**Susceptibility of *Trogoderma granarium* Everts and *Trogoderma inclusum* LeConte
(Coleoptera: Dermestidae) to Residual Contact Insecticides**

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Abstract

Commercial formulations of the pyrethroid insecticides β -cyfluthrin and deltamethrin were evaluated for delayed mortality of *Trogoderma granarium* (Everts), the khapra beetle, and *T. inclusum* (LeConte), the larger cabinet beetle. Ten 3-4-week-old larvae of either species were exposed on treated concrete arenas for 1, 2, 3, and 7 d then transferred into 175 ml diet cups containing 5 g of untreated rearing media for 30 d to evaluate delayed mortality. In a subsequent study, residual efficacy of β -cyfluthrin, deltamethrin, and chlorfenapyr was evaluated at 0-12 wks post-treatment against adults and larvae of a laboratory and a field strain of *T. granarium*. Ten adults or larvae of either strain were exposed on treated arenas at selected wks post-treatment and mortality was assessed after 4 and 30 d of exposure for adults and larvae, respectively. In the first study, delayed mortality of *T. granarium* and *T. inclusum* larvae was <26% when exposed on arenas treated with the two pyrethroids, and *T. inclusum* larvae were less susceptible than *T. granarium*. Adult mortality was 90-100% for both strains of *T. granarium* across post-treatment assays regardless of insecticide treatment and there was no difference between strains. All three insecticides produced 100% mortality for the laboratory strain of *T. granarium* larvae in initial post-treatment assays, but then efficacy declined. Field strain larvae were more tolerant to the insecticides than laboratory strain larvae, and larvae of both *T. granarium* strains were more tolerant than adults. Longer exposure times were required to produce complete mortality of larvae compared to adults. All three insecticides provided residual efficacy of adults for up to three months.

Key words: *Trogoderma*, quarantine pest, insecticides, residual efficacy, stored product insects.

1. Introduction

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) is recognized worldwide as a serious pest of stored products (Hagstrum and Subramanyam, 2009; Myers and Hagstrum, 2012). It is native to India and prefers a hot and dry climate where it frequently infests dried grains (Aitken 1975; EPPO, 2016). Larvae are scavengers that feed on a wide variety of stored products and dried foods (Athanasios et al., 2016). While the adults are short-lived, typically 7 to 10 days, larvae are long-lived and frequently enter a dormant state in response to adverse conditions such as overcrowding, low temperature, lack of food, or isolation (Bell, 1994). *T. granarium* larvae are capable of surviving for several years, but are able to break dormancy rapidly and continue regular development when favorable conditions return (Bell, 1994).

Trogoderma granarium is not presently known to be established in North America, and is considered a regulated pest that is a major threat to U.S. agriculture. In recent years detections of *T. granarium* in the U.S. have increased dramatically with increased interceptions at ports of entry (Myers and Hagstrum, 2012). When *T. granarium* is detected at U.S. ports of entry in commercial cargo, the US Department of Agriculture (USDA) mandates that the shipment must be destroyed, re-exported outside of North America, or fumigated with methyl bromide if the cargo is amenable to a fumigation treatment. *T. granarium* is also found in warehouses, cargo containers and other areas associated with international commercial shipments outside of the port environment (USDA-APHIS, 2015). These detections have renewed concern that *T. granarium* could become established in the U.S. (French and Venette, 2005). Previous infestations and establishments in the U.S. have resulted in lengthy and expensive control and eradication programs (Myers and Hagstrum, 2012). Similarly, infestations in Australia (Banks, 1977; Day and White, 2015) and Japan (Sonda, 1968) have also required significant resources to eradicate

60 this insect. Establishment of *T. granarium* in the U.S. would present concerns for domestic and
61 international trade markets. Australian estimates of the cost of *T. granarium* establishment are as
62 much as hundreds of millions of dollars annually (Day and White, 2015). The economic impact
63 on the U.S. grain industry could be even greater as the U.S. produces and exports more grain
64 compared to most developed countries (USDA-APHIS, 2015). It is therefore critical to have safe
65 and effective treatment options to eradicate this pest when it is intercepted at U.S. ports, or when
66 localized populations are detected elsewhere in the U.S.

