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ORIGINAL PAPER

Assessing repellency, movement, and mortality of three species of stored product insects after exposure to deltamethrin-incorporated long-lasting polyethylene netting

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Abstract
Long-lasting insecticide-treated netting (LLIN) has begun to be used in agricultural product protection. We investigated the effect of a deltamethrin-incorporated LLIN on three stored product insects, red flour beetle, *Tribolium castaneum* (Herbst), lesser grain borer, *Rhyzopertha dominica* (F.), and rice weevil, *Sitophilus oryzae* (L.). Long-distance repellency was assessed in a wind tunnel for adults of each species, but no repellency was observed. Insect movement and behavior was assessed by observing the movement of adult beetles on treated and untreated netting during a 5 min period for distance traveled, velocity, mobility, and time on each netting type. Among all netting combinations, *T. castaneum* spent the most time highly mobile, higher velocity, and traveled a greater distance. *Sitophilus oryzae* and *R. dominica* spent significantly more time immobile and lower velocity. Efficacy of LLIN was tested by exposing adults for 60 min, removing, and holding with or without food, and monitoring for mortality up to 7 d. All *S. oryzae* and *R. dominica* were either affected or dead at all post-exposure times. The number of affected and dead adult *T. castaneum* combined 7 d after exposure was 42% and 95%, with or without food, respectively, and 52% of affected adults were able to recover when food was present. Overall, LLIN had no repellency or negative effect on insect movement, which increases the probability of lethal exposures of adults from walking on the netting. LLIN can be adopted in existing integrated pest management (IPM) programs for food processing facilities to prevent stored product insect infestations.

Keywords Deltamethrin netting · Stored product insects · Delayed mortality · Integrated pest management

Key message

- No long-distance or contact repellency to the LLIN was observed among all species, suggesting that these species will not actively avoid the netting
- All of *S. oryzae* and *R. dominica* adults were either affected or dead 1 d after a 60 min exposure to LLIN, and *T. castaneum* adults ranged from 90 to 100%
- The presence of food significantly increased *T. castaneum* recovery after a 60 min exposure to the LLIN and the recovery was 58% after 7 d

Introduction

Commercially available long-lasting insecticide-treated nets (LLINs) were originally developed to provide long-term protection from mosquitos and other biting insects (Gerhardt 2009). These LLINs are treated with slow-release pyrethroid insecticides, often permethrin, deltamethrin, or α-cypermethrin that can remain effective over several washes and years of use (Gerhardt 2009). These pyrethroids quickly incapacitate and kill mosquitos on contact and can also be incorporated into textile materials for personal protection against biting insects. Recently, the use of LLINs has expanded outside the scope of mosquito and vector management into controlling multiple

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agricultural pests such as the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), the aphid *Lipaphis erysimi* (Kaltenbach) (Hemiptera: Aphididae), the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae), and the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) (Martin et al. 2006; Licciardi et al. 2008; Dader et al. 2014; Kuhar et al. 2017; Peverieri et al. 2018; Rumbos et al. 2018). *Halyomorpha halys* adults exposed to α-cypermethrin impregnated netting (Storanet®, BASF, Ludwigshafen, Germany) resulted in paralysis, defined as affected or knockdown in adult stored product insects, after less than 12-h post-exposure (Peverieri et al. 2018). As the post-exposure time increased up to 10 d, adult *H. halys* were less likely to recover with increasing exposure time on the α-cypermethrin netting resulting in an increase in morality. However, using a different active insecticide such as deltamethrin-incorporated LLIN (ZeroFly® netting, Vestergaard Frandsen Inc., Lausanne, Switzerland), *H. halys* adults exposed for only 10 min exhibited > 80% adult mortality 24 h post-exposure (Kuhar et al. 2017) compared to no adult mortality 24 h post-exposure to the α-cypermethrin netting at any exposure time (Peverieri et al. 2018).

Recent research into insecticide-treated materials to control stored product insects has focused on the use of insecticide-treated packaging to protect food products from infestations. Deltamethrin-treated packaging (ZeroFly® storage bags, 3 g/kg deltamethrin, Vestergaard Frandsen Inc.) has been shown to inhibit adult fecundity of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and the confused flour beetle *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), after ≥ 48 and 168 h of exposure, respectively (Scheff et al. 2018). However, when exposure periods were less than these times, females of both species produced viable progeny (Scheff et al. 2018). Kavallieratos et al. (2017) investigated knockdown and mortality of the same deltamethrin packaging on *T. castaneum,* lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), and the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Circulionidae). *Rhyzopertha dominica* and *T. castaneum* were knocked down on the treated packaging after 1 h of exposure, and after 5 d of continuous exposure mortality was 59% and 6%, respectively (Kavallieratos et al. 2017). Comparatively, 86% of *S. oryzae* were knocked down after 1 h of exposure and were all dead after 5 d (Kavallieratos et al. 2017). Scheff et al. (2018) and Kavallieratos et al. (2017) both demonstrated significant differences in susceptibility between stored product beetle species, but among all species there was delayed mortality after the adults were knocked down.

The use of LLIN is a new tool that can be incorporated into any integrated pest management (IPM) program at multiple focal points throughout the post-harvest supply chain. The LLIN could be used as a physical barrier to limit insect movement in, out, or around a food processing facility, grain bins, and coverings for palletized products. Insects are able to pass through the LLIN, but as the insects move on the LLIN they will have direct contact with the deltamethrin-treated surface. The LLIN can be used as a covering for the roof hatch, loading hatch, and roof vents in grain storage bins in which insects must traverse before entry. LLIN can also be used as a covering during transportation and long-term storage of packaged products or as a covering for stored agricultural commodities such as tobacco leaves. Recent investigations into the use of LLIN on stored product insects have shown a similar trend in delayed mortality after exposure as those observed with deltamethrin-treated packaging. Rumbos et al. (2018) reported *L. serricorne* adults exposed to a α-cypermethrin netting (Carifend®, BASF AG, Ludwigshafen, Germany), did not result in significant immediate mortality, despite exposures of 24 h, but as the post-exposure time increased *L. serricorne* mortality increased up to 55% after 7 d.

In addition to direct lethal effects of insecticides applied to grain, surfaces, packaging, and netting, insecticides can induce a range of sublethal behavioral changes in insects based on the class of insecticide. The sublethal effects of insecticides on the behavior of stored product pests have been historically neglected (Guedes et al. 2011). Sublethal effects can include changes in mating behavior, host-finding, feeding, dispersal, and locomotion (Haynes 1988; Desneux 2007). Commercial insecticide formulations of β-cyfluthrin (Tempo® SC Ultra, Bayer CropScience, Kansas City, MO), chlorfenapyr (Phantom® SC, BASF Chemical Corporation, Research Triable Park, NC), and pyrethrins (PyGanic Pro® SC, MGK Co., Minneapolis, MN) applied to concrete surfaces significantly reduced the total distance moved (cm) and velocity (cm/s) of two prevalent stored product psocids, *Liposcelis bostrychophila* Badonnel and *L. entomophila* (Enderlein) (Psocoptera: Liposcelidiae), over a 10 min period (Guedes et al. 2008). However, some insecticides increase movement or induce hyperactivity, which is often observed after exposure to pyrethroids. For example, locomotor activity of *Triatoma infestans* (Klug, 1834) (Hemiptera: Reduviidae: Triatominae) nymphs significantly increased with increasing concentrations of deltamethrin applied either topically or on filter paper (Alzogaray et al. 1997). But this is not always the case with pyrethroid exposures. Movement of adult *T. castaneum* and *R. dominica* after exposure to deltamethrin-incorporated netting at 0.4% (D-Terrence, Vestergaard Frandsen Inc.) reduced the average distance moved (cm) but increased the mean angular velocity (deg/s) over a two-hour observation period (Morrison et al. 2018). However, those authors did not evaluate insect movement directly on LLIN but observed movement after exposure to the LLIN.

