–F), dashed line and squares), possibly because the beetles in those original exposure dishes would continue to be affected by residues from the surface of the filter paper that were being absorbed by the flour during the 2-week holding period. At 2 d or later, at least 4 of 5 possible comparisons indicated significant differences ($P<0.5$, PROC t-test, indicated by an asterisk). The excessively high percentage of survivors in the treatments with no flour and where the exposed adults were transferred to new dishes, as compared to survival where beetles were exposed to flour and transferred, could possibly result from residue accumulation in the flour.

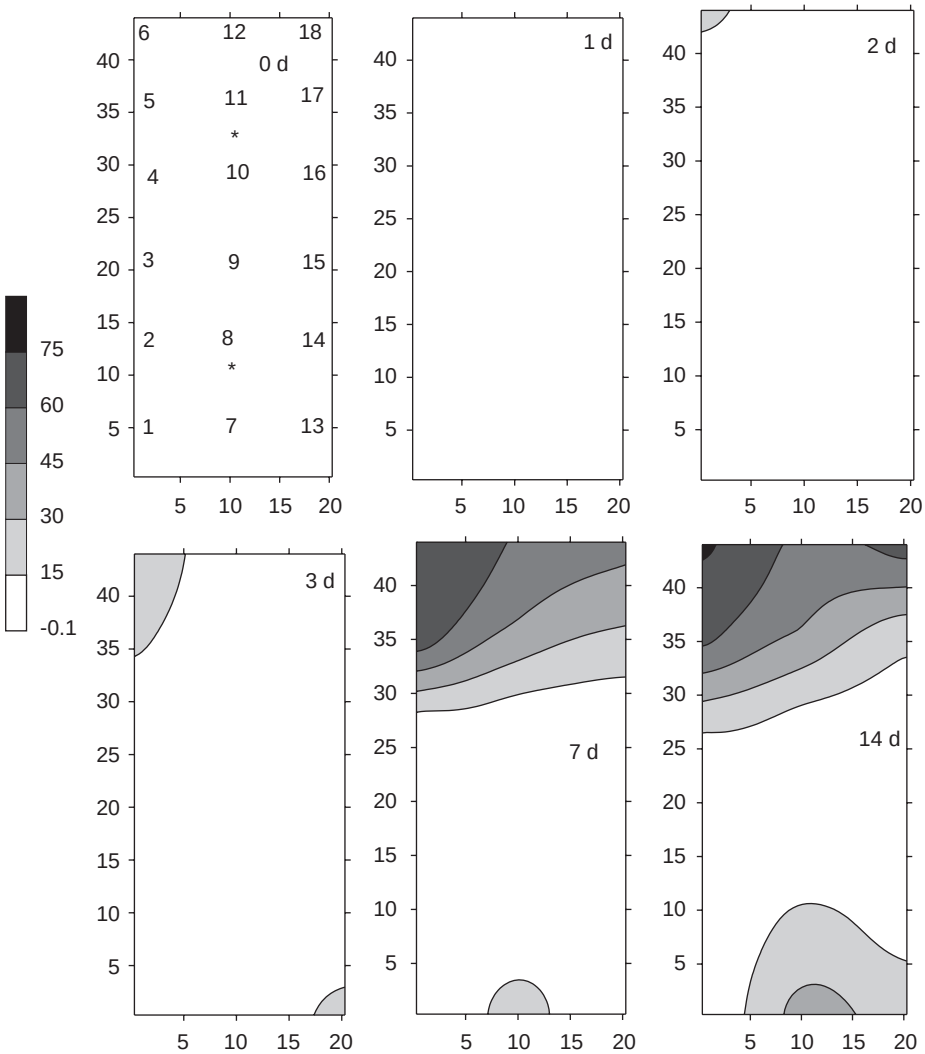

Fig. 2. Percentage of *T. confusum* survival 0, 1, 2, 3, 7, and 14 d after exposure to pyrethrin aerosol and held without flour in the Petri dishes in which they were exposed. Numbers 1–18 are the location of exposure dishes, Asterisks (*) indicate nozzle positions, *x* and *y* axes are warehouse dimensions in meters.

