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Laboratory Evaluation of Particle Size, Food Contamination, and Residual Efficacy of
Pyrethrin + Methoprene Aerosol

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Abstract

Efficacy of aerosol applications of pyrethrin+methoprene was assessed through emergence of morphologically-normal adults exposed as larvae and through a developmental index. Concrete arenas were treated with aerosol droplets (particles) averaging 2µm or 16µm, food material was placed on the arenas at 1-8 weeks post-treatment, and 3-4-week-old larvae of one of four stored product insect species placed on the arenas at the same time. No exposed *Lasioderma serricorne* (DeGeer) larvae reached the adult stage at either particle size, though they developed further when exposed residual deposits of the 2 µm aerosol particles compared to residual deposits of 16 µm particles. No exposed larvae of either *Tribolium confusum* Jacqueline duVal or *Trogoderma variabile* (Ballion) reached the adult stage when exposed to residues of the 16 µm particles, but most of the larvae exposed to the 2 µm residues completed development. Exposed *Dermestes maculatus* (DeGeer) larvae completed development to the adult stage after exposure to both particle sizes, indicating low susceptibility to methoprene residues. When food material in the arenas was also treated with the two particle sizes and bioassays conducted using only larvae of *L. serricorne*, *T. confusum*, and *T. variabile*, results were similar to those described above. In a final experiment, only the 16 µm particle size aerosol was used to treat arenas with or without food, with arenas without food having it added one day after treatment. At 1-8 weeks post-treatment, the food was transferred to new untreated arenas and food was replaced in the arenas originally treated with aerosol. Bioassays were conducted using only larvae of *T. confusum* and *T. variabile*. The food material blocked deposition of the aerosol on the arenas or absorbed some of the residues, indicating transference of the methoprene residues from the treated surfaces onto the food.

Key Words: aerosols; sanitation; pyrethrin; methoprene; control

1. Introduction

The use of aerosol insecticides can be an important component of insect pest management plans for stored product insects in mills, process plants, and food storages, and also a viable replacement for the fumigant methyl bromide in the United States (U.S.) (Arthur, 2012; Subramanyam et al., 2014). One aerosol insecticide used in the U. S. is pyrethrin alone or combined with the insect growth regulator (IGR) methoprene. The pyrethrin component offers direct initial kill of exposed immatures and adults (Tucker et al., 2015), while the methoprene component can give indirect residual control of immature life stages through deposition on different surfaces (Sutton et al., 2011).

One aspect of aerosols currently being investigated is how particle size affects distribution and efficacy of aerosols. While smaller particles may more readily disperse inside a facility, larger particles may more effectively adhere to the body surface of insects. Arthur et al. (2014) evaluated the response of adult *Tribolium confusum* Jacqueline DuVal, the confused flour beetle, when directly exposed to pyrethrin aerosol dispensed at either 2 μm or 16 μm . The smaller particle size did not kill *T. confusum* even when the actual concentration of insecticide dispensed at 2 μm was greater than or equal to concentration dispensed at 16 μm . In addition, flour mills and other indoor structures contain complex equipment, structural beams, and specific design configurations that can block or limit dispersal of aerosols, allowing some portions of the structure to receive less aerosol deposition than others (Campbell et al., 2014). Thus control of insect populations inside mills may occur through indirect measures such as contact with the treated surface rather than through direct contact with the dispensed particles.

Efficacy of IGRs on treated surfaces can be assessed through indirect exposure measures, such as emergence of morphologically-normal adults from larvae added to treated surfaces

versus untreated surfaces, or by examining progeny production from adults exposed on those treated and untreated surfaces (Arthur and Fontenot, 2012). However, exposure to an IGR can result in a range of morphological deformities that indicate effects of the insecticide but are not captured by just measuring adult emergence. Documentation of all the separate effects of exposure exhibited by immatures failing to complete development to the adult stage and adults that have morphological deformities and comparison of levels relative to untreated controls has also been used to assess IGR efficacy (Sutton et al., 2011). Another approach would be to use an efficacy index to simplify the categorization of the wide range of responses to treatment, while giving a more precise assessment of IGR efficacy than just adult emergence. Agrafioti et al. (2016) developed a lethality index to assess knockdown and recovery of adult *T. castaneum* (Herbst), the red flour beetle, and *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle, exposed on grains treated with different contact insecticides. Campbell et al. (2014) used an efficacy index to characterize knockdown and mortality of groups of adult *T. confusum* in an arena exposed to aerosol insecticides during a field trial. However, no index has been proposed to assess development of larvae of stored product insects exposed to an aerosol application involving an IGR.

There are no recent studies examining either residual efficacy of aerosols dispensed at precise particle sizes or variation among stored product insect species exposed to those aerosols. Recent studies indicate that members of the family Dermestidae may be less susceptible to contact insecticides and also less susceptible to cold temperatures relative to *Tribolium* spp. (Arthur et al. 2015b). Therefore, the objectives of this study were to: 1) determine residual efficacy of pyrethrin + methoprene aerosol dispensed at 2 μm and 16 μm , 2) examine potential variation in susceptibility of stored product insects to this aerosol combination, 3) examine the

impact of residual food material on residual efficacy, and 4) develop a susceptibility index based on the response of exposed larvae and compare that index with adult emergence data.

2. Materials and Methods

2.1. Experiment 1.

The target insect species used for this study were 4-week-old late stage larvae of *T. confusum*, *Lasioderma serricorne* (F.), the cigarette beetle, *Trogoderma variabile* (Ballion), the warehouse beetle, and *Dermestes maculatus* (DeGeer), the hide beetle. All species were obtained from pesticide-susceptible stock cultures maintained at the USDA-ARS Center for Grain and Animal Health Research. The *T. confusum* and *L. serricorne* colonies had been in culture for more than 30 years, and were reared on a diet of 95% whole-wheat flour and 5% brewer's yeast at 27°C-60% r.h. in continual darkness. The *T. variabile* colony had also been in culture for more than 30 years, was reared on a diet of 67% ground dog food and 33% dried powder vanilla protein shake, on a 16:8 hour light-dark cycle at 30°C-60% r.h. The *D. maculatus* colony was originally collected from a field site in 2008 and was reared on a diet of 100% ground dog food, at the same conditions as described for *T. variabile*.