Natural pyrethrins and some pyrethroids have been reported as being repellent to specific insects (Elliott et al. 1978). There have been anecdotal reports by pest management professionals that exposure to pyrethroids, and deltamethrin in particular, has a repellent effect on stored product pest behavior (Muntaha et al. 2017). This repellency could be a potential limitation for deltamethrin-incorporated netting, and it is important to evaluate whether there is repellency, avoidance behavior, or changes in movement by stored product insects after exposure to the netting. Based on previous studies with deltamethrin-treated packaging and netting, we investigated the potential efficacy and repellency of LLIN against three common stored product insect species, *T. castaneum, S. oryzae,* and *R. dominica.* These three species were chosen because they have previously shown ranges in susceptibility to contact insecticides and are commonly found in grain bins and food processing facilities where LLIN could be used as a pest management tool. The objectives of this study were to (1) determine if there is long-distance repellency to the LLIN, (2) monitor and measure insect movement and behavior directly on the treated and untreated netting, and (3) determine the mortality of *T. castaneum, R. dominica,* and *S. oryzae* after 60 min of exposure to LLIN.

Materials and methods

Long-lasting insecticide-treated netting, LLIN

Deltamethrin-treated and untreated netting were obtained from a commercial manufacturer (D-Terrance, Vestergaard Frandsen Inc.). The load rate of the treated netting was 0.4% or 4000 ppm of deltamethrin (active ingredient) incorporated into a polyethylene based net with a mesh size of 32 holes/cm^2. The control netting used was the same polyethylene netting and mesh size, but without the insecticide.

Long-distance repellency bioassay

All insects used in the wind tunnel assay were obtained from colonies maintained at the United States Department of Agriculture's Center for Grain and Animal Health Research (CGAHR) in Manhattan, Kansas, USA. *Tribolium castaneum* adults (field-derived strain from central KS in 2012) were reared on a diet consisting of 95% organic unbleached flour with 5% brewer's yeast added in 1-L glass containers and held at 27.5 °C, 60% RH, and 14:10 L:D. *Rhyzopertha dominica* (field-derived strain collected from central KS in 2012) and *S. oryzae* (laboratory strain) were reared at the same environmental conditions but fed on a diet of whole grain hard red winter wheat. For testing, 4–8-week-old adults of *T. castaneum*, *R. dominica* and *S. oryzae* were randomly selected from colony jars and used within 6 h for testing.

In order to evaluate whether there was any long-distance repellency to the LLIN, a wind tunnel assay based on the design of Miller and Roelofs (1978) was used. The wind tunnel consisted of a 130 × 86 × 86 cm (L:W:H) acrylic chamber, with the testing arena raised by 6 cm off the ground. Air flow was generated by a centrifugal fan (4C054B, Dayton Electrical Manufacturing Co., Naples, IL, USA), with air cleaned by an activated charcoal filter, then flattened into a laminar flow through an acrylic mesh (1.6 × 1.6 cm L:W) over a linear distance of 95.9 cm. Air speed was 0.43–0.53 m/s in the wind tunnel chamber, and flow was confirmed with a combination of a smoke test and anemometer. The odor source consisted of a 5 × 5 cm piece of LLIN or untreated netting that was placed inside an open 20-mL vial (7.5 × 2 cm H:D; Gerstel, Inc., Linthicum, MD, USA) flush with ground level of tested insects.

A 22 × 28 cm (W:L) testing arena was located 36 cm downwind from the odor stimulus, and either a single *T. castaneum, S. oryzae,* or *R. dominica* adult was released in the center of the arena and given a maximum of 2 min to respond. A response consisted of the adult leaving the arena either on the stimulus edge (edge closest to the odor source) or non-stimulus edge (any of the other three sides of the arena). The time to a decision was recorded using a stopwatch. Individuals that did not respond within the 2 min timeframe were excluded from the statistical analysis. A single individual was considered a replicate for each treatment, and 100 replicates per treatment (LLIN or untreated netting as odor source) were performed for all three species. The choices by individuals were analyzed with a χ^2-test against the null hypothesis that there was an equal likelihood of choosing non-stimulus and stimulus edges of the arena in R Software (R Core Team 2019). The time to decision between the treated and untreated netting was compared for each species with a *t*-test. For this, and all subsequent tests, significance was determined at $\alpha = 0.05$.

Insect movement and behavior on LLIN

In order to examine possible insect movement and behavioral changes when adults were exposed directly on the LLIN, the movement of 1–2-week-old mixed sex adults of *T. castaneum, S. oryzae,* and *R. dominica* were analyzed. Each species came from colonies that have been reared at the CGAHR, at 27° and 65% r.h. in continuous darkness, for more than 30 years.

Bioassay arenas were constructed using the bottoms of 100 × 100 mm square plastic Petri dishes (81 cm^2 surface area). The bottom of the Petri dish was first lined with filter paper (Whatman® Grade 1, GE Healthcare UK Limited, Buckinghamshire, UK). Then, two 80 × 40 mm rectangles of netting were placed on top. The netting was secured to the sides of the arena and the middle seam with a bead of caulking (DAP Kwick Seal, DAP Products Inc., Baltimore, MD, USA) and the sides coated with Fluon® (polytetrafluoroethylene, Sigma-Aldrich Co., St. Louis, MO, USA) to prevent insects from escaping the bioassay arenas during testing.

Three different Petri dish arena treatments or configurations were used. The first treatment had both halves of the dish covered in treated netting (treated–treated, hereafter), the second treatment had both halves covered in untreated netting (untreated–untreated, hereafter), and the third treatment had one half covered in treated netting and the other half covered in untreated netting (treated–untreated, hereafter). Orientation of the treated or untreated netting was reversed between every beetle assayed to account for positional effects. One adult *T. castaneum, S. oryzae,* or *R. dominica* was placed in the center of the caulking line in the middle of the arena and allowed 10 s to acclimate to the arena before 5-min video recording began (Sony Handycam HDR-XR520V, Sony Corp.). Afterward, the beetle was removed, discarded, and not used again. Three blocks of 20 adults from each species and on each Petri dish arena treatment were used.

Videos were scored using Ethovision XT (Version 8.0, Noldus Information Technology, Leesburg, VA) software for movement parameters, including total distance moved (cm), mean instantaneous velocity (cm/s), mobility classification (highly mobile, mobile, immobile), and time spent on each half of the netting (s). Mobility classification was based on the percent of an insect's body changing position between frames, at 10 frames per second. Mobility > 60% was classified as highly mobile, 20–60% was mobile, and 0–20% was immobile. For each video, the area of the treated or untreated netting was manually set and excluded all caulking. The proportion of time spent on each half of the netting in an arena was evaluated with the *lmer* package followed by the *car* ANOVA package in R. In addition, the proportion of time spent on the netting versus the caulking was also evaluated to determine if the beetles were actively avoiding the netting during the 5 min period and was evaluated with the *lmer* package followed by the *car* ANOVA package in R. Individual species were then compared with a post hoc *t*-test both for time spent on each half of the arena and for time spent on the caulking and time spent on the netting in R. All remaining analyses described below were performed using SAS (SAS Institute, version 9.4, 2012, Cary, NC, USA).

To examine differences between species and arena treatments, we used a generalized linear model with species, arena treatment, and the interaction between species and arena treatment as main, fixed effects. Replicate ($n = 60$) was used as a random effect in the model. Differences within each species were also examined using a generalized linear model with arena treatment as a main effect and replicate as a random effect with Tukey correction for multiple comparisons. To examine differences in mobility as an effect of exposure to the netting, we also analyzed differences in the first 2.5 min and last 2.5 min of the arena treatment exposure. Each species was analyzed individually with arena treatment, time of recording (first 2.5 min or last 2.5 min), and the interaction of those effects as main effects with a Tukey correction for multiple comparisons.

