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Aerosol concentration, deposition, particle size, and exposure interval as mortality factors *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae)

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ABSTRACT

A series of trials was conducted for an experiment. In the first trial, adults of *Tribolium confusum* Jacquelin du Val, the confused flour beetle, were exposed on concrete arenas and treated with a combination aerosol of pyrethrin + methoprene dispensed for 5, 10, and 20 min at particle sizes of 4, 8, 12, and 16 μm, inside an aerosol exposure chamber. Nearly all adult *T. confusum* were knocked down when immediately removed from the chamber. Among all the exposure time and particle size combinations, recovery increased as the post-exposure holding period increased from one to seven days and when adults were transferred to untreated dishes with flour. A second experiment evaluated the residual effect of the aerosol on concrete arenas at 1, 3, and 6 weeks using 3-4-week-old larvae of *T. confusum*. Adult emergence of exposed larvae decreased with increasing particle size and exposure time. A biological index that assessed development of exposed larvae to the pupal and adult stages was also related to particle size and exposure interval, and this index was correlated with adult emergence. A third set of experiments investigated effects of particle size on adult fecundity for the 10-min exposure time. Male and female adults were cross-mated: exposed female with exposed male, exposed female with unexposed male, exposed male with unexposed female, and unexposed female and unexposed male. Progeny production was reduced as particle size increased, and there were indications that females were affected more than males by the aerosol treatment. This research could be used to improve insect pest management programs by adjusting application equipment to dispense aerosols at particle sizes that give optimum control of exposed adults and residual control of immatures and would also benefit pest management programs.

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1. Introduction

An aerosol is a type of insecticides that are pressurized, atomized, and dispensed through an application system to give particle sizes ranging from hundreds of microns in size when dispensed from aircraft down to a range of about 20–50 μm when used in outdoor applications for mosquito control (Bonds, 2012). The effective range for aerosol use inside mills and warehouses to control stored product insects is generally much lower (Peckman and Arthur, 2006), and recent field testing has shown that the actual application rates for aerosols dispensed inside structures is in the range of 10–20 μm (Arthur et al., 2018). With the phase-out of the fumigant methyl bromide in the United States (US), aerosols are receiving increased attention for incorporation into pest management programs as spatial treatments inside mills and warehouses (Arthur, 2012; 2015; Boina and Subramanyam, 2012; Subramanyam et al., 2014). Unlike fumigants, aerosols do not penetrate through substances such as packaging material or large volumes of grain spillage. Aerosols only give immediate control of insects that come into direct contact during the treatment but will give control of juvenile stages through residual deposition of an insect growth regulator, IGR, when incorporated as a component in the aerosol insecticide mixture.

One common aerosol insecticide used in the US is synergized pyrethrins, and there have been several recent studies that examined efficacy toward different stored product insects and life stages,

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including *Tribolium confusum* Jacquelin du Val, the confused flour beetle (Arthur, 2008; Arthur and Campbell, 2008; Tucker et al., 2014). Incorporating an IGR along with synergized pryrethrins such as methoprene or pyriproxyfen, will contribute a residual aspect that controls immature stages of *T. confusum* and other stored product insects through deposition of residues on a treated surface (Arthur et al., 2014). When aerosols are dispensed in warehouses or milling facilities, the structural configurations will present obstacles to even dispersal of the aerosol particles, as shown by Campbell et al. (2014) and Scheff et al. (2018b), who examined distribution of pyrethrin combined with either methoprene or pyriproxyfen inside a pilot scale flour mill. Areas of the mill that were the farthest away from location where the aerosol was dispensed and/or areas blocked by large equipment generally received less insecticide, as assessed through knockdown (adult beetles on their backs and unable to right themselves or walk) and recovery of exposed adult *T. confusum*, compared to open areas closer to the application location (Campbell et al., 2014; Scheff et al., 2018b). In a study using TSI Aerodynamic Particle Sizers (APS Units, TSI Inc., Shoreview MN, USA) to measure aerosol particle size (reported by the APS Unit as Geometric Mean Diameter) and concentration, as the distance from the point of release of the aerosol application increased there was a corresponding decrease in concentration and estimated deposition on the flooring surface of pyrethrin plus methoprene and pyrethrin plus pyriproxyfen (Arthur et al., 2018). Furthermore, this latter study showed that aerosol particle size averaged about 10–12 μm when dispensed, but afterwards the particle size of both formulations was reduced to 4–6 μm within 20 min as the larger particles settled out of the air.

Previous laboratory tests using a specialized aerosol exposure chamber capable of regulating aerosol particle size (reported by the measuring instrument as Volume Median Diameter) established that particle size, and not necessarily concentration, was the most important factor in conferring mortality when adult *T. confusum* were exposed directly to a pyrethrin aerosol (Arthur et al., 2014). Aerosol particles dispensed at 2 μm apparently did not deposit on either the body surface of adult *T. confusum* or on the arena in which they were held, as evidenced by complete recovery from knockdown, but remained airborne. Adults exposed to particles dispensed at 16 μm did not recover even when provided with a food source. In the above test, the actual aerosol concentration dispensed was greater at 2 μm compared to 16 μm. Similarly, in tests in which late stage larvae of *T. confusum*, *Lasioderma serricorne* (F), the cigarette beetle, or *Trogoderma variabile* (Ballion), the warehouse beetle, were directly exposed in concrete treatment arenas with food, to a pyrethrin plus methoprene aerosol dispensed at 2 μm, they were able to complete development to the adult stage; however, when exposed to particles dispensed at 16 μm they could not complete development (Arthur et al., 2017). Similar results occurred when concrete surfaces were first treated with the same particle sizes, and then larvae and food material were added to the arenas.