The exposure arenas used for this study were created by filling the bottom portion of a plastic Petri dish, which was 62 cm² in area and 20 mm height, with a ready-mix dry concrete driveway patching material (Rockite, Heartline Products, Cleveland, OH, USA). This process has been described in detail in previous publications (Arthur et al., 2014; Arthur, 2015ab). It essentially involves mixing the dry powder with water to create a liquid slurry, and pouring the material into the Petri dish (hereafter referred to as arenas) to a height of about 1.25 cm. A total of 300 arenas was created in this manner. These arenas were allowed to dry for several days on a laboratory counter, and then the sides were coated with Fluon[®] (Northern Products, Woonsocket,

RI, USA) to minimize escape of larvae from the exposure surface by preventing larvae from crawling up the sides of the arena.

Approximately two weeks after the arenas were created, 220 of them were transported to a laboratory at MRIGlobal, Kansas City, MO, USA, which was where the exposure chamber was located. This was a vertical flow chamber that was utilized in a previously study and described in detail by Arthur et al. (2014). Briefly, this chamber provided for a continuous distribution of aerosol particles as long as the system was operating, and could produce particles within a narrow range of sizes with an average of either 2 μm or 16 μm , using a 6 or 24-jet Collison sprayer or a SprayTec sprayer, respectively (Arthur et al., 2014). The exposure times for the two sprayers was adjusted so that that the actual concentration of insecticide would be similar when two different particle sizes were used. The time intervals chosen to provide similar concentrations were 20 minutes with the Spraytec system at 16 μm and 15 minutes with the 24-jet Collison sprayer at 2 μm , based on results from the previous study (Arthur et al., 2014).

The actual spraying was done on 21 October 2015 as follows. The insecticide used was a 1% active ingredient (AI) pyrethrin formulation with a kerosene carrier (Entech[®] Fog 10, Entech Systems, Kenner, LA, USA), and a 33% AI methoprene formulation (Diacon[®] IGR, Central Life Sciences, Schaumburg, IL, USA). Label directions for using these products as an aerosol spray specify mixing the pyrethrin and methoprene in a 100:1 ratio. Thus, 60 ml of the pyrethrin formulation was pipetted into a graduated cylinder, and then 0.6 ml of methoprene was also pipetted into the cylinder. The solution was mixed and then poured into an injection cylinder connected to the 24-jet Collison sprayer. A total of 20 arenas were randomly placed on the false floor of the chamber for a spray treatment, with each spray considered a replicate treatment. After the exposure interval of 15 minutes was completed, the arenas were removed, and the

chamber vented for approximately 5 minutes to ensure that all aerosol particles were removed (Arthur et al., 2014). The analytical equipment used in the study continually measured the aerosol particles inside the chamber, and after this 5-minute venting period the aerosol particles inside the chamber were below the limits of detectability of the equipment; i. e. only background particle levels were present. Each of the arenas in a chamber during a replicate spray application were randomly assigned to different treatment combinations of insect species and post-treatment exposure times. This process was repeated four more times for a total of 5 replicates of each treatment combination, and then the spraying system was switched over to the 16 μ m SprayTec sprayer. The process of switching the sprayers required about 1.5 hours to complete. The exposure time for this sprayer was 20 minutes, and the actual exposure and labeling of the arenas was as described above. Also, it was necessary to complete replicates 4 and 5 the following morning, and these last two replicates were done between by 0930 hrs. A total of 200 arenas were treated in this manner.

All arenas were then transported back to the CGAHR facility, where they were held in a chamber set at 16:8 light-dark and 30°C-60 % r.h. One week after the spraying was done, the arenas labeled for evaluation at 1 week post treatment were removed from the chamber, and a small amount of food diet was placed in the respective arenas (~900 mg of the flour rearing diet for *T. confusum* and *L. serricorne*, 820 mg of ground dog food for *T. variabile*, and 1.5 g of ground dog food for *D. maculatus*). A companion set of four untreated arenas for each species were used as controls. The food diet amounts used were estimated to ensure enough food for normal development of 10 exposed larvae to the adult stage, without compromising the residual efficacy of the pyrethrin-methoprene treatment. The larger amount of food for *D. maculatus* was necessary because of the relative size of the larvae compared to the other three species and also

to limit larval cannibalism. Ten larvae of a species were introduced into all the corresponding treated and untreated arenas, the arenas were placed on a shelf in the chamber. This entire larval exposure process was repeated using new treated and untreated arenas at 2, 4, 6, and 8 weeks post-treatment.

The arenas were examined and classified as follows. About a week after each post-exposure bioassay was initiated, the arenas were removed from the chamber, and examined under a stereo microscope with a light attachment. Criteria for assessment were successful completion from the larval to the pupal stage, and from the pupal to the adult stage. For all species, there were various degrees of larval-pupal intermediates (individuals in some level of development between larvae and pupae) and pupal-adult intermediates (individuals in some level of development between pupae and adults). Intermediates were considered not to have advanced beyond their previous stage, i.e. larval-pupal intermediates were classified as larvae and pupa-adult intermediates were considered pupae. Successful completion of the adult stage was classified in three ways. First, adults were considered to have major deformities if they had rudimentary wings or severely split wings, unsclerotized portions of their abdominal segments, and impaired ability to move about the arena due to motor difficulties. Adults with wings that were split less severely than what is described above, wings that extended past the abdomen, slight unsclerotization of legs and antennae, along with normal motor movement, were considered to have minor deformities. Adults with uninhibited motor movement that were morphologically similar to untreated controls were considered as normal adults. An index was also created based on this classification, 1 for individuals that remaining as larvae, 2 for individuals that advanced to pupae, 3 for individuals that advanced to adult stage but with major deformities, 4 for adults with minor deformities, and 5 for morphologically normal adults.