67 Due to its status as a quarantine pest in the USA, laboratory cultures have not been
68 readily available to evaluate reduced-risk insecticides recently labeled for control of stored-
69 product insects in the U.S. Therefore, there are limited data regarding susceptibility of *T.*
70 *granarium* to these new insecticides. Current recommendations for *T. granarium* control rely
71 heavily on treatments with organophosphate insecticides and fumigation with methyl
72 bromide. Although international agreements currently allow for quarantine and pre-shipment uses
73 of methyl bromide, research on methyl bromide alternatives for quarantine insect pests remains a
74 priority (Myers and Hagstrum, 2012).

75 Recent efforts have improved knowledge regarding susceptibility of *T.*
76 *granarium* (e.g. Athanassiou et al., 2015; Kavallieratos et al., 2016), however; additional research
77 is still needed to assess efficacy and residual activity. It is particularly important to evaluate
78 susceptibility of *T. granarium* using insecticides already labeled in the U.S. to control other
79 stored-product insects. Developing a strong database for *T. granarium* control could help prevent
80 outbreaks and potential establishment should it be intercepted and/or detected. Therefore, the
81 objective of this study was to evaluate three commercial insecticides for control of *T.*
82 *granarium* adults and larvae. Results will provide state and federal regulatory agencies with data

to support treatment decisions for eradicating *T. granarium* in different settings. The larger cabinet beetle, *Trogoderma inclusum* (LeConte) is a closely related species to *T. granarium*, but it is not a quarantine pest in the U.S., thus comparisons may be beneficial for determining if *T. inclusum* can be used as a surrogate species for *T. granarium* to use in expanded experiments and avoid the necessity of a quarantine facility.

2. Materials and Methods

2.1. Delayed mortality of *T. granarium* and *T. inclusum* larvae exposed to β -cyfluthrin and deltamethrin for short durations: Experiment 1

This study was conducted at the USDA-APHIS-Center for Plant Health Science and Technology (CPHST), Otis Laboratory, Buzzards Bay, MA, USA. The insects used in this first phase of the study were a field strain of *T. inclusum* collected from a milling facility in the U.S. in 2012, and a field strain of *T. granarium* collected from Pakistan in 2011 and sent to the Otis Laboratory. Insects were reared in 0.95L glass jars using a diet of ~200 g of protein shake mix (IsaLean Shake Creamy French Vanilla, IsaGenix International LLC, Chandler, AZ, USA) with ~20 g of organic oatmeal (Heartland Mills, Marienthal, KS, USA) sprinkled on the surface. Both species were maintained in environmental growth chambers set to $29 \pm 1^\circ\text{C}$, $35 \pm 5\%$ r.h., and a photoperiod of 0:24 (L:D) h. The *T. granarium* cultures were held in a separate incubator and located in the Otis Laboratory insect containment facility.

Individual treatment arenas were constructed in the bottom portion of Plastic Petri dishes, 20 mm tall with an approximate area of 62 cm^2 , using Rockite[®] concrete mixture (Hartline Products, Cleveland, OH, USA). The Rockite[®] was mixed with water to form a liquid slurry, poured into a Petri dish to a depth of ~1.25 cm, then air dried for several days on a laboratory counter at $\sim 25^\circ\text{C}$, as described in recent publications (Arthur, 2013, 2015; Ghimire et al., 2016)

The insecticides used for the study were commercial formulations of the pyrethroids β -cyfluthrin (Tempo[®] SC Ultra, 11.8% active ingredient [AI], 120 mg AI/ml) and deltamethrin (Suspend[®] SC 4.75 % AI, 50 mg AI/ml). According to the insecticide label, β -cyfluthrin can be applied at either 10 or 20 mg AI/m², while deltamethrin can be applied at 4 to 24 mg AI/m². For this experiment, we chose the highest label rate for both insecticides. The volumetric spray application per area of the concrete arena was 0.27 ml of formulated spray per the area of the arena, which was rounded to 0.3 ml of spray. β -cyfluthrin was formulated by mixing 0.1 ml of the insecticide into 25 ml water, and applying the formulated solution at a rate of 0.3 ml/arena, in the same ratio as the label specifications of 3,784 ml of formulated solution per 94 m². Deltamethrin was formulated by mixing 0.3 ml of the insecticide into 25 ml water, and applying the same volume of 0.3 ml of formulated insecticide per arena, which is again equivalent to the label specifications for spray volume. A Badger 100 artist's airbrush (Badger Airbrush Co., Model 100, Franklin Park, IL, USA) connected to an air compressor, Badger Airstorm (Badger Airbrush Co., Model 180-15, Franklin Park, IL, USA) was used to spray the solution as a fine mist onto the concrete surface. Untreated controls were sprayed with the same amount of water. There were five replicates for each insecticide treatment and five for the untreated controls. Separate insecticide solutions were formulated for each of those replicates.