Another factor to consider is that when adult *T. confusum* are exposed to an aerosol insecticide, they can be temporarily incapacitated by the exposure, often described as knockdown or affected adults (Campbell et al., 2014; Arthur et al., 2014; Scheff et al., 2018b). This is a transitory stage, whereby the adults can recover when the dosage is not enough for mortality, adults recover when supplied a food source, the adults succumb to the insecticide and die, or remain in the knocked-down stage (Campbell et al., 2014). There are limited data on fecundity of adult insects after they recover from knockdown after being exposed to an aerosol. There are indications from studies with exposure of adult *T. castaneum* adults on packaging incorporating the pyrethroid deltamethrin, that once adults recover from knockdown they are capable of mating and females produce viable eggs, with little overall effect on fecundity when exposed for ≤48 h (Scheff et al., 2018a)

In our previous tests with aerosols, there was complete control of adult *T. confusum* or larvae exposed to pyrethrin aerosol at 16 μm but little or no control at 2 μm. However, to our knowledge, no tests have been conducted to examine the effect of particle sizes between 2 and 16 μm, which can be obtained during field applications nor on the effect of aerosol exposure time on *T. confusum* adults or larvae. Therefore, the objectives of these series of tests were to determine: 1) The effect on adult survival and residual effect on larval *T. confusum* when exposed to a range of particle sizes, 2) The relationship between particle size and aerosol exposure time on the survival of adults and residual effect on larvae, and 3) fecundity of adults after exposure to a range of aerosol particle sizes.

2. Materials and methods

2.1. General information

This study was conducted using the aerosol dispersal system and vertical-airflow chamber at MRIGlobal, Kansas City, MO, previously described in detail (Arthur et al., 2014). Briefly, the aerosol insecticide is introduced into the top of the chamber, dispensed through an air-assist nozzle (Spraying Systems, Wheaton, IL, USA), which atomized the insecticide by regulating the air pressure, and the aerosol particles drift downward in a continuous low air-flow. The chamber has a wire shelf on the bottom, and concrete exposure arenas also previously described by Arthur et al. (2014), were placed on the wire shelf to conduct bioassays. A Malvern Spraytec aerosol particle sizer (Malvern Instruments, Worcestershire, UK; Model: STP5342) is mounted on the outside of the chamber. This system measures particle size distributions (reported as Volume Median Diameter, hereafter termed particle size) using a laser and optical sensors and continuously collecting the data on a computer.

The insecticides used in this test were a 1% active ingredient [AI] pyrethrin formulation (Entech Fog 10, Kenner, LA, USA), and methoprene (Diacon® IGR, 288 mg AI/ml, Central Life Sciences, Schaumberg, IL, USA), that were utilized in Arthur et al. (2018). All experiments used both insecticides dispensed at a ratio of 100:1 pyrethrin to methoprene (as specified by the product labels) at particle sizes 4, 8, 12, and 16 μm as characterized by the Spraytec instrument. Untreated controls were held on a laboratory benchtop during experimentation in the aerosol chamber. The particle sizes were generated by adjusting the air-pressure of the air-assist spray nozzle to give the approximate desired particle size. The insecticide was introduced into the chamber at a rate of 1.8 cc of liquid per minute (increases in air pressure gave smaller particle sizes). In addition, the effect of aerosol exposure time was assessed by dispensing each particle size for either 5, 10, or 20 min (hereafter termed as exposure time). The particle size distributions and the amount of aerosol dispensed at each replicate was recorded on the computer system connected to the Spraytec particle sizing instrument. From the total concentration, an estimate could be made of the estimated amount of aerosol particles deposited on each exposure arena, as described in Arthur et al. (2018).

The test insect and life stage used for this experiment were one-to-two-week-old adults and three-to-four-week-old larvae of *T. confusum*, obtained from pesticide-susceptible colonies that had been reared at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), in Manhattan, KS, USA, for more than 30 years. All cultures were reared at 27 ± 1 °C, 60% r.h., inside a Percival incubator (Perry, IA, USA) on a diet of 95% whole-wheat flour and 5% brewer's yeast, in continual darkness.

2.2. Effect of aerosol particle size and exposure duration on T. confusum adults

Three different experimental days were used to assess the effect of aerosol particles size and exposure duration and thus resulting in three replicate trials. Trial one was done on 13 June 2017, trial two was 18 July 2017, and trial three was 2 February 2018. For the first trial, six concrete arenas, each containing 10 one-to-two-week-old mixed sex adult *T. confusum*, were placed on the bottom of the experimental chamber. An additional six concrete arenas containing adults were placed on a laboratory benchtop in the same room and represented untreated controls. Adults were exposed to the aerosol at either 4, 8, 12, or 16 μm for 5, 10, or 20 min (4 × 3 treatment factorial design). After the exposure times, adults were immediately assessed for the number of knocked down adults, those with uncoordinated movement or on their backs and unable to right themselves. Adults on three different concrete arenas, out of the six concrete arenas exposed, were transferred to new-untreated concrete arenas containing ~500 mg of diet. Adults on the remaining concrete areas were kept on the same treated arenas. This was repeated for all particle size and aerosol treatment time combinations.

At the completion of testing, all the arenas were transported back to the CGAHR, and then held in a walk-in environmental chamber set at 27 ± 1 °C, 60% r.h. on a 16:8 light/dark cycle. Recovery of adults from the knockdown state was assessed one, three, and seven days post-aerosol treatment. After the final seven-day assessment, the original exposed adults were discarded and a new set of ten one-to-two-week-old mixed sex adults were placed on the arenas without diet and knockdown and recovery was to be assessed after one, three, and seven days. However, after one day all exposed adults were alive and moving, which indicated that there was no residual efficacy of the pyrethrin on the concreted dishes that would adversely affect adult *T.* confusum.

2.3. Residual effect of aerosol particle size and exposure duration on T. confusum larvae

At the same time adult *T. confusum* were being exposed to the aerosol, an additional nine concrete arenas were also placed in the experimental chamber to be utilized in residual bioassay studies with larvae. After these arenas were exposed to the aerosol, at each particle size and exposure time combination, they were transported back to CGAHR, and held in the environmental chamber described above. At one, three, and six weeks post aerosol treatment 10 three-to-four-week-old larvae of *T. confusum* were placed in three different concrete arenas along with ~1.5 g of diet. Untreated arenas were held for about three to four weeks until all adult emergence was completed. The treated arenas were held for an additional week to ensure that adult emergence was completed and not just delayed.