Arenas were examined every two or three days, and the progression or lack thereof from the larval stage was recorded. The minimum value for an individual arena replicate would be 10, ten times the value of 1 for no advancement beyond the larval stage, while the maximum would be 50, ten adults times the value of 5 for morphologically-normal adults. Adults and obvious dead larvae, dead pupae, or larval-pupal or pupal-adult intermediates were removed from the arenas after they were recorded.

Data for adult emergence and the developmental index were first analyzed by species, using the PROC Mixed Procedure of the Statistical Analysis System (SAS Version 9.2, Statistical Analysis System, Cary, NC, USA) with bioassay week as the random effect, and treatment (particle size including untreated controls) as the main effect. Means for the replicated for adult emergence and developmental index were separated ($P < 0.05$) using LS means with Tukey adjustment (SAS Institute). Species were compared by summing all data for post-exposure times with replicate as the random effect.

2.2. Experiment 2

This next phase of the test was done using 4-week-old late-instar larvae of *L. serricorne*, *T. confusum* and *T. variabile*. In this test, the concrete arenas were created as described above, with the sides coated with Fluon® to limit escape of larvae from the treated surface. A total of 210 arenas were created, 30 for untreated controls and 210 for the treatments. The placement of insects, replications, and bioassay intervals for the three species were as described above. However, for this trial the food diet, in the same type and amounts for each species as described above, was placed in the arenas before being treated with either of the two aerosol particle sizes.

The actual spraying was done on 16 January 2016 using the same pyrethrin + methoprene formulations, mixed in the 100:1 pyrethrin-methoprene ratio, and following similar procedures

as described above. In this test, the 5 replicate sprays with the 16-micron Spray Tec Sprayer were done first. For each replicate aerosol spray, a total of 15 arenas were randomly placed on the false floor of the chamber (one for each of the insect species and post-exposure time combination). After the exposure interval of 20 minutes was completed, the arenas were removed, and the chamber vented for approximately 5 minutes as previously described. The arenas within the first replicate were randomly assigned to a species/post-exposure combination, as described above. This process was repeated four more times for a total of 5 more replicates, and then the spraying system was switched over to the 2-micron 24-jet Collison sprayer. This process is described above and required about 1.5 hours to complete. The exposure time for replicates treated with this sprayer was 15 minutes, and the actual exposure and labeling of the arenas was as described above. Two replicates for this sprayer were completed on 16 January 2016, and the remaining three on the following morning of 17 January.

After treatment the arenas were transported back to the laboratory at CGAHR. Residual testing was done at the same post-treatment intervals described above, using the same procedures. Bioassays were performed at the respective post-treatment intervals using the protocols described above. Statistical analysis of the data was also done using PROC Mixed in SAS for overall analysis, with replicate as a random effect, using LS Means for separation of data for the three treatments (untreated controls and the two particle sizes) at each bioassay time for each species.

2.3 Experiment 3

This experiment was initiated on 18 May 2016, and used the same protocols as described above, but only late-stage larvae of *T. confusum* and *T. variabile* and the Spray-Tec sprayer to generate the 16- μ m particles were used. The arenas were exposed to the aerosol for 20 minutes,

as described above for this particular sprayer. To test all treatment combinations (two species, presence or absence of food diet during exposure, and five post-exposure times) while maintaining the same number of dishes in the chamber as in Experiment 2, it was necessary to split the arena replicates across aerosol treatments. Four groups of arenas were exposed to aerosol spray, those with diet when treated (treated arenas with food, or TAF) for *T. confusum* and for *T. variable*, arenas with no diet when treated (treated arenas with no food, TANF) for both species as well. The arenas were exposed to the aerosol for 20 minutes, as described above for this particular sprayer. Treatments were divided so that the four groups of five dishes (e.g., one arena for each post-exposure time of 1, 2, 4, 6, and 8 weeks) and for each species, were split between two successive aerosol sprays. For example, if the first aerosol spray contained groups of arenas for the first replicates of *T. confusum* TAF, *T. variable* TAF, and *T. confusum* TANF, then the next aerosol spray would contain the first replicate of *T. variable* TANF, and second replicate for *T. confusum* TANF and second replicate of one of the TAF treatments. This process continued through seven aerosol sprays, with the last spray treatment having 10 arenas that will be used in experiments and five additional dishes added just so the numbers of arenas was kept constant. Due to limited variation between the aerosol sprays in the chambers, for statistical analysis there were five replicates of each treatment combination.

All the spray treatments were done in one day, and the arenas returned to the laboratory at CGAHR. The next day, the arenas without food during treatment (TANF), were separated by species and residual bioassay time. The standard amount of 900 mg of flour was placed in the arenas that were to have *T. confusum* added later, while 820 mg of ground dog food was placed in the arenas that were to have *T. variable* added later. At each bioassay time, for each set of five replicate arenas for each species, the food was removed from the original arenas treated with

food (TAF), and transferred to new untreated arenas, to create a new set for bioassays (transferred food from treated arenas, TAF→TTF, for transferred food). Similarly, the food was removed from the original arenas in which only the surface was treated (TANF) and food added immediately after treatment, and transferred to new untreated arenas (TANF→TTNF, for transferred food that was not treated). After the food material was transferred, the same amount of new food was added to each of the original arenas (TAF and TANF). Thus, the number of treatment combinations was doubled. There were also four sets of untreated controls for each species and bioassay time.

Larvae were added to these arenas and data collected and analyzed as described above. Data for week 6 had to be eliminated due to an error with the proper life stages being placed on the arenas and lack of available late-stage larvae for *T. variabile*. Overall analysis was through the Mixed Procedure of SAS, mean separations were now done for five treatments (untreated controls) plus the four treatment categories.