A total of 120 arenas were treated for each replicate of each of the three treatments (controls plus the two insecticides). For each replicate 10 three-four week-old larvae of either *T. inclusum* or *T. granarium* were placed into an arena, along with 2 g of the rearing diet, and exposed for either 1, 2, 3, or 7 d. The arenas containing *T. inclusum* and *T. granarium* were held in environmental chambers at $30 \pm 1^\circ\text{C}$ and $35 \pm 5\%$ r.h. in continual darkness.

Upon completion of the exposure interval, the arenas were placed under a stereomicroscope (Huvitz Model, HSZ-645, Geumjeong-dong, Republic of Korea), and the insects prodded with a probe. They were classified as knocked down if they were alive but not moving. Larvae from each arena were then transferred to 175ml diet cups containing 5 g of untreated rearing media to evaluate latent or delayed larval mortality. The cups with larvae were then returned to the original environmental chambers in which the exposure arenas were held. After 30 d these diet cups were removed from the environmental chamber, and larvae were categorized as dead if they were unable to respond when prodded. Any pupae that were black and discolored were also classified as dead. Larvae that were moving after being probed were considered live for the purposes of this study. Data were analyzed using the General LinearModels Procedure (PROC GLM) of the Statistical Analysis System (SAS Institute, version 9.3, Cary, NC, USA) to determine significant differences between treatments and exposure times. The percentage of affected and dead larvae were transformed using arcsine square root prior to analysis to meet the ANOVA assumption of homogeneity of variance. The untransformed data are presented in the text and figures.

2.2. Residual or post treatment assays of T. granarium adults and larvae exposed to β -cyfluthrin, deltamethrin, and chlorfenapyr: Experiment 2

Based on the results of the first experiment, a second experiment was initiated to determine susceptibility of *T. granarium* larvae to β -cyfluthrin and deltamethrin, plus the insecticidal pyrrole chlorfenapyr (Phantom™, 24.54% AI, 240 mg AI/ml). Larvae and adults of the lab and field strains of *T. granarium* were used in this study. The lab strain of *T. granarium* was brought into the U.S from Poland in June 2013. This strain had been maintained in a laboratory in Poland for over 30 years. The same field strain of *T. granarium* used in the first

study was also used here. A total of 480 concrete arenas were constructed, as described above, and the two strains (lab strain from Poland and the field strain from Pakistan) of *T. granarium* that were used for the first test were used here as well. β -cyfluthrin and deltamethrin were formulated and applied as described above. The insecticide label for chlorfenapyr specifies mixing 89 ml of the formulation in 3,784 ml water to make a 0.5% dilution, hence the equivalent amount of formulation in 25 ml water is 1.2 ml. This dilution is then used at a label rate of 35 ml to treat an area of 1,829 cm². This gives a volume rate of 1.2 ml of formulated spray per area of the concrete arena, which is 1,100 mg AI/m². Reducing the volume by 75% to give 0.3 ml per area of the arena reduces the AI by 75%, which gives a final calculated deposition of 280 mg/m². There were six replicates of each insecticide, plus an untreated control, with separate solutions of insecticide formulated for each replicate, sprayed with appropriate solutions using the airbrush system previously described.

For each replicate and treatment, either ten 3-4 week-old larvae along with 2 g of rearing diet, or ten 1-4 day-old adults without a food source, were placed in an individual arena. The residual efficacy assays were conducted at 1 d after treatment (time 0), and then 2, 4, 8, and 12 wks post-treatment, using different arenas each time. After the tests at the first post-treatment assay (time 0), the remaining untreated and treated arenas were boxed and placed on shelves in a laboratory room until they were used for subsequent post-treatment assays at 2, 4, 8, and 12 wk. At those times, exposure procedures were done as described above. Arenas containing adults or larvae of *T. granarium* were held in the environmental chamber at $30 \pm 1^\circ\text{C}$ temperature, $35 \pm 5\%$ r.h. in continuous darkness inside the containment facility until the evaluations of mortality were completed. The arenas containing adults were examined after 4 d, and they were classified as dead if they did not respond to being prodded with a probe. Larvae were continuously exposed

on the arenas for 30 d, and again classified as dead if they did not respond to being prodded with the probe.