The percentage of normal adult emergence, along with larval developmental index, was used to assess the residual efficacy of the pyrethrin plus methoprene aerosol against *T. confusum* larvae at one, three, and six weeks post-aerosol treatment. Larval development was classified based on a developmental index described by Arthur et al. (2017). Briefly, larvae that were unable to complete development beyond the larval stage (supernumerary larvae, half larvae-half pupae intermediates, or dead larvae) were scored as 1. Individuals which developed to the pupal stage or were half pupae-half adult intermediates were scored as 2. Emerged adults with major morphological deformities (deformed body parts, severely twisted wings, incomplete sclerotization) were scored as 3. Emerged adults with minor deformities (primarily twisted wings, unsclerotized legs and antennae, but otherwise morphologically the same as untreated controls) were scored as 4. Morphologically normal adults were scored as 5. Only those adults scored as 5 were considered as having emerged as normal adults. A score of 10 would mean that no larvae developed to the next stage, a score of 20 indicates not development beyond the pupal stage, and as follow for the remaining three categories as described above.

2.4. Effect of aerosol particle size on T. confusum adult fecundity

The experimental design used for the effect of aerosol particle size on adult *T. confusum* fecundity was based off that of Scheff et al. (2018a) and was as follows. Four concrete arenas, two of which contained twenty one-to-two-week-old virgin *T. confusum* adult males and two of which contained twenty virgin females, were placed inside the exposure chamber. An additional four concrete arenas, containing twenty virgin males or twenty virgin females, were placed on a benchtop outside of the testing chamber, and were used as control (unexposed) arenas.

The arenas containing adult beetles were exposed to each of the four aerosol particle sizes for a 10-min exposure time. Following the completion of the exposure interval, males and females were then paired in mating sequences described by Scheff et al. (2018a) and is as follows: one aerosol exposed male (EM) was paired with one aerosol exposed female (EF); an aerosol exposed male (EM) paired with and unexposed female (UF); an unexposed male (UM) paired with and aerosol exposed female (EF); and an unexposed male (UM) paired with an unexposed female (UF); (EM-EF; EM-UF; UM-EF; UM-UF). Mating pairs were placed into a 7-dram vial which contained ~3.5 g of the rearing diet and placed in a Percival environmental chamber at 27 ± 1 °C, 60% in complete darkness. Adults were held in vials for seven days before being removed. The vials were held in the environmental chamber for an additional eight weeks, then the contents of each vial were examined and the number of F_1 adults was tabulated. Assessments were not made for number of eggs laid or young larvae, because disturbing the 7-dram vials could have disrupted development of the progeny. There were twenty individual paired mating sequences made for all aerosol particle sizes tested. The particle size by paired mating sequences was repeated 3 times for a total of 960 mating sequences among all treatment combinations and replicates.

2.5. Statistical analysis

Since all three exposure trials were independent, the data for knockdown, adult recovery, and mortality of *T. confusum* after the direct exposures to the aerosol were combined for all trial dates and first analyzed using Mixed Model Procedures in the Statistical Analysis System (Version 9.2, SAS Institute, Cary, NC, USA), with exposure time, particle size, and presence of food as main effects. Data for adult emergence from the residual bioassay with *T. confusum* larvae were analyzed with exposure time, particle size, and residual bioassay time as main effects. The numerical values created by the developmental index were also analyzed with the same main effects. In addition, correlation analyses were done between adult emergence and index value. Means were separated using the Tukey Honestly Significant Difference test as an option under the Mixed Procedure at $P < 0.05$.

For the study in which fecundity of *T. confusum* was assessed, the

Table 1
Median aerosol particle size (mean ± SE, in μm) as measured by the Spraytec Particle Analyzer for exposure times of 5, 10, and 20 min.

Target Aerosol Particle Size (μm)	Measured Aerosol Particle Size (μm; mean ± SE)		
	5-min exposure	10-min exposure	20-min exposure
4	4.7 ± 0.07	4.1 ± 0.03	4.4 ± 0.21
8	8.7 ± 0.01	7.8 ± 0.07	8.1 ± 0.09
12	12.2 ± 0.01	11.5 ± 0.10	11.6 ± 0.18
16	17.1 ± 0.05	16.7 ± 0.06	16.7 ± 0.37

number of F_1 adult beetles were recorded and reported as counts. The data was transformed to log (x+1) scale for statistical analysis (Zar, 2010) and analyzed using a two-way analysis of variance (ANOVA) with the main effects of particle size and mating sequence under the Mixed Procedure. Adult counts were analyzed further across treatments at individual exposure times using ANOVA and significant differences were separated using a Bonferroni test for significance at $P < 0.05$.

3. Results

3.1. Aerosol characterization and deposition

The actual particle size, as measured by the Malvern Spraytec, was relatively consistent with the intended particle size at each exposure time of 5, 10, or 20 min (Table 1). Regarding the deposition of aerosol in the chamber relative to the exposure times, there are several factors to consider. First, the vertical flow chamber was operated as a continuous flow system, in which the aerosol is continually being introduced at a rate of 1.8 cc of liquid/minute and mixed with clean air that is pulled in from the top of the chamber. Aerosol is carried downward, some particles were deposited on the concrete surfaces, and the undeposited particles were vented out the bottom of the chamber (Fig. 1). Based on the Spraytec-measured particle size and particle dispersion within the vertical flow chamber, the amount of suspended particles reaches a "steady state" condition within several minutes. It is also evident that the concentration and deposition of the aerosol particles increased as exposure time increased, i.e. a proportional increase in deposition is seen when spray durations increase from 5 min to 20 min. Furthermore, deposition is expected to increase with increasing particle size. We estimated the amount of aerosol deposition on an individual exposure arena, using Equation 1 below. Values in Table 2 show how deposition increased with particle size and exposure time and thus can help explain the biological effects described below.

$$\textit{Estimated Deposition} = (\textit{rate of insecticide addition}) \times (\textit{exposure time}) \times \left(\frac{\textit{area of test arena}}{\textit{area of chamber floor}}\right) \times (\textit{impact efficiency})$$

Where the rate of insecticide addition was 1.8 cc of liquid/min; exposure time was 5, 10 or 20 min; area of test arena was 62 cm^2; area of chamber was 6075 cm^2; and impact efficiency (percentage of particles landing on the surface) were estimated as 40, 72, 84, and 96% for the 4, 8, 12, and 16 μm particles, respectively.