Effect of 60-min exposure to deltamethrin-treated netting

In order to determine the direct biological effect of the netting and delayed mortality on *T. castaneum, S, oryzae,* and *R. dominica,* we observed the number of affected adults immediately after a 60-min exposure to LLIN and mortality for up to 7-d post-exposure. A 60-min exposure period was chosen to serve as the benchmark for comparison between two previously published studies on deltamethrin-incorporated packaging (Kavallieratos et al. 2016) and alpha-cypermethrin netting (Rumbos et al. 2018).

The treated and untreated netting (control) was cut into 9-cm diameter circles and fitted on the bottom of a filter paper lined 100 × 20 mm plastic Petri dishes to create bioassay arenas, with ~ 62-cm^2 total surface area. The edges of the netting were secured down using adhesive caulking and the sides were coated with Fluon®. In addition, same-sized Petri dishes were fitted with filter paper only and were used as transfer arenas after insect exposure to netting. After all arenas were created, the dishes dried for 24 h at ambient conditions prior to use.

Twenty sets of 10, 1–2-week-old adults with mixed sexes from each species were obtained from colony jars and were exposed to either treated (10 arenas total) or untreated netting (10 arenas total) for 60 min. Immediately following the exposure, adults were observed in each arena and the number of affected adults was recorded. Affected adults were defined as adults that exhibited irregular movement, little to no movement when probed with an artist's paint brush, inability to walk or maintain an upright stature, or displayed erratic movement/behavior that was different from adults that had been exposed on the untreated netting (Scheff et al. 2019). Adults were then transferred from netting arenas to the transfer arenas. Half of the adults were transferred to arenas containing approximately 500 mg of species-specific rearing diet as described above and the remainder were transferred to arenas containing no diet. Therefore, experimental treatments were treated netting-food (trmt-F); treated netting-no food (trmt-NF); (untreated) control-food (cntl-F); and (untreated) control-no food (cntl-NF). *Tribolium castaneum* adults were observed at 1-, 2-, 3-, and 7-d post-exposure to netting arenas and the number of live, affected, and dead (no response when probed) were recorded. *Sitophilus oryzae* and *R. dominica* adults were observed at 1-, 2-, and 3-d post-exposure. The observations at 7 d post-exposure were not included in analysis because the mortality level of the food-deprived adults used in control arenas was > 20% for *R. dominica* and > 60% for *S. oryzae,* and therefore, it was not possible to certainly determine if this effect was caused by the LLIN or by starvation. The entire process was repeated three times on different days using individuals collected from different adult colonies, for a total of 15 replicates per treatment combination. Means and standard errors (SE) were calculated for percentage of live, affected, and dead adults and were transformed to angular values prior to analysis (Zar 2010). The main factors, treatment, diet, and post-treatment time, and their interactions were compared using a generalized linear model (GLM) analysis of variance (ANOVA) in SAS. To determine the effect of diet on the number of live, affected, and dead individuals for each species, a Tukey correction for multiple comparisons was used.

Results

Long-distance repellency

There was no significant preference by *T. castaneum* for exiting the arena on a stimulus or non-stimulus edge when LLIN ($\chi^2=0$; df = 1; $P=1.00$) or control netting ($\chi^2=1.72$; df = 1; $P=0.19$) was used as the odor source. *Tribolium castaneum* chose the stimulus edge 47% when LLIN netting was the odor source compared to 54% when the untreated netting was the source. Similarly, there was no significant difference in the length of time required to choose between the stimulus or non-stimulus edges ($t=0.73$; df = 280; $P=0.46$), when LLIN was the odor source (mean: 21 ± 2 s), or the control netting were the odor source (mean: 20 ± 2 s).

Sitophilus oryzae also had no significant preference for exiting on the stimulus or non-stimulus edge when LLIN ($\chi^2=0.07$; df = 1; $P=0.80$) or control netting ($\chi^2=0.07$; df = 1; $P=0.80$) was used as the odor source. *Sitophilus oryzae* chose the stimulus edge 45% regardless of whether LLIN netting or untreated netting was the odor source. There was also no statistical difference in the time to decision for *S. oryzae* adults leaving the arena ($t=0.43$; df = 120; $P=0.67$). It took adult *S. oryzae* an average of 22 ± 1 s and 21 ± 1 s to make a decision when control netting or treated netting was the odor source, respectively.

Finally, adult *R. dominica* also exhibited no significant preference in exiting the arena on the stimulus or non-stimulus edge when LLIN ($\chi2=2.56$; df = 1; $P=0.11$) or control netting ($\chi2=0.16$; df = 1; $P=0.69$) was the odor source. *Rhyzopertha dominica* chose the stimulus edge 41% when LLIN netting was the odor source compared to 39% when the untreated netting was the source. There was additionally no significant difference in the time it took for *R. dominica* adults to exit the arena ($t=0.73$; df = 280; $P=0.46$). It took adults an average of 87 ± 4 s and 85 ± 5 s to exit the arena when treated and untreated netting was used as the odor source, respectively. Compared to both *T. castaneum* and *S. oryzae*, *R. dominica* took nearly 4 × as long to make a decision and exit the arena regardless of the odor source.

Insect movement and behavior on LLIN

Evaluating the total distance moved during the 5-min exposures ($df_{Error}=529$), there were significant differences between species ($F=433.70$; df = 2; $P<0.0001$) and arena treatment ($F=7.74$; df = 2; $P<0.001$) and the interaction effect of species × arena treatment was significant ($F=6.55$; df = 4; $P=0.0001$). *Tribolium castaneum* moved the farthest distance in the treated–untreated arenas, compared to all other species and arena treatments (Table 1). The distance *S. oryzae* moved did not differ between the three arena treatments but was significantly less than *T. castaneum* and more than *R. dominica* (Table 1). Overall *R. dominica* moved 3 – 4 times shorter distance compared to *T. castaneum* and about half the distance *S. oryzae* traveled.

Velocity ($df_{Error}=529$) was significantly different among species ($F=11.32$; df = 2; $P<0.0001$) but arena treatment ($F=0.37$; df = 2; $P=0.69$) or the interaction between arena treatment and species ($F=1.49$; df = 4; $P=0.20$) were not significant. *Tribolium castaneum* (0.58 cm/s; $P<0.01$) moved at a significantly higher average velocity on all arenas treatments compared to both *S. oryzae* (0.30 cm/s) and *R. dominica* (0.25 cm/s), which did not differ in their velocity ($P=0.75$). The higher average velocity of *T. castaneum* could be one explanation why *T. castaneum* also moved a greater distance compared to *S. oryzae* and *R. dominica.*

Similar to distance moved, time spent highly mobile ($df_{Error}=529$) was significant among species ($F=113.54$; df = 2; $P<0.0001$) and arena treatments ($F=6.52$; df = 2; $P<0.01$), and the interaction ($F=6.81$; df = 4; $P<0.0001$) was also significant. *Tribolium castaneum* in the treated–treated and treated–untreated arenas spent the longest time classified as highly mobile, 8.6 and 8.1 s, respectively, followed by *R. dominica* in the treated–untreated *T. castaneum* in the untreated–untreated arenas (Table 1). *Sitophilus oryzae* spent the least amount of time in the highly mobile phase, averaging < 2.8 s. In contrast, time spent immobile was not significantly different ($df_{Error}=529$) for arena ($F=2.33$; df = 2; $P=0.10$) or arena treatment × species

Table 1 Overall summary of *T. castaneum, R. dominica,* and *S. oryzae* movement parameters captured during a 5 min exposure on three different arena treatments