The variation in efficacy among the 18 exposure positions in the warehouse is illustrated by the data for no flour, among insects held in the same dishes in which they were exposed (Fig. 2). Data show that recovery and eventual survival and activity in the back rear of the warehouse were greater than in the front portion. Approximately 6–80% of the adults in dishes that were set in positions 5, 6, 11, 12, 17, and 18 were classified as having survived the exposure at the 1-week assessment, which indicated considerable recovery from knockdown. During each of the individual replicate tests, the aerosol fog was directed toward the front of the room, and the percentage of survivors in these dishes in front positions 1, 2, 7, 8, 13, and 14 was generally $<20\%$.

Results from the pheromone monitoring indicated that pest levels within the warehouse were relatively low. In the Dome traps, only two *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle, were captured. *Plodia interpunctella* and *T. variabile* were the major species trapped inside the warehouse in the Pherocon II traps and their numbers tended to decline over the course of the study (Fig. 3). Outside the building large numbers of *T. variabile* were captured, but their numbers had also declined by the end of the study (Fig. 3). *Plodia interpunctella* captures outside the facility were low, but were relatively stable over the course of the study.

4. Discussion

The results of this study with pyrethrin aerosol show similar results to those obtained from trials with residual pyrethroid surface treatments: the presence of food materials often compromises the efficacy of insecticides (Arthur, 2000a, b). Sanitation and cleaning are considered to reduce insect pest populations in stored-product environments by eliminating food material that would support larval growth and development (Loschiavo and Okumura, 1979; Cuperus et al., 1990; Platt et al., 1998;

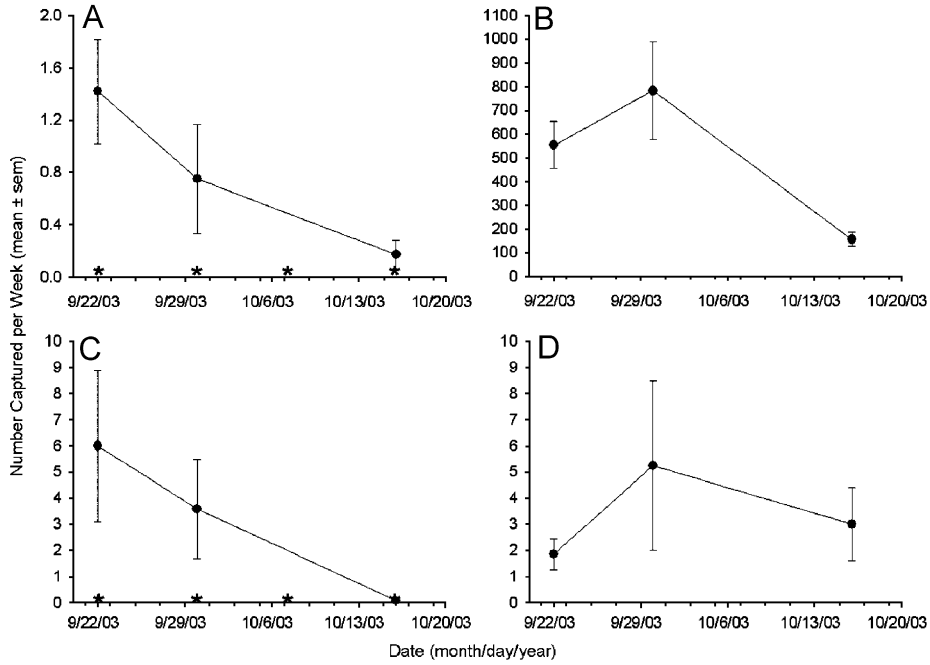

Fig. 3. Number of *Trogoderma variabile* and *Plodia interpunctella* captured in pheromone traps both inside (A and C, respectively) and outside the warehouse (B and D, respectively) over the course of the aerosol treatments in the warehouse. Asterisks (*) indicate the dates of aerosol application.