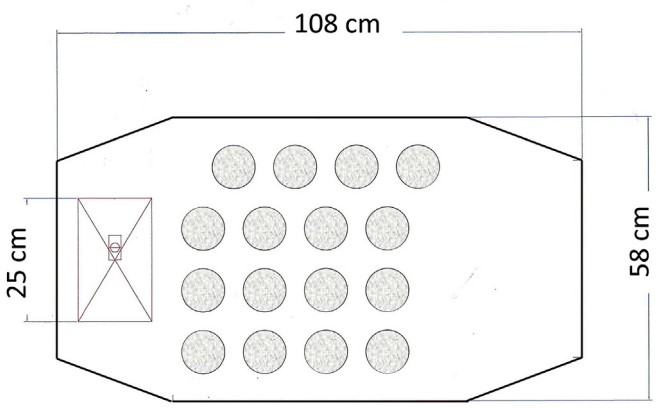

Fig. 1. Cross section of the continuous airflow-spray chamber holding 12 bioassay concrete exposure arenas. Each arena is 8.6 cm in diameter, the chamber is 58 cm × 108 cm.

3.2. Effect of aerosol particle size and exposure duration on T. confusum adults

Adult beetles were assessed for the number of knocked down adults immediately after the exposure times were completed. All the adults in the untreated control arenas for all exposure times and particle sizes were active and running and no adult knockdown was recorded, thus they were eliminated from analysis. The percentage of insects active after removal from the chamber was significant at $P < 0.001$ for main effects concentration and exposure time ($F = 9.1$, df = 3, 217; $F = 44.2$, df = 2, 217, respectively). Their interaction was significant at $P = 0.003$.

At the 5-min exposure time, more than 60% of the adults were active after exposure to the pyrethrin plus methoprene mixture at 4 μm, while the percentage of adults active after exposure to all other particle sizes did not exceed 29.5% (Table 3). There was an obvious decline in active adults as exposure time increased to 10 and 20 min at each specific particle size, with no significant difference between the 10 and 20-min exposure times for any particle size (Table 3).

The percentage of adult *T. confusum* active and running in the untreated controls at days one, three, and seven post-aerosol treatment was 98.7% and thus data for controls were eliminated from analysis. The main effects exposure time, days post aerosol-treatment, particle size, and presence of food were all significant at $P < 0.001$ ($F = 142.8$, df = 2537; $F = 387.4$, df = 2, 573; $F = 189.9$; df = 3, 573, $F = 378.0$, df = 1, 573, respectively). All interactions were significant at $P < 0.001$, except time by treatment, which was not significant ($P \geq 0.05$). One-way ANOVAs were then run in Proc Mixed to determine significance between particle size and exposure times and to determine significance of providing food to adults after the initial exposure versus no food. In all comparisons, significance was determined at $P < 0.05$.

Among all particle sizes tested, as the aerosol exposure time increased the percentage of adults recovering from knockdown correspondingly decreased regardless of the presence or absence of food. Additionally, there was also a general trend of adults recovering from knockdown as post aerosol-exposure time increased from one to seven days. For example, for the no-food scenario at the 5-min exposure time for the 4 μm treatment, the percentage of recovered adults was 48.8% after one day but 100% after three and

Table 2
Estimated deposition of aerosol on an individual treatment arena. The estimated "impact efficiencies" were 40, 72, 84, and 96% corresponding to target particle sizes of 4, 8, 12, and 16 μm, respectively. Calculations were based on the volume of insecticide introduced into the chamber (1.8 cc of liquid/minute) onto an individual arena (62 cm^2), which occupied an estimated 1% of the total cross-sectional area of the chamber floor. To obtain impact efficiency/cm^2, divide values in the Table by 62.

Exposure Time	cc insecticide	Particle Size	4 μm	8 μm	12 μm	16 μm
		Estimated Impact Efficiency	40%	72%	84%	96%
		cc/arena	cc/arena	cc/arena	cc/arena	cc/arena
5 min	9	0.090	0.034	0.062	0.072	0.082
10 min	18	0.170	0.069	0.124	0.145	0.163
20 min	36	0.340	0.138	0.248	0.289	0.327

Table 3
Percentage active (mean ± SE) of *Tribolium confusum* adults after an exposure time of 5, 10, or 20 min to pyrethrin plus methoprene aerosol dispensed at four particle sizes. Survival assessed after adults were removed from the chamber. Means within columns followed by different lower-case letters are significantly different, and means within rows followed by different capital letters, are significantly different ($P < 0.05$, LS Means under Proc Mixed in SAS)[a].

Particle Size (μm)	Initial Percent Active Adult *T. confusum* (mean ± SE)		
	5-min exposure	10-min exposure	20-min exposure
4	61.1 ± 3.7 aA	1.6 ± 1.6 aB	16.7 ± 7.1 aB
8	29.5 ± 8.7bA	0.6 ± 0.6 aB	1.0 ± 1.0 aB
12	25.0 ± 8.7bA	0.0 ± 0.0 aB	0.0 ± 0.0 aB
16	20.6 ± 9.4bA	0.0 ± 0.0 aB	0.0 ± 0.0 aB

[a] All adults in untreated controls were active at day 0.

seven days (Table 4). At the 16-μm treatment, no adults were active at one and three days but by day seven about 16% had recovered from knockdown. It was clear there was a strong interaction between particle size, exposure time, and recovery of adults. Furthermore, there was an effect when adults were provided with the flour food source, which was most noticeable at three and seven days post-treatment among all particle sizes and exposure times. There were 24 possible comparisons at those times, for each specific exposure time-particle size combination, and for 17 of those comparisons recovery was greater when the adults were given food (denoted by asterisks in Table 4 for means for the food scenario) compared to when the exposed adults were not provided with a food source.