Statistical analysis was done as described for the previous experiment, with the GLM and *t*-test procedures used to determine significant differences in mortality between adults and larvae. The percent mortality of larvae or adults was used as response variable to assess the residual efficacy of β -cyfluthrin, deltamethrin, and chlorfenapyr against the lab and field strain of *T. granarium*. Because the application rates for the three insecticides were not directly comparable, the data collected from each insecticide were analyzed separately. Similarly, the data collected for adults and larval mortality were also analyzed separately. Insecticide, insect strain, and residual or post treatment assay time were fixed effects, and replications were random factors for the analysis of the response variables. Data for adult and larval mortality from both insecticide experiments were arcsine square root transformed before analysis to meet the assumptions of normality and homogeneity of variance. Means were separated using Tukey's studentized honestly significant difference (HSD) test ($\alpha=0.05$) under the GLM procedure, and original mean values from untransformed data are presented in the text and figures.

3. Results

3.1. Delayed mortality of T. granarium and T. inclusum larvae exposed to β -cyfluthrin and deltamethrin treated arenas: Experiment 1

The overall experiment-wide delayed mortality for larvae exposed on untreated arenas was significantly lower ($F = 39.06$, $df = 2, 102$, $P < 0.001$) as compared to those larvae exposed on treated arenas. Adult emergence of larvae exposed on untreated arenas (74.3%) was significantly greater ($P < 0.001$) than those that emerged from the arenas treated with β -cyfluthrin

(46%) and deltamethrin (44%), regardless of exposures times and insect species. Affected larvae and delayed mortality of larvae from the untreated control arenas at all exposure times was less than 1%, hence no corrections or adjustments were necessary for treatment data, and data from untreated controls were not included in the subsequent statistical analysis (Robertson et al., 2007).

The percentage of affected larvae of *T. granarium* and *T. inclusum* that were exposed on arenas treated with β -cyfluthrin ranged from 94.0 ± 6.0 to 100% across the four exposure times (Fig. 1a), but did not vary with the insect species ($F = 1.85$, $df = 1$, 32 , $P = 0.183$) or exposure time ($F = 0.72$, $df = 3$, 32 , $P = 0.549$). In contrast, larval mortality was much lower (ranging from 6.0 ± 2.45 to $26.0 \pm 5.1\%$ for *T. granarium* and 4.0 ± 4.0 to $10.0 \pm 4.5\%$ for *T. inclusum*), compared to affected larvae (Fig. 1b), varied significantly with the insect species ($F = 8.23$, $df = 1$, 32 , $P = 0.007$), but did not differ with the exposure time ($F = 2.32$, $df = 3$, 32 , $P = 0.094$). The interaction of insect species by exposure time was non-significant ($P \geq 0.05$). The only significant difference of larval mortality between the two species (26.0 ± 5.1 for *T. granarium* and $10.0 \pm 4.5\%$ for *T. inclusum*) occurred at 3 d of exposure time (Fig. 1b).

Similar results occurred for *T. granarium* and *T. inclusum* larvae that were exposed on arenas treated with deltamethrin. The affected larvae ranged from 94.0 ± 6.0 to 100% for *T. granarium* and 92.0 ± 3.7 to 100% for *T. inclusum* (Fig. 2a). The main effects insect species and exposure time were non-significant ($P \geq 0.05$), but the interaction of insect species by exposure time was significant ($F = 3.57$, $df = 3$, 32 , $P = 0.025$). The larval mortality was much lower (ranging from 10.0 ± 6.3 to $34.0 \pm 6.8\%$ for *T. granarium* and 6.0 ± 2.4 to $18.0 \pm 3.7\%$ for *T. inclusum*) as compared to affected larvae (Fig. 2b). Mortality of *T. granarium* larvae was always greater than mortality of *T. inclusum* larvae across the four exposure times and varied with exposure time (F

= 4.53, df = 3, 32, $P = 0.009$). The only significant difference of larval mortality between two species ($34.0 \pm 6.8\%$ for *T. granarium* and $18.0 \pm 3.7\%$ for *T. inclusum*) occurred at 7 d exposure time (Fig. 2b).