Arena treatments	Species	Distance moved (cm) mean (±SE)	Avg. velocity (cm/s) mean (±SE)	Duration highly mobile (s) Mean (±SE)	Duration mobile (s) mean (±SE)	Duration immobile (s) mean (±SE)
Treated–untreated	*R. dominica*	47.1 ± 3.2 d	0.1 ± 0.01	6.0 ± 0.5 b	4.3 ± 0.3 d	281.4 ± 4.5
Treated–untreated	*T. castaneum*	195.0 ± 9.4 a	0.7 ± 0.03	8.1 ± 0.7 a	38.4 ± 2.7 a	249.3 ± 3.3
Treated–untreated	*S. oryzae*	88.8 ± 4.7 c	0.3 ± 0.02	2.8 ± 0.2 d	14.4 ± 0.9 c	278.7 ± 3.4
Treated–treated	*R. dominica*	49.0 ± 4.2 d	0.2 ± 0.01	3.9 ± 0.3 d	4.8 ± 0.5 d	284.7 ± 3.7
Treated–treated	*T. castaneum*	159.0 ± 6.6 b	0.5 ± 0.02	8.6 ± 0.5 a	30.5 ± 1.9 b	260.3 ± 2.4
Treated–treated	*S. oryzae*	98.0 ± 4.3 c	0.3 ± 0.01	2.8 ± 0.2 d	13.4 ± 0.9 c	281.7 ± 1.3
Untreated–untreated	*R. dominica*	43.4 ± 2.8 d	0.4 ± 0.32	5.3 ± 0.5 bc	5.8 ± 0.5 d	278.6 ± 5.4
Untreated–untreated	*T. castaneum*	158.0 ± 6.1 b	0.5 ± 0.02	6.0 ± 0.4 b	31.0 ± 2.1 b	261.4 ± 2.6
Untreated–untreated	*S. oryzae*	81.3 ± 4.6 c	0.3 ± 0.02	2.2 ± 0.2 d	12.9 ± 0.9 c	279.5 ± 3.5

Different letters within the column indicate significant differences between each species and arena configuration combinations. If no letters are in a column, the species and arena comparisons were not significant ($P > 0.05$)

interaction ($F = 1.55$; df = 4; $P = 0.19$) but was significantly different for species ($F = 47.94$; df = 2; $P < 0.0001$). *Rhyzopertha dominica* and *S. oryzae* spent more time immobile, 24.9 s and 22.9 s, respectively, compared to *T. castaneum* ($P < 0.0001$) across all arena treatments (Table 1).

In addition to assessing long-distance repellency, we assessed contact repellency by assessing differences in time spent on the treated versus untreated netting. There was no significant difference ($\mathrm{df}_{\mathrm{Error}} = 116$) in the frequency with which *T. castaneum* visited either side of the arena ($F = 0.45$; df = 1; $P = 0.50$; Fig. 1a) or time ($F = 3.02$; df = 1; $P = 0.085$; Fig. 1d) on either treated or untreated netting in the treated–untreated arenas. Similar to the wind tunnel, the treated netting showed no direct contact repellency on *T. castaneum* and adult did not actively avoid the treated netting. Further evaluation of the treated–treated arenas showed there was a slight (about 1.17 magnitude difference) preference ($\mathrm{df}_{\mathrm{Error}} = 116$) in the number of times *T. castaneum* went to the left side (17.2 ± 1.06 events) rather than the right side (14.6 ± 1.03 events) of the arena ($F = 4.15$; df = 1; $P = 0.044$), although the time spent on either left (77.2 ± 4.4 s) or right side (75.9 ± 5.5 s) did not significantly differ ($F = 0.04$; df = 1; $P = 0.85$). *Tribolium castaneum* on the untreated–untreated arena had a preference to frequent the left side (18.1 ± 1.1 events) more than the right side (14.6 ± 0.9 events) of the arena ($F = 7.92$; df = 1; $P < 0.01$) but the time spent on either the left (76.8 ± 4.3 s) or right (69.7 ± 4.6 s) side did not differ ($F = 1.30$; df = 1; $P = 0.26$).

Sitophilus oryzae did not have more frequent visits ($\mathrm{df}_{\mathrm{Error}} = 116$) to untreated netting when compared to treated netting in the treated–untreated arena ($F = 3.79$; df = 1; $P = 0.054$; Fig. 1b), but individuals spent significantly more time on the treated netting ($F = 20.98$; df = 1; $P < 0.0001$; Fig. 1e) compared to the treated netting. In general, on average *S. oryzae* spent more time on the treated netting compared to *T. castaneum* and *R. dominica*. If the arenas had both sides with treated netting, there was no difference ($\mathrm{df}_{\mathrm{Error}} = 116$) between frequency of visits to either the left (7.4 ± 0.6 events) or right (7.9 ± 0.9 events) side ($F = 0.27$; df = 1; $P = 0.60$) or time spent on left (104.0 ± 11.9 s) or right (102.0 ± 11.2 s) side ($F = 0.01$; df = 1; $P = 0.93$). In the untreated–untreated arena, there was no preference in the frequency of visits to either the left (8.1 ± 1.0 events) or right (7.8 ± 0.8 events) side ($F = 0.05$; df = 1; $P = 0.82$) nor any preference for time spent on the left (95.4 ± 10.5 s) or right (125.0 ± 11.5 s) side of the arena ($F = 3.64$; df = 1; $P = 0.06$).

Similar to *T. castaneum, R. dominica* did not significantly ($\mathrm{df}_{\mathrm{Error}} = 116$) frequent either side in the treated–untreated arenas ($F = 1.84$; df = 1; $P = 0.18$; Fig. 1c) nor preferentially spend time on either side ($F = 0.30$; df = 1; $P = 0.58$; Fig. 1f). When *R. dominica* was placed on the treated–treated arenas, there was no preference ($\mathrm{df}_{\mathrm{Error}} = 116$) in frequency of visits to the left (6.2 ± 0.8 events) or right (6.5 ± 0.8 events) side ($F = 0.10$; df = 1; $P = 0.75$) or time spent on the left (95.2 ± 13.1 s) or right (121.0 ± 14.4 s) side ($F = 1.82$; df = 1; $P = 0.18$). In untreated–untreated arenas, there was also no preference ($\mathrm{df}_{\mathrm{Error}} = 116$) in the frequency of visits to the left (3.5 ± 0.6 events) or right (4.3 ± 0.6 events) side ($F = 0.72$; df = 1; $P = 0.40$) or preference in time spent on either the left (119.0 ± 16.0 s) or right (110.0 ± 14.7 s) side ($F = 0.18$; df = 1; $P = 0.82$).

Upon observing no significant differences in frequency of visits between the treated and untreated netting among all species, we further calculated the proportion of time spent on the netting within the arena and compared it to the time spent on the caulking, in another comparison analysis to determine if there is contact repellency to the netting itself. The main effect of species was significant for proportion of time spent on the netting ($\chi^2 = 32.97$; df = 2; $P < 0.0001$) but the arena type ($\chi^2 = 1.60$; df = 2; $P = 0.45$) and interaction