3.3. Residual of aerosol particle size and exposure duration on *T. confusum* larvae

The main effects treatment (the four particle sizes plus the

Table 4
Percent recovery (Mean ± SE) of *Tribolium confusum* adults one, three, and seven days after exposure for 5, 10, or 20 min to pyrethrin plus methoprene aerosol dispensed at four particle sizes and held with (Yes) or without a flour food source (No). Means within columns for each day followed by different lower-case letters and means within rows for each day followed by different capital letters are significantly different ($P < 0.05$, LS Means under Proc Mixed in SAS). Means for recovery in those adults provided with a food source denoted by an asterisk indicates greater recovery compared to recovery without a food source[a].

Particle size (μm)	Day Post-Treatment	Food Source	Percent Recovery of Adult *T. confusum* (mean ± SE)		
			5-min exposure	10-min exposure	20-min exposure
4	1	No	48.8 ± 14.2 aA	6.7 ± 3.3 aB	0.0 ± 0.0 aB
8			33.3 ± 16.7 aA	0.0 ± 0.0bB	0.0 ± 0.0 aB
12			0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0 aA
16			0.0 ± 0.0bA	0.0 ± 0.0bA	0.0 ± 0.0 aA
4	1	Yes	66.7 ± 16.7 aA	56.7 ± 11.4 aA*	33.3 ± 16.3 aA*
8			62.2 ± 15.8 aA	15.6 ± 6.3bB*	0.0 ± 0.0bB
12			26.7 ± 10.9abA*	1.1 ± 1.1 cB	0.0 ± 0.0bB
16			0.0 ± 0.0bA	0.0 ± 0.0 cA	0.0 ± 0.0bA
4	3	No	100.0 ± 0.0 aA	100.0 ± 0.0 aA	24.4 ± 11.4 aB
8			64.4 ± 8.0bA	48.9 ± 11.8bA	0.0 ± 0.0bB
12			7.8 ± 6.6 cA	10.0 ± 5.3 cA	0.0 ± 0.0bA
16			0.0 ± 0.0 cB	24.4 ± 11.6 cA	0.0 ± 0.0bB
4	3	Yes	100.0 ± 0.0 aA	91.1 ± 3.1 aB	96.7 ± 2.4aAB*
8			100.0 ± 0.0 aA*	96.7 ± 1.7 aA*	63.3 ± 6.2bB*
12			90.0 ± 5.3 aA*	88.9 ± 3.5 aA*	53.3 ± 14.2bB*
16			96.7 ± 2.1 aA*	51.1 ± 13.2bB	4.4 ± 3.7 cC
4	7	No	100.0 ± 0.0 aA	100 ± 0.0 aA	87.8 ± 5.5 aB
8			90.0 ± 3.3 aA	8.9 ± 7.2bB	2.2 ± 1.5bC
12			85.0 ± 5.6 aA	6.7 ± 7.3bB	0.0 ± 0.0bC
16			16.7 ± 8.8bA	3.3 ± 2.3bB	0.0 ± 0.0bB
4	7	Yes	100.0 ± 0.0a	100.0 ± 0.0a*	100.0 ± 0.0a*
8			100.0 ± 0.0a*	98.9 ± 2.6a*	91.1 ± 4.5 ab*
12			100.0 ± 0.0 aA*	98.9 ± 1.1 aA*	76.7 ± 4.7bB*
16			70.0 ± 15.2bB	87.8 ± 3.6 aA*	22.2 ± 3.7 cB*

[a] Average of percent active untreated controls over all particle sizes, days post treatment, and presence of food was 98.7 ± 0.3.

untreated control), exposure time and post-treatment bioassay week were significant at $P < 0.001$ for percentage of adult emergence from exposed *T. confusum* larvae ($F = 398.6$, df = 4, 354; $F = 132.0$, df = 2, 354; $F = 75.4$, df = 2, 254, respectively). All interactions were significant at $P < 0.001$ except for exposure time by week, which was significant at $P = 0.013$. Main effects treatment, exposure time, and bioassay week were also significant at $P < 0.001$ for the index value ($F = 318.7$, df = 4, 354; $F = 146.5$, df = 2, 354; $F = 86.9$, df = 2, 354, respectively). All interactions were significant at $P < 0.001$, except for exposure time by week, which was significant at $P = 0.004$. Analyses were then done by week for adult emergence and index value.

At one week post-treatment, there was no difference in emergence of morphologically normal adults (hereafter termed "adult emergence") from exposed *T. confusum* larvae in untreated controls and the 4 μm particle size at exposure times of 5 and 10 min (range of 85.5–100%) (Fig. 2A). Increasing the 4 μm exposure time to 20 min, resulted in a decrease in adult emergence to 15.5% (Fig. 2A). The percentage of adult emergence was significantly lower for particle sizes of 8, 12, and 16 μm at all exposure times compared to the untreated controls. The 16 μm particle size had the greatest effect on adult emergence, which was less than 5% for all exposure times. In general, there was a non-linear decrease in adult emergence as particle size and exposure time increased.

The mean index values generally followed the same non-linear decreasing trend with increasing particle size and exposure time as adult emergences. There was no difference between untreated controls and the 4 μm particle size at exposure times of 5 and 10 min (index levels ranging from 45.6 to 49.7 with a maximum level of 50 if every larva emerged as a morphologically normal adult), and a decrease to 25.8 when exposure times increased to 20 min (Fig. 2B). The index levels for 8, 12, and 16 μm were significantly lower compared to the untreated control for all exposure times. No advancement to the adult stage occurred for any exposure time at the 12 and 16 μm particle sizes, except for one index level score above 30 at the 12 μm at 10-min exposure time. Some advancement to the adult stage occurred at the 8 μm particle size treatment, as indicated by the range of index values.