3.2. Residual or post treatment exposure of *T. granarium* adults and larvae exposed to β -cyfluthrin, deltamethrin, and chlorfenapyr treated arenas: Experiment 2

In all of the post treatment assay times with β -cyfluthrin, deltamethrin, and chlorfenapyr, mortality of the lab and field strains of *T. granarium* adults on untreated control arenas ranged from 3.3 to 13.3% and varied with exposure time, hence the mean adult mortality for corresponding treatments was corrected for control mortality using Abbott's formula (Abbott, 1925). However, the larval mortality of both strains on untreated control arenas was $<1.0\%$ for all three insecticide assays, hence no corrections or adjustments were made for treatment data and untreated control data were not included in the statistical analysis (Robertson et al., 2007). The overall experiment-wide effect of insecticides was significant for larval mortality ($F = 9.18$, df = 2, 150; $P < 0.001$), but it was non-significant for the adult mortality ($P \geq 0.05$). Larval mortality was greater on the arenas treated with chlorfenapyr or β -cyfluthrin than on the arenas treated with deltamethrin, regardless of post-treatment assay time or insect strains.

3.2.1. Exposure to β -cyfluthrin

The overall ANOVA, which included main effects post-treatment assay time ($F = 5.5$, df = 4, 100; $P < 0.001$), insect strain ($F = 12.2$, df = 1, 100; $P < 0.001$), life stage ($F = 35.9$, df = 1, 100; $P < 0.001$), were significant for larval mortality of *T. granarium*. The interaction of bioassay time by insect strain was significant ($F = 13.9$, df = 1, 100; $P < 0.001$), but all other associated interactions were non-significant ($P \geq 0.05$). Mortality of field strain adults was always greater

than mortality of field strain larvae across all of the post-treatment assay times, but the only significant difference occurred at 0, 2, and 12wk post-treatment assays (Fig. 3ab). Similarly, mortality was always higher for lab strain adults as compared to lab strain larvae across all of the post-treatment assay times, but the only significant difference in the mortality between adults and larvae occurred at 4wk post treatment (Fig. 3ab).

The subsequent two-way ANOVA, analyzed separately for adult and larval mortality, showed no significant difference for adult mortality for post-treatment assay time or with insect strain ($P \geq 0.05$). The interaction of assay time by insect strain was also non-significant ($P \geq 0.05$). The mortality of *T. granarium* adults ranged from 89.5 ± 4.7 to 100% for the field strain and 89.5 ± 5.4 to $98.3 \pm 1.7\%$ for the lab strain (Fig. 3a). In contrast, larval mortality varied significantly with residual or post-treatment assay time ($F = 6.1$, $df = 4$, 50; $P < 0.001$) and insect strain ($F = 28.1$, $df = 1$, 50; $P < 0.001$) and the interaction of assay time by insect strain ($F = 3.1$, $df = 4$, 50; $P < 0.001$). The larval mortality ranged from 66.7 ± 6.1 to $88.3 \pm 3.1\%$ for the field strain and 76.7 ± 6.7 to 100% for the lab strain of *T. granarium* (Fig. 3b). Mortality of the lab strain larvae in the first two post-treatment assay times, 0 (1day) and 2wk, was significantly greater (100%) than mortality of field strain larvae (Fig. 3b).

3.2.2. Exposure to deltamethrin

The overall ANOVA, which included main effects residual or post-treatment assay time ($F = 5.3$, $df = 4$, 100; $P = 0.001$), insect strain ($F = 53.2$, $df = 1$, 100; $P < 0.001$), life stage ($F = 121.7$, $df = 1$, 100; $P < 0.001$) were all significant including associated interactions ($P < 0.05$) for adult or larval mortality of *T. granarium*. Mortality of the field strain adults was significantly greater than mortality of field strain larvae across the post-treatment assay times except at the

4wk assay (Fig.4ab). Similarly, mortality of lab strain adults was always greater compared to the mortality of lab strain larvae, but the only significant difference in the mortality between adults and larvae occurred at the 4 and 12wk post-treatment assay times (Fig. 4ab).