Arthur and Phillips, 2003). However, it is often difficult to correlate the effects of sanitation and cleaning in commercial storage facilities with insect control. Studies conducted in commercial grain elevators document extensive insect populations in spilled grain, residual grain inside elevator silos, and accumulated material throughout the elevator facility, but it is difficult to relate these populations to those found inside the silo because of the huge difference in the volume of grain in silos versus the areas with spillage and accumulation (Reed et al., 2003; Arthur et al., 2006). However, in food processing facilities the impact of pests persisting in residual material and moving into commodities is likely to be more important (Arbogast et al., 2002; Campbell and Arthur, 2007).

Our study also showed recovery of *T. confusum* from knockdown by the pyrethrin aerosol, which has also been documented in laboratory studies (Arthur, 1988, 1993). In this test, the material was applied at the label rate, in accordance with standard procedures, yet control was not complete. This could relate to the relative tolerance of *T. confusum* to aerosols and other insecticides, as shown in other studies (Arthur, 2000a, b), or to the practical difficulties of insect control in large storage facilities. However, there are no recent field tests where comparable data were obtained for the aerosol formulation and system used in our trials, and more data are needed before making any conclusions regarding efficacy of the specific aerosol tested in our study. Some laboratory studies show an increase in final mortality of *T. confusum* and other stored-product insects when additional aerosol applications are made immediately after the initial application, while adults are at the stage where they are knocked down by the aerosol (Arthur, 1993). However, this effect has not been verified in field trials.

With more emphasis on targeted control, sanitation should be emphasized when using pesticides with less penetration ability than fumigants and reduced insecticide applications as part of integrated management programs. This is particularly important because flour mills, processing plants, and food warehouses contain structural components that provide sites where insect exposure to aerosols could be either reduced or minimized. Examples of these areas include areas underneath wooden pallets, pieces of equipment, and storage shelves. There are no studies, which show the penetration of aerosols and their efficacy on infestations that might occur in these obstructed areas. Our study was conducted in an empty warehouse, and all exposure positions were in the open, which provided maximum exposure to the aerosol.

The presence of stored-product insects in and around storage facilities has been documented in a number of relatively recent field studies (Doud and Phillips, 2000; Arbogast et al., 2002; Campbell and Arbogast, 2004; Campbell and Mullen, 2004). Immigration of insects into areas where management tactics are targeted can impact the long-term effectiveness of a treatment. Therefore, when using pheromone traps to monitor the effectiveness of insecticide treatment, it is important to consider the overall population at the site, not just in the area targeted for insecticide treatment. Even when whole-plant treatments such as fumigation and heat are used to disinfest the facility, data from pheromone traps show a sharp decline and often a quick rebound and recovery to levels that existed before the treatment (Roesli et al., 2003; Campbell and Arbogast,

2004). *Trogoderma variabile* is a mobile species that is often found in large numbers in the outside environment around flour mills and food-storage facilities (Campbell and Mullen, 2004). In our tests, *T. variabile* numbers inside the test room receiving the aerosol applications tended to decline, but a similar pattern was observed outside. This decline could be due to the tendency for pest flight activity to decline in the fall due to decreasing temperatures and/or the onset of diapause. *Plodia interpunctella* trap captures also declined in the warehouse during the study, but few moths were captured outside. Interestingly, the outside captures did not dramatically change over the course of the study. It is difficult to draw conclusions from single trapping studies because populations are dynamic even in the absence of treatments, but our findings are encouraging in that they suggest that repeated applications could impact populations.

Acknowledgments

The authors thank J. Vardeman for technical assistance with the study, and the Industrial Fumigants Company for providing the testing facility. We also thank G. Daglish and V. Stejskal for reviewing the publication prior to journal submission.

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