At three weeks post-treatment, adult emergence generally began increasing at all particle sizes at the 5 and 10-min exposure times with the 4 and 8 μm particle size not significantly different from untreated control adult emergence at the exposure time of 5 min, 87.9–94.4% respectively (Fig. 3A). Adult emergence for 4 and 8 μm particles sizes decreased with increasing exposure time, and at the 20-min exposure time they were significantly lower than the untreated controls. There were no significant differences in adult emergence between the 12 and 16 μm particle sizes at any exposure time. Adult emergence did not exceed 30% at the 5 and 10-min exposure time and at the 20-min exposure time adult emergence was less than 2.2% at either of the particle sizes.

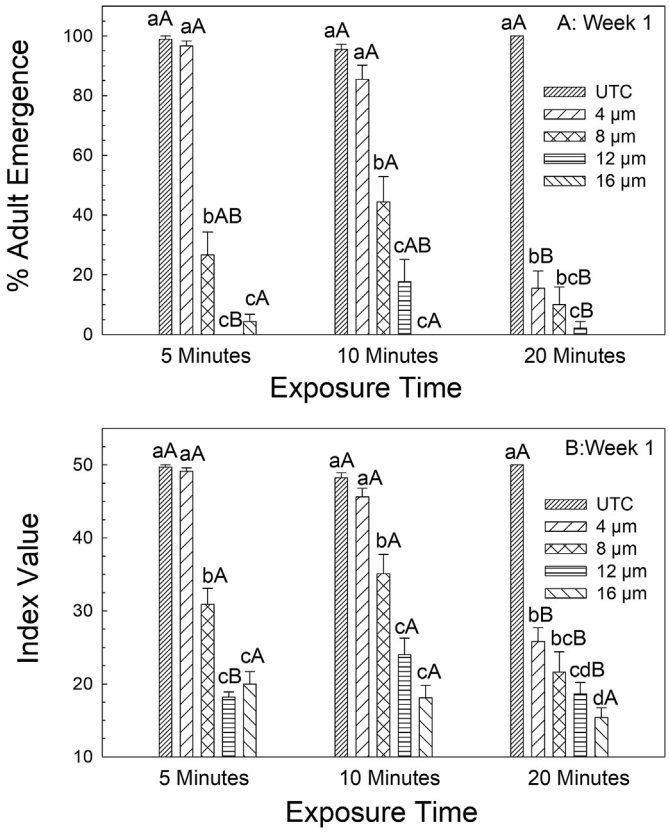

Fig. 2. A, Percent adult emergence of morphologically normal adults of *T. confusum* from exposure of larvae on concrete arenas exposed to pyrethrin + methoprene aerosol dispensed at 4, 8, 12, and 16 μm for 5, 10, or 20 min, and on untreated control arenas (UTC) one week after aerosol treatment; B) Mean (±SE) index values, ranging from a minimum of 10 to a maximum of 50, from the larval exposures at the same conditions above. Means within each exposure time followed by different lower-case letters and means for each particle size followed by different capital letters, are significantly different ($P < 0.05$, LS Means under Proc Mixed in SAS).

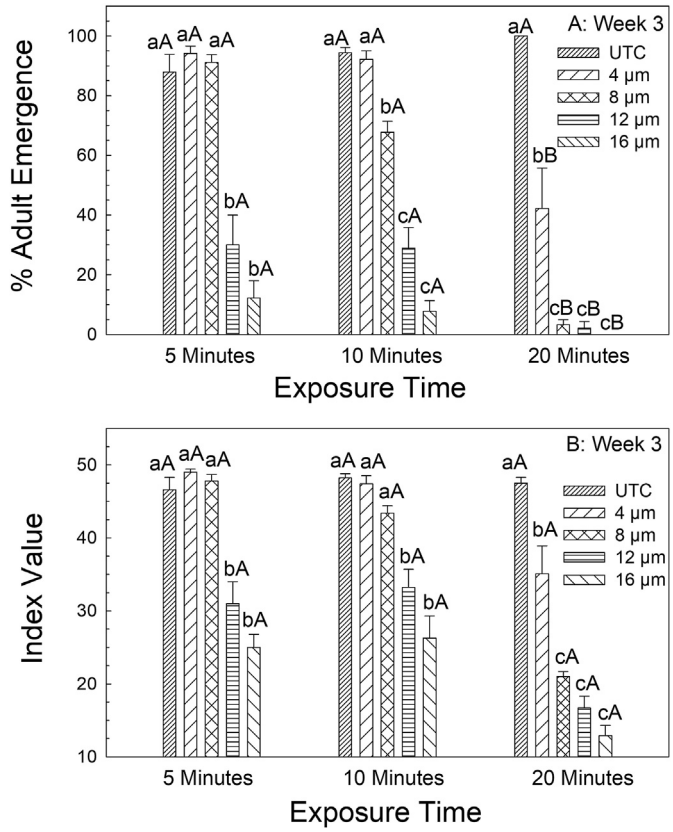

Fig. 3. A, Percent adult emergence of morphologically normal adults of *T. confusum* from exposure of larvae on concrete arenas exposed to pyrethrin + methoprene aerosol dispensed at 4, 8, 12, and 16 μm for 5, 10, or 20 min, and on untreated control arenas (UTC) three weeks after aerosol treatment; B) Mean (±SE) index values, ranging from a minimum of 10 to a maximum of 50, from the larval exposures at the same conditions above. Means within each exposure time followed by different lower-case letters and means for each particle size followed by different capital letters, are significantly different ($P < 0.05$, LS Means under Proc Mixed in SAS).

The index values at the 5 and 10-min treatment times were similar to the adult emergence data for untreated controls and the 4 and 8 μm particle sizes, with index level ranging from 43.2 to 49.0 respectively, and were not significantly different from the controls, but reduced significantly at the 20-min exposure time (Fig. 3B). The 12 and 16 μm particle sizes, were both significantly lower compared to the untreated controls but did not significantly differ from one another. he 20-min exposure time using 12 and 16 μm particle sizes was most effective, with mean index values < 20.