The subsequent two-way ANOVA analyzed separately for adults and larval mortality showed no significant effects ($F = 0.7$, $df = 4$, 50 ; $P = 0.563$) of post-treatment assay time for adult mortality, but insect strain was significant ($F = 5.4$, $df = 1$, 50 ; $P = 0.025$). The interaction of post-treatment assay time by insect strain was non-significant ($P \geq 0.05$). In general, mortality of lab strain adults was always greater than mortality of field strain adults (98.2 ± 1.8 to 100% and 92.9 ± 4.5 to 100% , respectively, Fig. 4a). Larval mortality was significant for post treatment assay time ($F = 6.0$, $df = 4$, 50 ; $P < 0.001$), insect strain ($F = 53.1$, $df = 1$, 50 ; $P < 0.001$), and the interaction between post treatment assay time and insect strain ($F = 6.32$, $df = 4$, 50 ; $P < 0.001$). Mortality of the lab strain larvae was significantly greater than mortality of field strain larvae across all of the post treatment time except at 4wk, where slightly lower mortality occurred for lab strain larvae (Fig. 4b). Mortality ranged from 35.0 ± 6.7 to $81.7 \pm 6.0\%$ for the field strain and 73.3 ± 4.9 to 100% for the lab strain (Fig. 4b).

3.3.3. Exposure to chlorfenapyr

The overall ANOVA, which included main effects residual or post-treatment assay time were all significant for adult vs larval mortality of *T. granarium* ($F = 42.9$, $df = 4$, 100 ; $P < 0.001$), insect strain ($F = 9.2$, $df = 1$, 100 ; $P = 0.003$), and life stage ($F = 70.7$, $df = 1$, 100 ; $P < 0.001$). The associated interactions post-treatment assay time by life stage ($F = 14.0$, $df = 4$, 100 ; $P < 0.001$) and insect strain by life stage ($F = 5.8$, $df = 1$, 100 ; $P = 0.017$) were significant, but the interaction between post treatment assay time by insect strain was not ($P \geq 0.05$). Mortality of

field strain adults and larvaewas 100% at the first two post-treatment assay times, 0 and 2wk, but varied significantly at the last three post-treatment assay times, 4, 8, and 12wk (Fig. 5ab). Similarly, the mortality ofthe lab strain adults and larvae was 100% at the first two assays, but the only significant difference in the mortality between adults and larvae occurred at the 4, 8, and 12wkpost-treatment assays (Fig. 5ab).

The subsequent two-way ANOVA analyzed separatelyfor adults and larval mortality showed significant effects of post treatment assay time ($F = 12.4$, $df = 4$, 50 ; $P < 0.001$) for adult mortality, but the effect of insect strain was non-significant ($F = 0.3$, $df = 1$, 50 ; $P = 0.583$). The associated interaction was also non-significant ($P \geq 0.05$). Mortality of the both lab and field strainadults was 100% across all of the post-treatment assay timesexcept forone,with no significant different ($P \geq 0.05$) between the two strains. Larval mortality was significant for post treatment assay time ($F = 35.9$, $df = 4$, 50 ; $P < 0.001$), and insect strain ($F = 10.9$, $df = 1$, 50 ; $P = 0.002$), but the interaction of post-treatment assay time byinsect strain was non-significant ($F = 2.3$, $df = 4$, 50 ; $P = 0.09$). The mortality for the field and lab strain larvae was 100% at the first two post- treatment assay times (Fig. 5b). At the last three assays, 4, 8, and 12wk, mortality of the lab strain larvae was always greater than field strain larvae,with marginal difference at4wk post-treatment. Mortalityat the last three assay times, 4, 8, and 12wkranged from 41.7 ± 3.1 to 83.3 ± 5.6 % for the field strain larvae and 60.0 ± 9.3 to 95.0 ± 2.2 % for thelab strain larvae (Fig. 5b).

4. Discussion

Two of the three insecticides, β -cyfluthrin and deltamethrin, evaluated in this study are synthetic neurotoxic pyrethroids. Theydisrupt the voltage sensitive sodium channel that causes

hyperexcitation of the nervous system, which leads to convulsions and ultimately death (Corbett et al., 1984; Narahashi, 2002). These two products are registered with the U.S. Environmental Protection Agency (EPA) Reg. No. 432-1363 and 432-763, respectively for surface application to the interior of the empty bins or storage structures, or as a residual treatment to flooring and wall surfaces (U.S. EPA, 2010ab; Arthur and Subramanyam, 2012). β -cyfluthrin (Tempo[®] SC Ultra) suspension concentrate (SC) formulation is comparable in efficacy to the older cyfluthrin wettable powder (WP) and emulsifiable concentrate (EC) formulations (Arthur et al., 2015). Deltamethrin and β -cyfluthrin (Suspend SC[®]) are both registered in the U. S. as residual surface treatments to control stored-product insects inside structures.