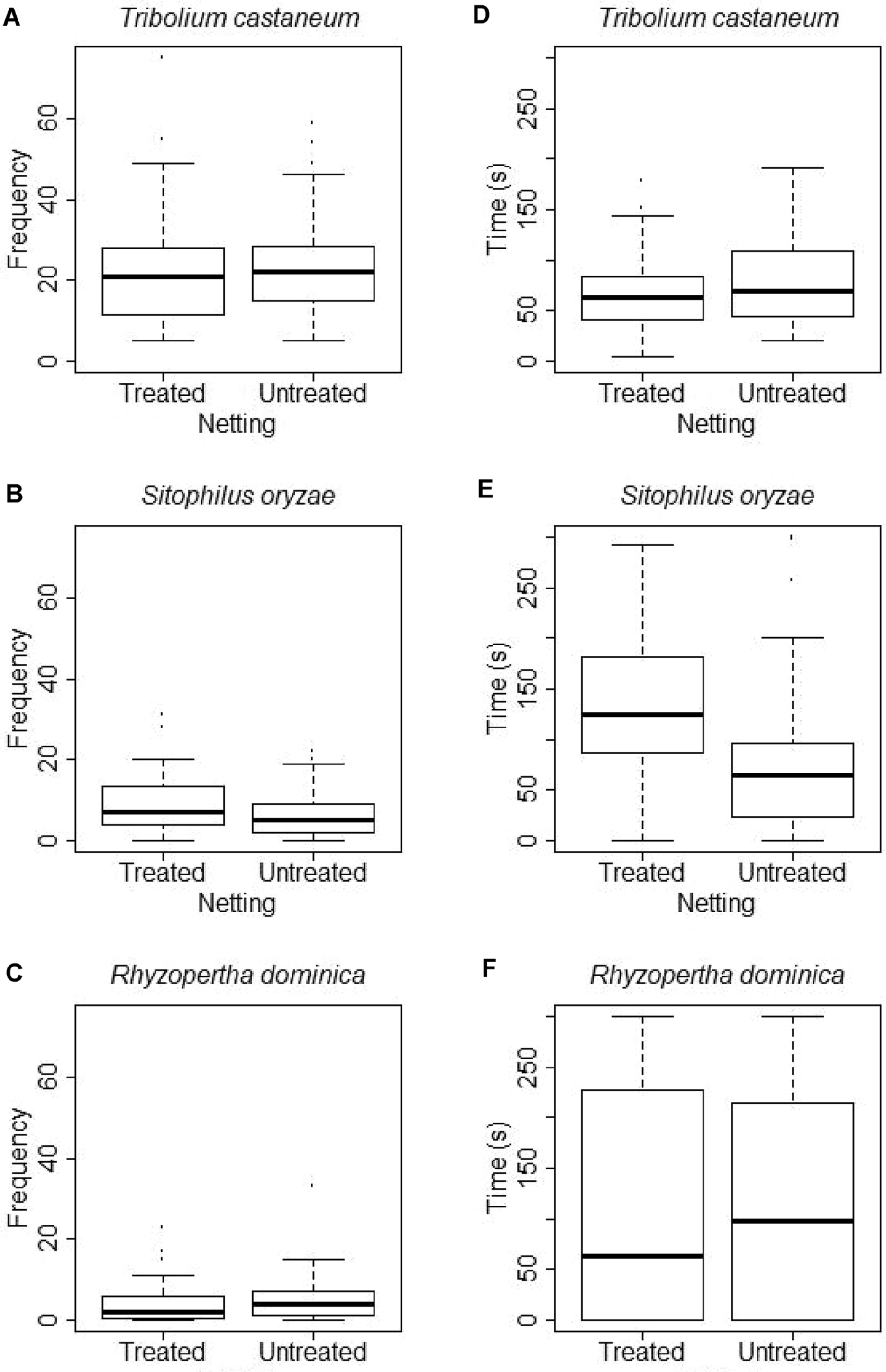

Fig. 1 Box plots of the mean percentage for the frequency of visits on the treated–untreated netting arena for A) *T. castaneum*, B) *S. oryzae*, and C) *R. dominica* and the total time spent on either treated or untreated netting material for D) *T. castaneum*, E) *S. oryzae*, and F) *R. dominica*

of species and arena were not significant ($\chi^2 = 3.29$; df = 4; $P = 0.51$). *Tribolium castaneum* spent a significant proportion of time off of the netting (0.5 ± 0.02 s), which was significantly more than both *R. dominica* (0.7 ± 0.2 s; $t = 11.33$; df = 547; $P < 0.0001$) and *S. oryzae* (0.7 ± 0.2 s; $t = -9.48$; df = 547; $P < 0.0001$). *Rhyzopertha dominica* and *S. oryzae* did not significantly differ from each other ($t = 1.84$; df = 547; $P = 0.16$). Further analysis shows the time spent on the netting and the time spent on the caulking did not significantly differ across all arenas for *T. castaneum* ($t = -1.46$; df = 362; $P = 0.14$) but was significantly different for *S. oryzae* ($t = 16.59$; df = 358; $P < 0.0001$) and *R.*

dominica ($t = 18.39$; df = 358; $P < 0.0001$). The increase in time *T. castaneum* spent off the netting and on the caulking could be due to the tendency of *T. castaneum* to move around the edges of a room or in this case the edges of the arena. Both *S. oryzae* and *R. dominica* spent more time on the netting (211.0 ± 5.2 s and 223.0 ± 5.6 s, respectively) than on the caulking (89.2 ± 5.2 s and 76.7 ± 5.6 s, respectively) compared to *T. castaneum* which spent nearly equal time on and off the netting, 147.0 ± 3.4 s and 153.0 ± 3.4 s on the caulking, respectively.

We also examined the difference in velocity for each species separately and for each arena configuration in the first 2.5 min (early) and second 2.5 min (late) time frame (Table 2). There were significant differences in the mean instantaneous velocity of *T. castaneum* adults by the arena treatment ($F = 82.43$; df = 2; $P < 0.0001$), time ($F = 73.83$; df = 1; $P < 0.0001$), and arena treatment × time interaction ($F = 28.32$; df = 2; $P < 0.0001$). The same significant differences occurred in the mean distance moved (cm) of *T. castaneum* adults; arena treatment ($F = 18.28$ df = 2; $P < 0.0001$), time ($F = 42.25$; df = 1; $P < 0.0001$), and arena treatment × time interaction ($F = 13.55$; df = 2; $P < 0.0001$). *Tribolium castaneum* had the fastest velocity in the second 2.5 min of the assay (0.4 cm/s) compared to all other time and arena treatments and this corresponded to the farthest distance moved (Table 2).

The mean velocity for *S. oryzae* was significantly different for arena treatment ($F = 1546.37$; $df_{Num} = 2$; $df_{Den} = 0$; $P < 0.0001$) and time ($F = 27.87$; $df_{Num} = 1$; $df_{Den} = 0$; $P < 0.0001$) but not for the interaction between arena treatment and time ($F = 1.44$; $df_{Num} = 2$; $df_{Den} = 0$; $P = 0.24$). The mean distance moved was significantly different for both arena treatment ($F = 639.97$; $df_{Num} = 2$; $df_{Den} = 0$; $P < 0.0001$) and time frame ($F = 33.95$; $df_{Num} = 1$; $df_{Den} = 0$; $P < 0.0001$) but not their interaction ($F = 1.66$; $df_{Num} = 2$; $df_{Den} = 0$; $P = 0.19$). *Sitophilus oryzae* overall had significantly higher velocity on the treated–untreated arenas regardless of the time frame (Table 2). Similar to *T. castaneum*, the higher velocity resulted in significantly greater distanced moved on the untreated–treated arena treatments.

Comparing mean velocity for *R. dominica* ($df_{Den} = 0$), time frame ($df_{Num} = 1$), arena configuration ($df_{Num} = 2$), and their interaction (df = 2) were all significant ($F_{range} = 31.35 – 218.55$; $P < 0.0001$). All arenas were significantly different between the first 2.5 min and last 2.5 min of the assays, with the latter 2.5 min of the assay having a higher velocity than the early half. Distance moved also showed similar patterns with time frame ($df_{Num} = 1$), arena configuration and their interaction ($df_{Num} = 2$) were all significant ($F_{range} = 4.08 – 35.79$; $P < 0.0001$). As with *S. oryzae* and *T. castaneum* higher mean velocity resulted in a greater distance traveled and the farthest distance traveled was in the last 2.5 min of the assay.