At six weeks post-treatment, adult emergence was same for untreated controls and at the 4 μm particle size for all exposure times. The 5 and 10-min exposure times for the 8 μm particle size was not significantly different from the untreated controls, but the 20-min exposure time reduced adult emergence to about 15% (Fig. 4A). There was significantly lower adult emergence at the 5, 10, and 20-min exposure times for the 12 and 16 μm particle sizes, with the 20-min exposure time being the most effective. Comparing all particle size and exposure times, there was a general increase in adult emergence from one-week post-treatment to six weeks post-treatment, which indicates degradation of the methoprene on treated surfaces.

Index values for untreated controls and the 4 and 8 μm particle sizes ranged from 47.9 to 50, indicating complete development to morphologically normal adults (Fig. 4B). Index values for 12 and 16 μm were generally significantly lower than the untreated controls at all exposure times and, was generally the lowest at the 20- minute exposure time. Comparing all aerosol particle sizes and exposure times, there was an increase in index level from one to six weeks post-treatment. This indicates that the larvae were progressing further along the developmental pathway even though adult emergence remained low.

A further analysis was done to correlate adult emergence with index value by combining data for all particle sizes. Percentage adult emergence was highly positively correlated with the index value (Proc Corr in SAS, $P < 0.001$, $r = 0.97$). Table Curve software (Version 5.2, SPSS, San Jose, CA, USA) was used to fit a non-linear curve (R^2 of 0.97) to the raw data (Fig. 5). The closer the index level was to the maximum score of 50 correlated to a high percentage of adult emergence.

3.4. Effect of aerosol particle size on T. confusum adult fecundity

In this test male and female adult *T. confusum* were exposed for 10 min to all particle sizes and were paired in the following combinations: exposed male-exposed female (EM-EF), exposed male-unexposed female (EM-UF), unexposed male-exposed female (UM-EF), and unexposed male-unexposed female (UM-UF). The two-way ANOVA showed that main effects particle size and exposure combination were significant at $P < 0.001$ ($F = 7.8$, df = 3.338, $F = 37.8$, df = 3, 818, respectively), along with the interaction, for the number of progeny adults produced from the four combinations. One-way ANOVAs were then done in Proc Mixed in SAS to determine significance between particle size and the exposure combinations with respect to progeny production. In general, there appeared to be a mild sublethal affect in that fewer progeny were produced from adults exposed to all particle sizes, but the 16-μm particle size appeared to produce fewer progeny compared to the other three sizes (Table 5). Also, there was an evidence of a sex effect; at each particle size fewer progeny were produced with the female exposed to the aerosol, except for 8-μm.

4. Discussion

One of the difficulties in explaining the biological effects of aerosols applications on stored product insects is estimating the actual deposition of the insecticide on both the insect and treated surface. In our first publication describing tests conducted using the MRIGlobal chamber (Arthur et al., 2014), we estimated aerosol concentration by using ppm of aerosol dispensed per minute, and

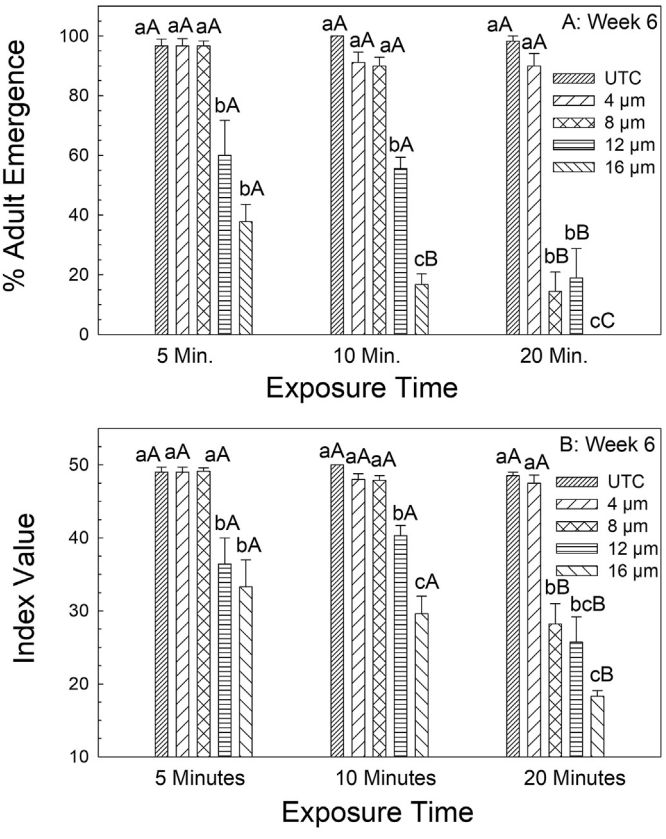

Fig. 4. A, Percent adult emergence of morphologically normal adults of *T. confusum* from exposure of larvae on concrete arenas exposed to pyrethrin + methoprene aerosol dispensed at 4, 8, 12, and 16 μm for 5, 10, or 20 min, and on untreated control arenas (UTC) six weeks after aerosol treatment; B) Mean (±SE) index values, ranging from a minimum of 10 to a maximum of 50, from the larval exposures at the same conditions above. Means within each exposure time followed by different lower-case letters and means for each particle size followed by different capital letters, are significantly different ($P < 0.05$, LS Means under Proc Mixed in SAS).

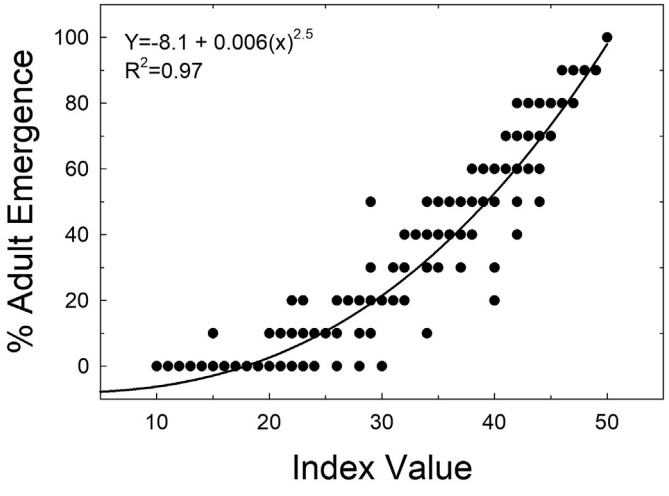

Fig. 5. Correlation between normal adult emergence and index values for combined data.