3. Results

3.1 Experiment 1

For adult emergence of exposed *L. serricorne* larvae, the main effect treatment was significant ($F=960.8$, $df = 2, 52$, $P < 0.001$), but neither main effect bioassay week ($F = 1.6$, $df = 4, 52$, $P = 0.18$) nor the interaction was significant ($F = 1.3$, $df = 8, 52$, $P = 0.25$). The lack of a bioassay week effect indicates that this species was susceptible to the insecticide residues, presumably from the methoprene component of the mixture. Adult emergence of larvae in untreated controls ranged from 85.5 ± 0.5 to $95.0 \pm 5.0\%$, but there was no emergence of larvae exposed to residues from the two aerosol particle sizes (Fig. 1A). For the developmental index, main effects, treatment and bioassay week, and the interaction were significant for *L. serricorne*

larvae ($F = 2043.8$, $df = 2$, 52, $P < 0.001$; $F = 3.0$, $df = 4$, 52, $P = 0.028$; and $F = 3.6$, $df = 8$, 52, $P < 0.001$, respectively). The index values for untreated controls ranged from 47 ± 1 to 48.9 ± 0.5 , which approached the maximum value of 50. The developmental index was always greater for controls than for larvae exposed to residues of either particle size, while the index was greater for larvae exposed to residues of the $2 \mu\text{m}$ particle size compared larvae exposed to residues of the $16 \mu\text{m}$ particles (Fig. 1B). The index for the $2 \mu\text{m}$ treatment ranged from 16.6 ± 1.7 to 20.8 ± 0.8 , indicating some advancement to the pupal stage, compared to 10 ± 0 to 10.2 ± 0.2 in the $16 \mu\text{m}$ particle size treatment, indicating little or no advancement beyond the larval stage (Fig. 1B).

For adult emergence of exposed *T. confusum* larvae, both main effects, treatment and bioassay week, and the interaction were significant at $P < 0.001$ ($F = 1153.3$, $df = 2$, 52; $F = 23.9$, $df = 4$, 52; and $F = 27.4$, $df = 8$, 52, respectively). Adult emergence ranged from 90.5 ± 5.8 to 100% in untreated controls, there was no adult emergence in larvae exposed to residues of the $16 \mu\text{m}$ particles. Adult emergence ranged from 26.0 ± 8.1 to $90.0 \pm 2.3\%$ for larvae exposed to residues of the $2 \mu\text{m}$ particles, with the only significant difference from controls occurring at week 1 (Fig. 2A). For the developmental index both main effects, treatment and bioassay week, and the interactions were significant at $P < 0.001$ ($F = 1153.3$, $df = 2$, 52; $F = 12.6$, $df = 4$, 52; and $F = 6.4$, $df = 8$, 52, respectively). The index values for untreated controls and the $2 \mu\text{m}$ particle size treatment ranged from 47.0 ± 1.7 to 50 and 31.8 ± 3.8 and 49.8 ± 0.2 , respectively, again indicating little differences between these two treatments. The index values for the $16 \mu\text{m}$ particle size treatment ranged from 10 to 15.9 ± 1.6 , indicating little advancement of *T. confusum* beyond the exposed larval stage.

For adult emergence of exposed *T. variabile* larvae both main effects treatment and bioassay week and the interaction were significant at $P < 0.001$ ($F = 960.8$, $df = 2$, 52; $F = 26.7$,

df = 4, 52; and $F = 27.0$, df = 8, 52, respectively). Adult emergence in untreated controls and of larvae exposed to the residues of the 2 μm particles ranged from 90.0 ± 6.0 to 100% and 94.0 ± 4.0 to 100%, respectively, and there were no significant differences between untreated controls and this particle size at any time (Fig 3A). There was no adult emergence of larvae exposed to residues from the 16 μm particle size treatment, except at the 4 week bioassay ($68.8 \pm 8.6\%$), which was appeared to be an anomaly (Fig. 3A). For the developmental index both main effects, treatment and bioassay week, and the interaction were significant at $P < 0.001$ ($F = 1153.3$, df = 2, 52; $F = 12.6$, df = 4, 52; and $F = 6.4$, df = 8, 52, respectively). The index values for untreated controls and the larvae exposed to residues of the 2 μm particles ranged from 47.0 ± 1.7 to 50 and 48.2 ± 1.2 to 50, respectively, with no differences between these two treatments at any bioassay time. The index values for the larvae exposed to the 16 μm particle size treatment were below 20 (no advancement to the pupal stage), except for the anomalous value of 43.2 ± 2.1 at week 4.

Dermestes maculatus was the most tolerant of the four species that were used in this experiment. There was some cannibalization by the larvae at each bioassay time, and the 8-week bioassays had to be discarded because of excessive cannibalization in untreated controls. For adult emergence, the main effect treatment was not significant ($F = 0.1$, df = 2, 43, $P = 0.87$), but both week and the treatment by week interaction were significant ($F = 21.7$, df = 3, 43, $P < 0.01$ and $F = 27.0$, df = 6, 43, $P = 0.005$, respectively), showing some variation at the residual bioassays but no differences with respect to adult emergence of larvae exposed on untreated controls or larvae exposed to residues of the two particle sizes. Adult emergence in untreated controls and the two particle size treatments ranged from 60.0 ± 6.6 to 100 %, with the variation primarily due to effects of larval and adult predation on pupae (Fig 4A). Similarly, for the

development index there was no difference between untreated controls and the two particle size treatments ($F = 0.02$, $df = 2$, 43, $P = 0.98$), with index values ranging from to 38.0 ± 0.9 to 50 (Fig. 4B), but both week and the treatment by week interaction were significant ($F = 22.3$, $df = 3$, 43, $P < 0.01$ and $F = 3.9$, $df = 6$, 43, $P = 0.004$, respectively).

Differences between species for untreated controls and the two particle size treatments were done by combining all bioassay data and re-analyzing using Proc Mixed in SAS with Tukey adjustment. There was a significant difference in adult emergence of untreated controls, with lower emergence of *D. maculatus* compared to the other three species (Fig. 5A). This was presumably because of cannibalism of pupae by larvae and adults. Adult emergence was lower in *L. serricorne* exposed to residues from the 2 μm particle size compared to the other species, and greatest in *T. variabile* (Fig. 5A). At the 16 μm particle size treatment, adult emergence of exposed larvae was greatest in *D. maculatus*, followed by *T. variabile*, with no difference between *T. confusum* and *L. serricorne* (Fig. 5A). Differences in the developmental index values for untreated controls and each of the two particle size treatments were the same as those described for adult emergence (Fig. 5B).