Efficacy after exposure to treated netting

There was no mortality of *T. castaneum* exposed to the untreated netting, and no affected adults were observed. Therefore, no corrections were made. All main factors (netting treatment, diet, and time) and their interactions were significant for the percentage of live and affected *T. castaneum* (Table 3). The main factor of diet and the interaction between diet and treatment were not significant for the percentage of dead *T. castaneum.*

Immediately following the 60 min exposure to the LLIN, > 96% of adult *T. castaneum* were affected. The percentage of affected *T. castaneum* held without food decreased significantly ($F = 6.11$; df = 4; $P = 0.001$) from 1 to 7 d post-exposure to the LLIN, 97 to 83%, respectively (Fig. 2A). The decrease in affected adults correlated to an increase in adult mortality from 3 to 12% by 7 d post-exposure. There was also an increase in live adults, 5% at 7 d post-exposure, but was not significantly different at any post-exposure time period ($F = 1.32$; df = 4; $P = 0.2723$). On the contrary, the presence of food played a significant role in *T. castaneum* recovery. As expected affected *T. castaneum*

Table 2 Tukey adjusted differences between early (first 2.5 min) and late (last 2.5 min) time frame for mean velocity (LS-mean ± SE) and mean distance moved (LS-mean ± SE) for each species for each arena treatment, (Trt-Trt) treated–treated; (Trt-Unt) treated–untreated; (Unt-Unt) untreated–untreated

Arena trmts	time	*T. castaneum*	*S. oryzae*	*R. dominica*	*T. castaneum*	*S. oryzae*	*R. dominica*
		Velocity (cm/s)			Distance moved (cm)		
Trt-Trt	Early	0.30 ± 0.004 bc	0.54 ± 0.003 b	0.14 ± 0.002 d	0.011 ± 0.0002 b	0.019 ± 0.0001 b	0.0095 ± 0.0004 bc
Trt-Unt		0.29 ± 0.004 bc	0.67 ± 0.003 a	0.15 ± 0.002 c	0.011 ± 0.0002 b	0.023 ± 0.0001 a	0.0092 ± 0.0004 c
Unt-Unt		0.27 ± 0.004 d	0.54 ± 0.003 b	0.14 ± 0.002 d	0.011 ± 0.0002 b	0.019 ± 0.0001 b	0.0099 ± 0.0004 bc
Trt-Trt	Late	0.36 ± 0.004 a	0.52 ± 0.003 c	0.19 ± 0.002 a	0.014 ± 0.0002 a	0.018 ± 0.0001 c	0.012 ± 0.0004 a
Trt-Unt		0.31 ± 0.004 b	0.66 ± 0.003 a	0.17 ± 0.002 b	0.012 ± 0.0002 b	0.023 ± 0.0001 a	0.022 ± 0.0004 bc
Unt-Unt		0.28 ± 0.004 cd	0.52 ± 0.003 c	0.15 ± 0.002 c	0.012 ± 0.0002 b	0.018 ± 0.0001 c	0.011 ± 0.0004 c

Different letters within the column indicate significant differences between each arena configuration and time frame combinations. If comparisons were not significant ($P > 0.05$)

Table 3 Three-way ANOVA for the percentage of live, affected, and dead *T. castaneum* adults after exposure on the treated or untreated netting and the main effects of treatment (LLIN or untreated netting), diet (food vs. no food), and post-exposure length (time), and their interactions

Factor—Live	*F*	df	*P*
Treatment	1228.28	1	< .0001
Diet	43.36	1	< .0001
Time	5.61	3	0.0010
Treatment × Diet	43.36	1	< .0001
Treatment × Time	5.61	3	0.0010
Diet × Time	2.94	3	0.0342
Treatment × Diet × Time	2.94	3	0.0342
Affected			
Treatment	1272.91	1	< .0001
Diet	44.81	1	< .0001
Time	12.83	3	< .0001
Treatment × Diet	44.81	1	< .0001
Treatment × Time	12.83	3	< .0001
Diet × Time	2.73	3	0.0448
Treatment × Diet × Time	2.73	3	0.0448
Dead			
Treatment	51.86	1	< .0001
Diet	0.12	1	0.7313
Time	3.49	3	0.0164
Treatment × Diet	0.12	1	0.7313
Treatment × Time	3.49	3	0.0164
Diet × Time	2.97	3	0.0329
Treatment × Diet × Time	2.97	3	0.0329

decreased significantly ($F = 12.71$; df = 4; $P < 0.001$) from 88 to 35% by 7 d post-exposure. The percentage of live adults, those who recovered from the affected state, increased significantly from 1 d post-exposure, 10%, to 7 d post-exposure, 58% ($F = 6.85$; df = 4; $P = 0.0001$) while mortality only reached 7% at 7 d post-exposure. There were no affected *S. oryzae* observed after exposure to the untreated netting at any timepoint. Overall control mortality was < 6% when held without food and < 4% when held with food, and no corrections to mortality were made. The main effect of treatment and time was significant for the percent live, affected and dead (Table 4), but their interaction was only significant for the percent dead. All other interactions were not significant.

Immediately after the 60 min exposure on the LLIN, 77% of *S. oryzae* were affected compared to the 96% of *T. castaneum* adults. The major difference between *T. castaneum* and *S. oryzae* is that there was no adult recovery and 100% of adults were either affected or dead at every time point regardless of whether food was present (Fig. 2C, D). In addition, food had no effect on *S. oryzae*. As the post-exposure period increased from 1 to 3 d, the percentage of affected adults significantly decreased ($F = 1.33$; df = 2; $P < 0.0001$) from 95 to 58% without food and 92–51% with food but the percentage of dead adults increased ($F = 21.33$; df = 2; $P < 0.0001$) to 42% and 49% when held without food or with food, respectively. This is roughly 11 times greater mortality compared to *T. castaneum* at 3 d post-exposure and 5 times greater mortality at 7 d post-exposure.

Similar to both *T. castaneum* and *S. oryzae,* mortality of *R. dominica* on control netting was < 1% and there were no affected individuals, and therefore, no corrections were made. The main effect of treatment was significant for live, affected, and dead *R. dominica* (Table 5) but there was no significant effect of diet on live, affected, or dead *R. dominica*.

Immediately after the 60 min exposure on the LLIN, 100% of *R. dominica* adults affected. Similar to *S. oryzae,* all adult *R. dominica* were either affected or dead at all observation periods after exposure (Fig. 2E, 2F) and there was no adult recovery. One day after exposure to the treated netting, ~ 99% of adults were affected when held with or without food. Over the next 3 d, the percentage of affected adults significantly decreased ($F = 54.69$; df = 2; $P < 0.0001$) and the percentage of dead adults significantly increased ($F = 54.70$; df = 2; $P < 0.0001$) to 29% of adults, regardless of the presence of food. In comparison *T. castaneum* at 3 d post-exposure, this is roughly 7 times greater mortality but *S. oryzae* had 1.5 times greater mortality.