Table 5
Progeny production (mean ± SE) from adult *Tribolium confusum* adults after exposure to the pyrethrin plus methoprene aerosol for 10 min at different particle sizes, and paired immediately after exposure as follows: exposed male-exposed female (EM-EF), exposed male-unexposed female (EM-UF), unexposed male-exposed female (UM-EF), and unexposed male-unexposed female (UM-UF). Means within columns for particle size followed by different lower-case letters and means within rows for combination followed by different capital letters, are significantly different from each other ($P < 0.05$, LS Means under Proc Mixed in SAS).

Particle size (μm)	Mean (±SE) F_1 Progeny Produced from Mated *T. confusum* Adults			
	EM-EF	EM-UF	UM-EF	UM-UF
4	40.8 ± 1.4 aB	46.4 ± 1.6 aA	39.7 ± 1.5bB	46.7 ± 1.6 aA
8	41.1 ± 1.2 aB	40.4 ± 1.5bB	44.0 ± 1.7 aA	46.2 ± 2.0 aA
12	35.3 ± 1.4bB	45.5 ± 1.4abA	38.5 ± 1.4bB	46.6 ± 1.6 aA
16	31.3 ± 1.5bC	41.7 ± 1.8bB	34.5 ± 1.7bC	48.2 ± 1.5 aA

then doing straight multiplication by spray times to get a total estimate of concentration. Although this method gave a reasonable approximation of effects of increasing concentration when conducting tests in the chamber, it may not have accounted for the continuous outflow of aerosol from the chamber and the chamber reaching a steady state with concentration, or differential deposition depending on particle size. Our method of estimation of deposition on a treatment surface, rather than calculation dispensed aerosol concentration, as described here may be more reflective of actual conditions and will be utilized in future studies assessing aerosol deposition in the exposure chamber.

The pyrethrin component of the tested aerosol will kill all life stages of *Tribolium* spp. but has little residual efficacy (Arthur, 2010). In previous studies with pyrethrin aerosol and exposed adult *Tribolium* spp. as the target, two factors have been noted: a tendency for generalized recovery after exposure (Campbell et al., 2014; Scheff et al., 2018b) and decreased efficacy when adults were provided with a food source during or immediately after exposure (Arthur, 2008; Arthur and Campbell, 2008). In our previous study with the MRIGlobal exposure chamber, in which a pyrethrin aerosol was applied at 16 versus 2 μm, we showed increased recovery when adults were exposed to the 16-μm particle size for 2.5, 5, and 10 min and then were transferred to untreated arenas with food compared to transfer to untreated arenas without food (Arthur et al., 2014). In the current study, we showed the percentage of adults recovering from knockdown increased with decreasing exposure time, regardless of the presence of food.

An alternative adulticide aerosol is the organophosphate dichlorvous, which has vapor toxicity and disperses underneath barriers and equipment and into open areas to a greater extent than a pyrethrin aerosol, as shown in simulated field studies (Campbell et al., 2014; Subramanyam et al., 2014). However, dichlorvos has more restrictive application requirements compared to pyrethrin; in the US a 24-h holding time is required before re-entry, and new regulations could limit use to timed releases without worker or applicator presence. Also, dichlorvos does not give residual control (Subramanyam et al., 2014). The methoprene component of the aerosol combination tested in this study will give residual control of immature life stages that come into contact with the residues. This is an important factor, because in natural populations in actual and simulated field studies adult *Tribolium* spp may comprise less than 15% of the total population (Toews et al., 2009), so some level of residual control is necessary when considering aerosol applications to control stored product beetles within a large facility.

In the current study, we observed a reduction of adult emergence from larvae exposed to treated surfaces at one, three, and six weeks post-treatment. The longest exposure time, 20 min, was the most effective at reducing adult emergence for all particle sizes at all residual bioassay weeks. In this study we also used a developmental index for larval exposure. The idea of the developmental index for larvae exposed to IGRs was based on a lethality index first proposed by Agrafioti et al. (2015), and then adapted for use in larval exposure studies with IGRs (Arthur et al., 2017; Arthur and Hartzer, 2018). In the current study the developmental index values correlated well with percentage adult emergence, but the index is subject to bias from classifications of pupal/adult intermediates and determining major versus minor deformities in emerged adults. However, it is still a reliable indicator of effects, as the index values tended to decrease as concentration, deposition, and particle size increased, and for the all particle sizes the index values generally increased with the progressive bioassays from one to three to six weeks after the arenas were treated.

When fecundity was examined for adults exposed to an aerosol treatment, results showed some indications of decreased fecundity with increasing particle size. Although the general trend was that if the adults were not killed by the pyrethrin exposure, some level of oviposition still occurred, but at three of the four particle sizes progeny production from the mated pairs was less when the female was exposed compared to male exposure only or when neither sex was exposed. In studies in which *T. castaneum* or *T. confusum* was exposed on deltamethrin treated packaging or grain short term exposures did not lead to reduced fecundity (Kavallieratos et al., 2015; Scheff et al., 2018b). Thus, it appears that if adult *T. confusum* or *T. castaneum* recover from knockdown after exposure to pyrethrins or pyrethroids, they can still oviposit and produce progeny. Further investigations may be warranted for adults of other stored product beetle species that may be targets for aerosol application in milling and processing facilities.

Research studies have repeatedly documented reduced efficacy of aerosol insecticides when adult flour beetles have access to food material. This study and other recent studies show that aerosol particle size directly affects residual efficacy when insects are exposed directly to an aerosol or indirectly through exposure on a treated surface. Deposition of aerosols can be calculated by assessing concentration in the airspace, which provides a means of predicting residual aerosol efficacy at different particle sizes. Results can be used to optimize insect pest management plans utilizing aerosols to control insect pests in milling and processing facilities.

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