3.2. Experiment 2

For adult emergence of exposed *L. serricorne* larvae the main effects treatment and bioassay week, and the interaction, were all significant at $P < 0.01$ ($F = 907.2$, $df = 2$, 54, $F = 10.5$, $df = 4$, 54; and $F = 9.7$, $df = 8$, 54, respective). Adult emergence in of larvae in untreated controls ranged from 65.5 ± 13.0 to $95.5 \pm 5\%$, but there was no adult emergence of larvae exposed to residues from the two aerosol particle size treatments (Fig. 6A). For the development index, the main effects treatment and bioassay week, and the interaction, were also significant at $P < 0.001$ ($F = 1440.0$, $df = 2$, 54; $F = 10.5$, $df = 4$, 54; and $F = 9.7$, $df = 8$, 54, respectively).

Index values for untreated controls ranged from 47.0 ± 10 to 48.5 ± 0.9 (Fig. 6B). The index values for larvae exposed to residues of the $2 \mu\text{m}$ particles ranged from 16.6 ± 1.7 to 20.8 ± 0.8 , indicating that most of the larvae advanced to the pupal stage. However, most all of the larvae exposed to residues of $16 \mu\text{m}$ particles were not able to develop to the pupal stage (maximum value of 10.2 ± 0.2 , Fig. 5B).

For adult emergence of exposed *T. confusum* larvae, the main effect treatment and the interaction between treatment and bioassay week were both significant ($F = 1202.0$, $df = 2, 54$, $P < 0.001$ and $F = 2.8$, $df = 8, 54$, $P = 0.009$, respectively), but the main effect week was not ($F = 2.4$, $df = 4, 54$, $P = 0.058$). The interaction term was likely significant, because at 1 and 2 weeks post treatment there was significantly lower adult emergence of larvae exposed to the residues of the $2 \mu\text{m}$ particle size compared to the untreated controls, and significantly greater than adult emergence of larvae exposed to the residues of the $16 \mu\text{m}$ particle size (Fig. 7A). However, at all other bioassay times there was no difference between larvae exposed on untreated controls compared to the $2 \mu\text{m}$ aerosol treatment. Adult emergence in untreated controls ranged from 97.5 ± 2.5 to 100% and from 70.0 ± 8.4 to $94.0 \pm 4.4\%$ at $2 \mu\text{m}$, with the only significant differences occurring at weeks 1 and 2 (Fig. 7A). There was no adult emergence of larvae exposed to residues of the $16 \mu\text{m}$ particles. Comparing the results for *T. confusum* in this experiment to those of Experiment 1, adult emergence was much greater in this experiment at 1 week (70.0 ± 8.4 and $26.0 \pm 8.1\%$), indicating some potential loss of efficacy when the flour food source was present during treatment. For the developmental index both main effects and the interaction were significant at $P < 0.001$ ($F = 2619.7$, $df = 2, 54$; $F = 9.3$, $df = 4, 54$; and $F = 6.8$, $df = 8, 54$, respectively). The index values for untreated controls and the $2 \mu\text{m}$ particle size treatment ranged from 49.3 ± 0.7 to 50 and 41.6 ± 2.1 and 49.4 ± 0.6 , respectively, again indicating little effect of

the 2 μm particles (Fig. 7B). The index values for the 16 μm particle size treatment exceeded 10 on only one bioassay date, indicating little or no advancement of *T. confusum* beyond the exposed larval stage.

For adult emergence of *T. variabile* larvae, the main effect treatment was significant ($F = 680.5$, $df = 2, 48$, $P < 0.001$), but neither bioassay week nor the interaction were significant ($F = 0.7$, $df = 4, 48$, $P = 0.61$ and $F = 0.4$, $df = 8, 48$, $P = 0.90$, respectively). Adult emergence of larvae in untreated controls and in the residues from the 2 μm particle size treatment ranged from 92.5 ± 4.8 to 100% and from 88.0 ± 8.0 to $98.0 \pm 2.0\%$, respectively, and there were no significant differences between the two at any time (Fig. 8A). There was no adult emergence of larvae exposed to the residues of the 16 μm particles at any time (Fig. 8A). Similarly, for the development index value the main effect treatment was significant ($F = 665.0$, $df = 2, 48$, $P < 0.001$), but bioassay week and the interaction were not significant ($F = 2.4$, $df = 4, 48$, $P = 0.065$ and $F = 1.1$, $df = 8, 48$, $P = 0.40$, respectively). The index values for untreated controls and the 2 μm particle size treatment ranged from 47.7 ± 1.4 to 50 and 46.2 ± 0.8 to 19.4 ± 0.5 , respectively, with no differences at any time (Fig. 8B). The index values for the larvae exposed to residues from the 16 μm particles ranged from 10.8 ± 0.6 to 18.4 ± 0.8 , indicating some advancement beyond the larval stage, which was greater than what was observed for *T. confusum*.

Differences among the three species for untreated controls and the two particle size treatments were again evaluated by combining all bioassay week data and re-analyzing using Proc Mixed in SAS with Tukey adjustment. There was no difference in adult emergence of exposed larvae of the three species in untreated controls (Fig. 9A). No adults emerged from larvae of *L. serricornis* exposed to residues of the 2 μm particles, while emergence of larvae of *T.*

confusum and *T. variabile* was 87.2 ± 2.9 and $92.4 \pm 2.5\%$, respectively, with slightly greater emergence in *T. variabile* (Fig. 9A). At the 16 μm particle size, there was no emergence of larvae of any of the three species (Fig. 9A). There was no difference in developmental index values of untreated controls, while index values at the 2 and 16 μm particle sizes followed similar patterns as for adult emergence (Fig. 9B). There was little development of larvae exposed to residues from the 16 μm particle size.