Chlorfenapyr is a pyrrole insecticide that has a novel mode of action as a mitochondrial electron transport inhibitor. The active ingredient is converted into the toxic form within the insect body by mixed function oxidases, which act to uncouple oxidative phosphorylation in the mitochondria. The eventual result is disruption of energy production, leading to cell dysfunction and death of the insect (Black et al., 1994; Hunt and Tracey, 1998). Chlorfenapyr (Phantom[®]) is registered with the US-EPA (EPA Reg. No. 241-392) for termite control and against a variety of field crop insect and mite pests, as well as for general pest control including selected stored-product beetles, with specific usage defined on the product label (U.S. EPA, 2008; Arthur, 2013). It is effective as a surface treatment at lower rates than specified for outside perimeter sprays or as a spot treatment against the red flour beetle, *Tribolium castaneum* (Herbst) (Arthur, 2013, 2015).

The results of the current study where larvae of *T. granarium* and *T. inclusum* were exposed on treated arenas for short time durations indicate that although most of the larvae may be affected by initial exposure to an insecticide, they could recover if they are transferred to

untreated areas. This could easily occur during the course of normal cleaning, for example if these affected larvae are swept away or moved by air cleaning of the floors. Thus, the scenario of larvae coming into contact with an insecticide-treated surface for short time durations is realistic and could occur in a food warehouse, flour mill or food processing facility. Cracks, crevices, and areas covered by equipment could remain untreated, and larvae that are affected by insecticide exposure could be transported or moved into untreated areas, and recover from exposure and emerge as adults.

Based on the results from the latent mortality or short exposure assays, *Trogoderma* larvae are more tolerant than adults to β -cyfluthrin and deltamethrin when exposed for a short duration. Also, *T. inclusum* larvae are less susceptible than *T. granarium* larvae to these pyrethroids. In the current study, the variation in larval mortality between the two species, *T. granarium* and *T. inclusum* is not evident, possibly because of low larval mortality when they were exposed for short durations of 1 and 2-d. One possible explanation is that in short exposure times, larvae may have remained close to or on the food source, thus limiting exposure. However, in longer exposure times, the food particles may have absorbed some of the insecticides, and the larvae came into contact with those particles when moving through or ingesting the treated food source. In longer exposure times (ex. 3 and 7-d), *T. inclusum* larvae appeared to be more tolerant than *T. granarium* larvae, which could perhaps explain the species differences, or alternatively our source of field collected *T. inclusum* may have been exposed more frequently to the insecticides tested in the current than the *T. granarium*. The low larval mortality obtained in our short exposure experiments are similar to those of Kavallieratos et al. (2016), who also showed low larval mortality (4 to 12% and 4 to 18% for immediate and delayed mortality, respectively) when late-stage *T. granarium* larvae were exposed for 1-7 d on a surface

353 treated with deltamethrin. In the same study, they also reported about 50% mortality for exposed
354 early-stage larvae. In a previous study by Athanassiou et al.(2015), the authorsreported that late-
355 stage larvae of *T. granarium*were more tolerant (larval mortality of < 5%) compared tolate-stage
356 larvae of *Tenebrio molitor* L. (larval mortality of 26.7-32%) when they were exposed for 7 d on a
357 surface treated with alpha-cypermethrin and thiamethoxam. Thus, although *T. granarium* larvae
358 are affected once they contactaninsecticide-treated surface,they may not necessarily die. One
359 explanation is that the presence of long dense larval hairs reduce the direct body contact to the
360 treated surface. In addition, the presenceof the food source provided when larvae are exposed on
361 treated surface may have helped to reduce larval exposure to the toxicant or provideda means for
362 larvae to limit or escape exposure (Toews et al., 2010; Kharel et al., 2014).

363 In the secondstudy where larvae and adults from the lab and field strains of *T.*
364 *granarium*were exposed to the insecticides, *T. granarium* adults from the both strains were
365 generally more susceptible than larvae.These results are similar to those obtained in a recent
366 study using three species of dermestid beetles;*T. granarium*, the warehouse beetle, *T.*
367 *variabile*(Ballion) and *T. inclusum*. Results show consistently that adults are more susceptible to
368 insecticides compared to larvae (Athanassiou et al., 2015; Ghimire et al., 2016). However, the
369 greater mortality obtained for adults could be due to the fact that they were exposed directly on
370 the treated surface without a food source. The reasons behind not providing a food source for
371 adults isbecause they are short lived and may rarely feed (Hill, 1990).In contrast, larvae had to be
372 provided with a food source to deter the larvae from going into diapause or dying outright, but
373 they were exposed for 30 days compared to the 4-day exposure for adults, a much longer time
374 period.