Discussion

The results of our study showed that there was no significant repellent or attractive attributes of the LLIN among these species when observed in a wind tunnel or by comparing the frequency of visits between adults of each species on an untreated–treated arena. This attribute positively affects assessment of its use as a physical and chemical barrier treatment through which insects must cross before entering a facility. Our study did show that there are significant differences between species in terms of distance moved and mobility on the netting itself. One hypothesis to explain differences in the movement parameters observed among the species is that they likely arise as a result of anatomical and physiological differences between the species. For example, *T. castaneum* moved faster, spent the most time highly mobile, and moved the greatest distance on the netting compared to *S. oryzae* and *R. dominica*. The larger physical size of *T. castaneum,* 1.5–1.8 mg weight, 3.4 mm long, 1.0 mm wide (Park 1934), compared to *R. dominica,* 1.0–1.4 mg weight, 2–3 mm long, 0.8–1.0 mm wide (Edde 2012), could make this beetle more adept at moving across the netting surface. The hole openings in the netting are approximately 1.2–1.4 mm in diameter, which allow each of our insect species to traverse through, but the smaller

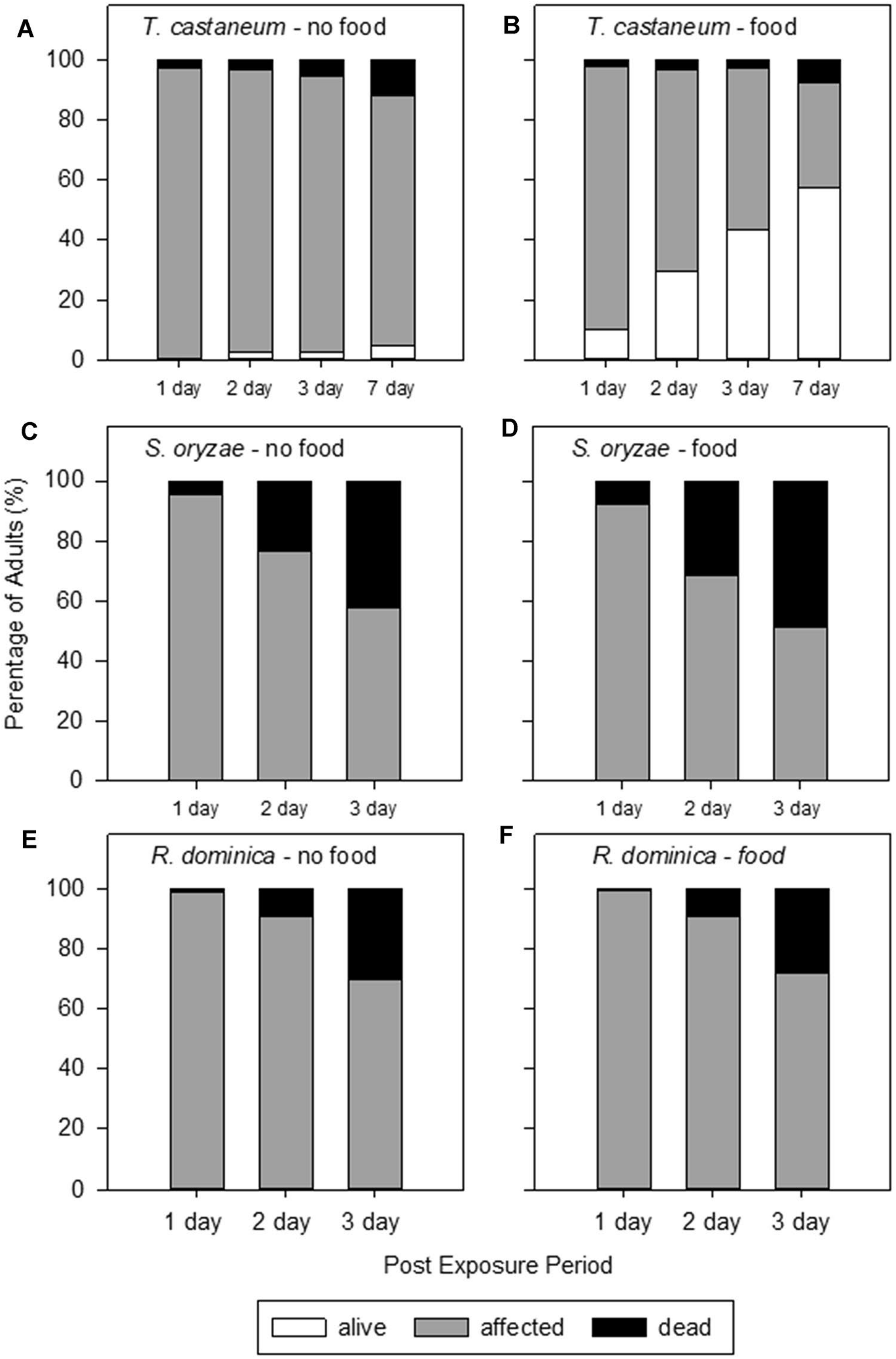

Fig. 2 Mean percentage of adults **A** *T. castaneum* held without food; **B** *T. castaneum* held with food; **C** *S. oryzae* held without food; **D** *S. oryzae* held with food; **E** *R. dominica* held without food; **F** *R. dominica* held with food, classified as alive, affected, or dead at 1-, 2-, 3- or 7-d post-exposure to LLIN

body with of *R. dominica* (0.8–1.0 mm; Edde 2012) could increase the amount of time *R. dominica* spent moving in and out of the netting holes compared with *T. castaneum* and *S. oryzae* which could move over or around the holes more easily (1.0 mm and 0.9–1.0 mm, respectively; Park 1934; Devi et al. 2017). Since *T. castaneum* spent more time highly mobile and at a greater velocity, this could account for the greater distance traveled during the testing period. Generally speaking, each species moved a greater distance, had higher velocity, and increased time spent immobile on arena treatments that had at least one half containing the LLIN. We can conclude that the direct exposure on the netting does not appear to limit the movement of the beetles, but it appears as if the adults were behaving normally. This

Table 4 Three-way ANOVA for the percentage of live, affected, and dead *S. oryzae* adults after exposure on the treated or untreated netting and the main effects of treatment (LLIN or untreated netting), diet (food vs. no food), and post-exposure length (time), and their interactions

Factor—Live	*F*	df	*P*
Treatment	7458.07	1	< .0001
Diet	0.44	1	0.5081
Time	2.15	2	0.1193
Treatment × Diet	0.44	1	0.5081
Treatment × Time	2.15	2	0.1193
Diet × Time	0.04	2	0.9625
Treatment × Diet × Time	0.04	2	0.9625
Affected			
Treatment	1063.57	1	< .0001
Diet	2.31	1	0.1303
Time	21.24	2	< .0001
Treatment × Diet	2.31	1	0.1303
Treatment × Time	21.24	2	< .0001
Diet × Time	0.16	2	0.8491
Treatment × Diet × Time	0.16	2	0.8491
Dead			
Treatment	84.33	1	< .0001
Diet	1.14	1	0.2875
Time	22.59	2	< .0001
Treatment × Diet	2.74	1	0.0994
Treatment × Time	12.35	2	< .0001
Diet × Time	0.08	2	0.9200
Treatment × Diet × Time	0.19	2	0.8234

Table 5 Three-way ANOVA for the percentage of live, affected, and dead *R. dominica* adults after exposure on the treated or untreated netting and the main effects of treatment (LLIN or untreated netting), diet (food vs. no food), and post-exposure length (time), and their interactions

Factor—Live	*F*	df	*P*
Treatment	63,476.7	1	< .0001
Diet	3.00	1	0.0851
Time	0.00	2	1.000
Treatment × Diet	3.00	1	0.0851
Treatment × Time	0.00	2	1.000
Diet × Time	0.00	2	1.000
Treatment × Diet × Time	0.00	2	1.000
Affected			
Treatment	3956.98	1	< .0001
Diet	0.11	1	0.7409
Time	53.07	2	< .0001
Treatment × Diet	0.11	1	0.7409
Treatment × Time	53.07	2	< .0001
Diet × Time	0.15	2	0.8596
Treatment × Diet × Time	0.15	2	0.8596
Dead			
Treatment	140.83	1	< .0001
Diet	0.66	1	0.4167
Time	48.73	2	< .0001
Treatment × Diet	0.03	1	0.8593
Treatment × Time	48.73	2	< .0001
Diet × Time	0.14	2	0.8698
Treatment × Diet × Time	0.14	2	0.8698

is important because the insects are moving on the treated netting, and they will continue to be exposed to the deltamethrin and potentially at long enough exposure times to have 100% mortality.