3.3, Experiment 3

For adult emergence of *T. confusum* the main effects treatment and bioassay week were significant ($F = 226.9$, $\text{df} = 4$, 90, $P < 0.001$ and $F = 4.4$, $\text{df} = 3$, 90, $P = 0.006$, respectively), but the interaction was not significant ($F = 1.6$, $\text{df} = 12$, 90, $P = 0.09$). Adult emergence of larvae in untreated controls ranged from 84.0 ± 6.0 to $99 \pm 1\%$ (Fig 10A). Adult emergence of larvae in untreated controls was always greater than adult emergence of larvae in any of the arenas exposed directly or indirectly to the aerosol. The two treatments with food that was either treated directly in the arena with aerosol or added to the treated arena a day after aerosol treatment, but subsequently transferred to new untreated dishes (TTF and TTNF) had the strongest suppression of development to the adult stage with no adult emergence at any post-treatment time (Fig. 10A). Adult development on arenas that had been treated with aerosol but had the food present during or immediately after treatment removed and new untreated food added (TAF and TANF) was more variable, with some exposed larvae able to complete development to the adult stage. These results indicate that the food material absorbed the aerosol insecticide and/or blocked its deposition on surfaces.

For the development of *T. confusum* main effects treatment and bioassay week, and the interaction, were all significant ($F = 203.78$, $\text{df} = 4$, 90, $P < 0.001$; $F = 3.4$, $\text{df} = 3$, 90, $P =$

0.020), and $F = 1.9$, $df = 12, 90$, $P = 0.049$, respectively). The index values for untreated controls ranged from 44.9 ± 1.8 to 49.6 ± 0.4 , and was always greater than the index in the four treatments (Fig. 10B). The developmental index for the larvae exposed in the arenas originally treated in the chambers, (TAF for those with food, TANF for those in which food was added a day later), tended to have higher developmental indexes on each bioassay date, ranging from 19 ± 2.4 to $38.4 \pm 3.9\%$, than those exposed on flour moved from treated arenas (TF for the flour moved from the TAF arenas, TNF for flour added one day later to the treated arenas), which ranged from 12.6 ± 1.1 to $15.8 \pm 1.5\%$ (Fig. 10B). This indicated less advancement past the larva stage when exposed to the food treated or added to treated arenas immediately after treatment, suggesting that the flour absorbed some of the residues from the aerosol application.

For *T. variabile* adult emergence, the main effects treatment and bioassay week and the interaction were significant at $P < 0.001$ ($F = 285.8$, $df = 4, 91$; $F = 6.2$, $df = 3, 91$; and $F = 3.5$, $df = 12, 91$, respectively). The overall trend was similar to results described above with *T. confusum*, except that there were more times post-treatment where adult emergence was significantly less in treatments where flour was added to treated arenas the day after treatment (TANF) than in treatments where flour was present when aerosol was applied to the arenas (TAF) (Fig. 11A). Adult emergence of larvae on untreated controls ranged from 84.0 ± 6.0 to $99 \pm 1\%$, which was similar to emergence of control larvae of *T. confusum*. No adults emerged at any time from larvae exposed on the flour that was originally treated with the arena then moved to untreated arenas (TTF), or on the flour placed on the arenas one day after treatment then moved to new arenas (TTNF), again indicating that biologically active residues were contained in the transferred flour (Fig 11A).

For the *T. variabile* development index, the main effects treatment and bioassay week, and the interaction, were all significant for the developmental index values as well ($F=247.2$, $df = 4$, 91 ; $P < 0.001$; $F = 3.4$, $df = 3$, 90 , $P = 0.004$; and $F = 3.3$, $df = 12$, 90 , $P < 0.001$, respectively). The index values for untreated controls ranged from 46.0 ± 1.1 to 50, and was always greater than the index in the four treatments (Fig 11B). The developmental index for the larvae exposed in the TAF and TANF treatments, arena originally treated with aerosol, ranged from 19.0 ± 0.8 to $38.4 \pm 1.9\%$. On two occasions the index values were greater in the TAF compared to TANF treatments, and on two occasions the lowest index values were in the TTF compared to the TTNF treatments (Fig. 11B). Overall, there was little advancement past the larval stage in those latter two treatments.

4. Discussion

This is the first report of susceptibility of larval *D. maculatus* to an aerosol insecticide, and our results showed that the late larval stage was not susceptible to the methoprene residues from the aerosol application, which is consistent with recent studies in which adult *D. maculatus* have been exposed on surfaces treated with contact insecticides. Kavallieratos et al. (2017) conducted a study in which adult stored product beetles were exposed on packaging with the pyrethroid deltamethrin incorporated into the laminate layers of the packaging. Maximum mortality of *D. maculatus* was 5.6% after 5 days of exposure. Similarly, Athanassiou et al. (2013), again using the same parent strain of *D. maculatus*, exposed adults of this species and also *T. castaneum*, and *T. confusum*, among other species, on concrete surfaces treated with Temprid[®], an insecticide that is a mixture of imidacloprid and the pyrethroid cyfluthrin. Mortality of these species after 7 days of exposure did not exceed 57, 25, and 17%, respectively, but in that study *Tribolium* spp. were more tolerant than *D. maculatus*. Hence, the specific

response of *D. maculatus* to an insecticide may depend on the testing procedures, the life stage, the specific insecticide, and the particular strain being used for the studies. One difficulty in evaluating the current study is that larvae of *D. maculatus* are extremely cannibalistic (Fontenot et al., 2015), which creates a potential bias in the results. In our study, groups were exposed on the treated arenas, and a better approach may be to expose individual larvae. The drawback in this approach is that dermestids tend to go into diapause at low densities (Wilches et al., 2016), which may also create issues in evaluating the data.