Mortality of adults in both the lab and field strains of *T. granarium* ranged from 90-100% across the post-treatment assays for all insecticides. Any significant differences were more evident in larval mortality, particularly at the 4, 8, and 12-wk post-treatment assays. The recently field-collected Pakistani strain larvae were more tolerant to the tested insecticides compared to the lab strain from Poland. This difference in mortality could be caused by genetic changes after long-term laboratory rearing (> 30 years) of the lab strain of *T. granarium* as opposed to the recently collected field strain. This suggests potential exposure of the field strains to insecticides and thus greater natural tolerance. There are number of studies that report reduced susceptibility to insecticides in field strains of stored-product insects compared to laboratory strains (Sehgal et al., 2013, 2014; Kljajic and Peric, 2006).

In the present study, chlorfenapyr was more effective than β -cyfluthrin and deltamethrin. For both strains of *T. granarium*, 100% adult mortality was obtained across the post-treatment assays except for the last one at 12 weeks. Similarly, 100% larval mortality was obtained for the both laboratory and field strain larvae at the 0 and 2 wk post-treatment assays. These results are in agreement with Kavallieratos et al. (2016), who reported chlorfenapyr provided the greatest delayed larval mortality (76.6% for early stage-larvae and 61.5% for late-stage larvae) compared to deltamethrin, pirimiphos-methyl, pyriproxyfen and spinosad. β -cyfluthrin provided greater control for adults and larvae of laboratory and field strains of *T. granarium* across the post-treatment assays, and had greater residual persistence compared to deltamethrin. Based on the results of our study and those recent studies cited above, management strategies for evaluating susceptibility of *T. granarium* should be focused on the larval stage in addition to the adult stage.

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Fig. 1. Affected (A) and dead (B) larvae (% mean $\pm$ SE) of *T. granarium* (black bar) and *T. inclusum* (grey bar) exposed on concrete treated with 20 mg/m² active ingredient [AI] β -cyfluthrin for 1, 2, 3, and 7 d. At each time interval significant differences between species are denoted by asterisks ($P < 0.05$, *t*-test, SAS).

Fig. 2. Affected (A) and dead (B) larvae (% mean $\pm$ SE) of *T. granarium* (black bar) and *T. inclusum* (grey bar) exposed on concrete treated with 24 mg/m² active ingredient [AI] deltamethrin for 1, 2, 3, and 7 d. At each time interval significant differences between species are denoted by asterisks ($P < 0.05$, *t*-test, SAS).

Fig. 3. Adult (A) and larval (B) mortality (% mean $\pm$ SE) of *T. granarium* field strain (black bar) and lab strain (grey bar) exposed on concrete treated with 20 mg/m² active ingredient [AI] β -cyfluthrin at 0, 2, 4, 8, and 12 wk post-treatment, with adults exposed for 4 days and larvae exposed for 30 days. At each time interval significant differences between strains are denoted by lower case letters, differences between adults and larvae denoted by asterisks ($P < 0.05$, *t*-test, SAS).

Fig. 4. Adult (A) and larval (B) mortality (% mean $\pm$ SE) of *T. granarium* field strain (black bar) and lab strain (grey bar) exposed on concrete treated with 24 mg/m² active ingredient [AI] deltamethrin at 0, 2, 4, 8, and 12 wk post-treatment, with adults exposed for 4 days and larvae exposed for 30 days. At each time interval significant differences between strains are denoted by lower case letters, differences between adults and larvae denoted by asterisks ($P < 0.05$, *t*-test, SAS).

Fig. 5. Adult (A) and larval (B) mortality (% mean $\pm$ SE) of *T. granarium* field strain (black bar) and lab strain (grey bar) exposed on concrete treated with 280 mg/m² active ingredient [AI] chlorfenapyr at 0, 2, 4, 8, and 12 wk post-treatment, with adults exposed for 4 days and larvae

519 exposed for 30 days. At each time interval significant differences between strains are denoted by
520 lower case letters, differences between adults and larvae denoted by asterisks ($P < 0.05$, t -test,
521 SAS).

Fig. 1. Short exposure to β -cyfluthrin

Fig. 2. Short exposure to Deltamethrin

526

527 Fig. 3. β -cyfluthrin

Fig. 4. Deltamethrin

Fig. 5. Chlorfenapyr