The active ingredient in the LLIN is deltamethrin, which acts on the insect's central nervous system by disrupting the gating mechanism of voltage-gated sodium channels; sufficient exposure to the compound causes tremors, involuntary extremity movements, and reduced coordination (Narahashi 1971), otherwise defined as affected when observed in the laboratory. The mode of action of the deltamethrin LLIN is by contact on surfaces of the insect cuticle or tarsi, and upon extend exposure results in affected adults and mortality. Directly comparing treated–treated and untreated–untreated netting, there is little differences in movement parameters within each species during our 5 min trial. However, Morrison et al. (2018) observed *R. dominica* and *T. castaneum* movement decreased, distance moved decreased, but angular velocity increased 2 h after exposures to the same LLIN for 1, 5 or 10 min. These same effects can also be observed immature *T. castaneum* and warehouse beetle, *Trogoderma variabile* (Ballon) (Coleoptera: Dermestidae) (Wilkins et al. 2020a) when observed after exposure to the LLIN. In these cases, the reduction in insect movement after exposure to the LLIN is a direct result of the deltamethrin toxicity in the insects' central nervous system over time. Deltamethrin toxicity also causes insect mortality. We observed differences in susceptibility between each species and differences in recovery when food was present. Our results show that *R. dominica* was affected at a faster rate and to a greater degree than *S. oryzae* and *T. castaneum* since all of *R. dominica* adults were affected after 60 min compared to 77% of *S. oryzae* and 96% of *T. castaneum*. Yet, *S. oryzae* had great mortality 3 d after exposure compared to *T. castaneum* and *R. dominica*. However, all of *S. oryzae* and *R. dominica* were either affected or dead at each post-exposure interval. It should be noted that affected individuals may not mechanically be able to locate and travel to a novel food source, but if the current food patch is adequate for the next generation there is no driving force for the insects to exploit a new food resource. The fact that there are still *T. castaneum* individuals that survived, even without a food source, could be enough to maintain the insect population. Previous research into the effect of deltamethrin-treated packaging has shown that *T.*

castaneum adults will still produce viable progeny if they are exposed for sublethal time periods (Scheff and Arthur 2018). Benchtop bioassays performed by this laboratory group have also shown *T. castaneum* adults exposed to treated LLIN for a short 5 min exposure had a decrease in progeny production but is not expected to decrease the population (unpublished data, Scheff and Gerken). Considering that, to the best of our knowledge, the effect of shorter exposure intervals on population dynamics has not been fully investigated and some trials are currently underway. One potential way to increase exposure to deltamethrin-treated netting is by inducing multiple exposures (Gerken et al. 2020), which could be done by pairing it with a food bait or pheromone lure to attract the insects away from a building or mill, for example in interception traps (Wilkins et al. 2020b). A food bait or lure could attract the insects, and upon walking on the treated material multiple times or for longer periods, delayed effects including increased mortality could ensue.

Each species tested here showed a delayed mortality after netting exposure, ranging from 7 to 48%, which is consistent with the results observed from bioassays conducted with deltamethrin-treated packaging (Scheff et al. 2018; Kavallieratos et al. 2017) and α-cypermethrin-coated netting (Rumbos et al. 2018). As time elapsed after exposure to the LLIN, there was also an increase in mortality. The same effect was observed for adult *L. serricorne* and the tobacco moth, *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae), exposed to α-cypermethrin-treated netting for up to 24 h (Rumbos et al. 2018). There was no significant difference in mortality of *L. serricorne* compared to untreated netting initially, but as the post-exposure time increased from 1 to 7 d, mortality increased to 55% by 7 d (Rumbos et al. 2018). However, it is important to note that only a 24-h exposure period to the α-cypermethrin netting resulted in a significantly higher mortality compared to a 24-h exposure period on untreated netting, 55% and 24%, respectively. Similarly, *E. elutella* also had 100% mortality by 7 d after a 24 h exposure to the treated netting and only the 12- and 24-h exposure periods were significantly different from the control mortality (Rumbos et al. 2018). There were no significant differences in mortality between the treated and untreated netting in exposure times < 12 h for both *L. serricorne* and *E. elutella*. In our study, we chose a 60 min exposure period to the LLIN as a benchmark to determine knockdown and delayed mortality for our three species of insects based on the results obtained from Rumbos et al. (2018) and Kavallieratos et al. (2017). A 60 min exposure to the LLIN is reasonably likely to occur, depending on the implementation technique the LLIN is used. The LLIN we investigated has the potential for many uses throughout bulk grain storage, food processing facilities, storage warehouses, shipping, and transportation. Longer exposures, such as 60 min, could occur when the LLIN is utilized as a pallet covering for packaged food products during transportation and shipping between cities, states, and countries. The LLIN could be used for the protection of agricultural commodities such as tobacco or peanuts during storage and dry down. Thus, it is important to determine the knockdown and mortality of stored product insects at a longer exposure period that could occur in field applications as well as shorter intervals.

It should be noted that in both the Rumbos et al. (2018) and our study provided evidence that high and rapid knockdown or being classified as affected, does not necessarily translate into insect mortality for some species of stored product insects. The presence of food can significantly affect insect mortality. Both Gerken et al. (2020) and our study demonstrated that the addition of a food source appeared to aid in the recovery of affected *T. castaneum* adults after exposure to LLIN. *Tribolium castaneum* showed > 55% recovery from exposure when given a food source and only 5% when no food was provided. The impact of food on insect recovery has also been observed and documented in previous studies involving pyrethrin/pyrethroid-based aerosols and contact insecticide (Arthur 1998a,b, 2000a,b, 2009, 2013, 2014; Sehgal et al. 2014) for both *T. castaneum* and *T. confusum* adults. Therefore, before combining and reporting affected and dead individuals into one category, the biology of the insect in relation to recovery after removal from a treated surface must be fully understood because researchers could be overstating the effect of the treatment. Being knocked down or being affected by an insecticide is a transitory state, whereby adult beetles will either succumb to the toxic effects or they will recover (Arthur 2015) and *T. castaneum* adults were the only species in our study that recovered after exposure when given a food source.

The recovery and maintenance of populations after exposure to LLIN is important because the recovery from pyrethroids could lead to the development of resistance, and the pace of resistance development could increase with even shorter exposures if those exposure do no kill *T. castaneum, S. oryzae* and *R. dominica*. In this context, the rotation of the active compound or mode of action is the key to limiting the development of insecticide-resistant populations and reliance on one single treatment technique, active ingredient, or mode of action, will increase the chances for a resistant population. Understanding that LLINs must be used in a comprehensive IPM program that employs multiple hurdles or pest mitigation tools, inside and outside of a facility, will be the key to preventing future resistance issues. This includes using mechanical tools such as food or pheromone baited traps, physical tools like shutting doors and windows, and monitoring pest populations within a space to determine if additional chemical treatments are needed. Additional studies on a larger suite of stored product species of concern are also needed to understand how the use of LLIN could affect competing populations found in food processing and storage facilities. Finally, LLINs with other active ingredients should also be evaluated to understand whether modes of action could be rotated for netting as well as how resistant populations of stored product insects may respond to the netting.

Conclusions

Warehouse, storage facilities, food processing facilities, and grain bins contain food volatiles and present many opportunities for insect colonization of spillage, packaged food, or raw grain to become infested. The use of deltamethrin-treated netting utilizes two methods of protection for stored products, mechanical and chemical protection. We observed delayed mortality in three species of stored product insects and changes in insect movement on the netting. Importantly, no long-distance or contact repellency was observed among the tested species to the insecticide netting. In preventing the development of resistance, multiple hurdles or IPM methods, each with their own mode of action, are the keys to a successful IPM program. Using this longstanding technology in a new and innovative manner delivers another tool in the IPM toolbox.

Author contributions

All authors conceived and designed the research. DSS, ARG, and WRM conducted experiments and analyzed data. DSS, ARG, and WRM wrote the manuscript. All authors read and approved the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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