This is also the first published study evaluating susceptibility of larval *L. serricornis* to residues from an aerosol application involving the current formulation of methoprene that is labeled in the USA. Also, there are no recent studies evaluating *L. serricornis* in comparison with other stored product beetles. *L. serricornis* is known to be a serious pest of tobacco, but it can develop on a wide range of food products even though it does not feed as an adult (Abdelgahny et al., 2016). The adults live only 1-2 weeks (Sağlam et al., 2015), hence an aerosol with a residual IGR may be more useful for management of this species compared to a surface contact adulticide. The strain of *L. serricornis* used in this study was extremely susceptible to methoprene, as larvae exposed to residues from the 2µm application could not develop past the pupal stage, in contrast to development in the other species.

In previous tests in which *T. castaneum* and *T. confusum* larvae were exposed on surfaces treated with methoprene, *T. castaneum* was the more susceptible of the two species (Arthur and Fontenot, 2012). Larvae of *T. confusum* were used in this test to select the least susceptible species, and because adults of *T. confusum* were used in the previous tests in the chambers at MRIGlobal (Arthur et al., 2014). In experiments 1 and 2, adult emergence in larvae exposed to 2µm was less than emergence in untreated controls, but greater than the zero emergence in the

16 μ m treatment. Values for the developmental index followed the same pattern. Adult emergence and developmental index values were greater after this week 1 bioassay in experiment 2 compared to experiment 1, indicating a slight loss in efficacy when the flour food source was treated directly. However, this was the only time bioassay where there was a difference between the two experiments, as thereafter there was high adult emergence and high index values in the 2 μ m treatment.

There are currently no published data in scientific journals regarding susceptibility *T. variabile* larvae to methoprene. However, results from recent laboratory research suggests that *T. variabile* may be more tolerant to methoprene compared to *T. castaneum* (Scheff, 2016). In this research, a series of studies in which larvae of both species were exposed on packaging material with 0.1% active ingredient methoprene incorporated into the laminate layers. Larvae of *T. castaneum* continuously exposed for 1-4 days on the outside and inside of the packaging surface did not emerge as normal adults, while *T. variabile* larvae could not complete development on the outside surface. However, some development did occur with larvae exposed in the inside surface. When larvae of both species were exposed for short times of 1-4 days, emergence of exposed *T. variabile* larvae was generally lower than that of *T. castaneum* larvae exposed on the outside and inside bag surfaces. A cautionary note is that these studies were done with single strains of each species, and results may or may not be transferrable to other strains of the same species.

The developmental index utilized in this study provided an additional method to assess efficacy of the IGR component of the aerosol mixture, which can have effects on insect fitness beyond just adult emergence. The developmental index created for this study is the first to incorporate the range of morphological defects and delays in development associated with larval

exposure to an IGR, including the presence of intermediate life stages, were first described by Hoppe (1981), in studies with methoprene and *T. castaneum*. These defects have been categorized in Sutton et al. (2011), which included the separation of minor and major deformities in emerged adults. Failing to advance from the larval stage or deformities that occur during the larval pupal transition are predicted to be associated with a higher level of exposure to the IGR or a greater level of sensitivity than deformities exhibited during the pupa to adult transition, and morphological effects exhibited in adults only indicate an even lower level of exposure or less sensitivity to the IGR. The index as calculated captures this gradation and enables these wide range of IGR effects to be quantified and analyzed using a single value. The index clearly showed higher values for all species exposed to the 2 μm particles compared to the 16 μm particles in experiments 1 and 2, indicating that the larger particles resulted in less development along the larval-adult continuum. However, although there was variation with respect to week, there was not a clear corresponding increase in index values as the weeks progressed, which would seem to indicate a relative stability of the methoprene residues during the 8-week residual testing period. It is likely that a longer residual exposure period could have yielded more variation in the index over time.

Prior tests have documented reduced efficacy of contact surface treatments and aerosols when adult *T. confusum* or *T. castaneum* are provided with a food source either during or after exposure (Arthur, 2009, 2013, 2015ab). The current test also shows how the presence of extraneous food material can reduce deposition of insecticides on a concrete surface, or lead to removal of residues through absorption of those residues by the food material. In a previous test, concrete arenas were treated with the pyrethroid cyfluthrin, and a flour food source was allowed to remain on the arenas for up to eight weeks before removal (Arthur et al., 2015a). The food

source absorbed residues from the treated surface, as shown by increased time to 100% knockdown on the surface after the flour was removed, compared to the time required for 100% knockdown on a clean treated surface. In the current test, when the food source was treated along with the arena, deposition of the aerosol was reduced. This was evident by the increased adult emergence and increased index values of both *T. confusum* and *T. variabile* exposed on the surface with a new food source after the treated food source was removed. The amount of food in the dish was a thin layer that covered virtually all of surface of the arena but was only about 1-2 mm in depth. No larvae exposed on the transferred treated flour were able to emerge as adults. Similar effects occurred when the food source was placed on the arenas after they were treated; in this case the food source absorbed some of the residues, similar to what was reported in the Arthur et al. (2015a) study for cyfluthrin. These results show how inside food facilities the presence of food residues during an aerosol application can reduce efficacy, but also how the continual deposition of food materials on the flooring surface during the manufacturing process and the regular removal of food materials during sanitation programs can potentially reduce the residual efficacy of aerosol treatments. Thus, the importance of cleaning and sanitation in conjunction with insecticide applications needs to be continually emphasized as an important component of integrated management programs involving aerosol applications. But is also needs to be emphasized that even with a good sanitation program, residual efficacy under real world conditions is likely to be less than can be obtained under more controlled laboratory conditions.

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549 of Agriculture or MRIGlobal. The US Department of Agriculture and MRIGlobal are equal
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Figure Captions

Fig. 1. Percentage of adult *L. serricorne* (A, mean $\pm$ SE) that emerged from late-stage larvae and Developmental Susceptibility Index values (B, mean $\pm$ SE) exposed on concrete arenas treated with a 100:1 ratio of pyrethrin+ methoprene at 2 μ m and 16 μ m. Bioassays conducted at 1, 2, 4, 6, and 8 weeks after initial treatment. Means at each week for adult emergence and index values followed by the same letter are not significantly different ($P \geq 0.05$, Tukey test under PROC Mixed in SAS).

Fig. 2. Percentage of adult *T. confusum* (A, mean $\pm$ SE) that emerged from late-stage larvae and Developmental Susceptibility Index values (B, mean $\pm$ SE) exposed on concrete arenas treated with a 100:1 ratio of pyrethrin+ methoprene at 2 μ m and 16 μ m. Bioassays conducted at 1, 2, 4, 6, and 8 weeks after initial treatment. Means at each week for adult emergence and index values followed by the same letter are not significantly different ($P \geq 0.05$, Tukey test under PROC Mixed in SAS).

Fig. 3. Percentage of adult *T. variabile* (A, mean $\pm$ SE) that emerged from late-stage larvae and Developmental Susceptibility Index values (B, mean $\pm$ SE) exposed on concrete arenas treated with a 100:1 ratio of pyrethrin+ methoprene at 2 μ m and 16 μ m. Bioassays conducted at 1, 2, 4, 6, and 8 weeks after initial treatment. Means at each week for adult emergence and index values followed by the same letter are not significantly different ($P \geq 0.05$, Tukey test under PROC Mixed in SAS).

Fig. 4. Percentage of adult *D. maculatus* (A, mean $\pm$ SE) that emerged from late-stage larvae and Developmental Susceptibility Index values (B, mean $\pm$ SE) exposed on concrete arenas treated with a 100:1 ratio of pyrethrin+ methoprene at 2 μ m and 16 μ m. Bioassays conducted at 1, 2, 4,

and 6 weeks after initial treatment (8-week bioassays discarded due to high predation).

Treatments were not significantly different ($P \geq 0.05$, Tukey test under PROC Mixed in SAS).

Fig. 5. Species differences in adult emergence (A, mean $\pm$ SE) and developmental index values (B, means $\pm$ SE), summed for the 8-week residual testing period. Means for adult emergence and index values between the four species followed by the same letter are not significantly different ($P \geq 0.05$, Tukey test under PROC Mixed in SAS).

Fig. 6. Percentage of adult *L. serricornis* (A, mean $\pm$ SE) that emerged from late-stage larvae and Developmental Susceptibility Index values (B, mean $\pm$ SE) exposed on concrete arenas containing a flour food source and treated with a 100:1 ratio of pyrethrin+ methoprene at 2 μ m and 16 μ m. Bioassays conducted at 1, 2, 4, 6, and 8 weeks after initial treatment. Means at each week for adult emergence and index values followed by the same letter are not significantly different ($P \geq 0.05$, Tukey test under PROC Mixed in SAS).

Fig. 7. Percentage of adult *T. confusum* (A, mean $\pm$ SE) that emerged from late-stage larvae and Developmental Susceptibility Index values (B, mean $\pm$ SE) exposed on concrete arenas containing a flour food source and treated with a 100:1 ratio of pyrethrin+ methoprene at 2 μ m and 16 μ m. Bioassays conducted at 1, 2, 4, 6, and 8 weeks after initial treatment. Means at each week for adult emergence and index values followed by the same letter are not significantly different ($P \geq 0.05$, Tukey test under PROC Mixed in SAS).

Fig. 8. Percentage of adult *T. variabile* (A, mean $\pm$ SE) that emerged from late-stage larvae and Developmental Susceptibility Index values (B, mean $\pm$ SE) exposed on concrete arenas containing a flour food source and treated with a 100:1 ratio of pyrethrin+ methoprene at 2 μ m and 16 μ m. Bioassays conducted at 1, 2, 4, 6, and 8 weeks after initial treatment. Means at each

week for adult emergence and index values followed by the same letter are not significantly different ($P \geq 0.05$, Tukey test under PROC Mixed in SAS).

Fig. 9. Species differences in adult emergence (A, mean $\pm$ SE) and developmental index values (B, means $\pm$ SE), summed for the 8-week residual testing period. Means for adult emergence and index values between the three species followed by the same letter are not significantly different ($P \geq 0.05$, Tukey test under PROC Mixed in SAS).

Fig. 10. Percentage of adult *T. confusum* that emerged (A) and the developmental index (B) (mean $\pm$ SE) from late-stage larvae exposed on concrete arenas treated in the following manner. The aerosol was the 100:1 ratio of pyrethrin+ methoprene at 16 μ m, with bioassays conducted at 1, 2, 4, and 8 weeks after initial treatment. Five treatment categories as follows: untreated controls containing a flour food source (UTC), arenas treated with a flour food source and then the treated flour was transferred to new untreated arenas with an untreated food source (TTF), while untreated flour was placed in the original treated arena (TAF); or clean arenas were treated with the aerosol and then the flour was added one day later, then transferred to untreated arenas (TTNF) while new flour was placed on the original treated arenas (TANF). Means at each week for adult emergence and index values followed by the same letter are not significantly different ($P \geq 0.05$, Tukey test under PROC Mixed in SAS). No adult emergence at any time on TTF or TTNF arenas, no bars for these treatments on Fig. 10A.

Fig. 11. Percentage of adult *L. serricornis* that emerged (A) and the developmental index (B) (mean $\pm$ SE) from late-stage larvae exposed on concrete arenas treated in the following manner. The aerosol was the 100:1 ratio of pyrethrin+ methoprene at 16 μ m, with bioassays conducted at 1, 2, 4, and 8 weeks after initial treatment. Five treatment categories as follows: untreated controls containing a flour food source (UTC), arenas treated with a flour food source and then

the treated flour was transferred to new untreated arenas with an untreated food source (TTF), while untreated flour was placed in the original treated arena (TAF); or clean arenas were treated with the aerosol and then the flour was added one day later, then transferred to untreated arenas (TTNF) while new flour was placed on the original treated arenas (TANF). Means at each week for adult emergence and index values followed by the same letter are not significantly different ($P \geq 0.05$, Tukey test under PROC Mixed in SAS). No adult emergence at any time on TFF or TTNF arenas, no bars for these treatments on Fig. 10A.

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