Research Article

Received: 24 July 2020 Revised: 30 September 2020 Accepted article published: 13 October 2020 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.6134

Effectiveness of long-lasting insecticide netting on *Tribolium castaneum* is modulated by multiple exposures, biotic, and abiotic factors

Alison R Gerken,* Erin D Scully, James F Campbell and William R Morrison III

Abstract

BACKGROUND: Prevention is the first line of defense in mitigating losses of post-harvest crops. Long-lasting insecticide treated (LLIN) could be used in food facilities to expose insects to insecticide at different areas within a facility. Prior research has shown that single short exposures reduce movement and longer exposures increase mortality for stored-product insect pests, but we do not know how multiple short duration exposures and biotic and abiotic conditions affect insects exposed to LLIN. Here, we repeatedly exposed red flour beetles, *Tribolium castaneum*, to LLIN to assess the cumulative effects. We also examined the effects of beetle age and time of day during exposure, and temperature, humidity and food availability during recovery after a single exposure to LLIN.

RESULTS: We found that four repeated 10-min exposures had similar knockdown effects as a single 30-min exposure. We also found that beetles were more affected when aged 1–6 days versus 14–20 days or were exposed at mid- or late in the day versus earlier in the day. Higher recovery levels were observed with food and at higher relative humidity. In addition, older beetles were more active than younger beetles during exposure, which could reduce time in contact with netting and partially explain why older beetles tended to be less affected.

CONCLUSION: Some individuals can recover after exposure to LLIN, dependent on exposure duration and environmental factors, but our study shows that sublethal effects likely persist and future work should consider the physiology of *T. castaneum* before, during, and after exposure to LLIN.

Supporting information may be found in the online version of this article.

Keywords: red flour beetle; stored products; integrated pest management; bed nets; sublethal effects; indirect toxicity

1 INTRODUCTION

After harvest, insect pests infest and ruin millions of dollars' worth of whole or processed grain.[1] Damage caused by insect pests ranges in type and severity, reducing the quantity and quality of products, triggering a need for remedial treatments, and may render products unsuitable for consumption.[2–5] Insect infestations can occur in relatively small amounts of food, such as spillage accumulations inside or outside structures,[6,7] and can contribute to product infestation. Managing populations of stored product pest (SPP) insects requires significant investments of time and monetary resources, and prevention is a key initial step in integrated pest management (IPM) strategies in post-harvest supply chains.[8,9] Prevention techniques such as good facility design and sanitation are important parts of an effective IPM program.[10,11] Another newer prevention technique is incorporation of insecticides within packaging to prevent insects from entering or reproducing inside finished products.[12–14]

Sealing grain bins and food processing and storage structures is an important component of pest prevention programs, and openings needed for air exchange can be covered with screening as a physical barrier to insect movement. However, typical window screening is not fine enough to exclude all insect species and fine mesh screening necessary to physically exclude insects can be expensive and become blocked with fine particulates present in dusty food facilities.[15] Netting or screening impregnated or treated with insecticide can reduce insect movement through a chemical barrier while also maintaining air flow and reducing dust blockage and cost. Long-lasting insecticidal netting (LLIN) was originally used as bed netting for management of mosquitos in malaria-endemic regions,[16] but has also gained attention for use against other insects in agricultural and urban settings.[17–21] Recent research has demonstrated the potential of this netting

---

* *Correspondence to: AR Gerken, USDA, Agricultural Research Service, Center for Grain and Animal Health Research, 1515 College Ave, Manhattan, KS 66502, USA. E-mail: alison.gerken@usda.gov*

Center for Grain and Animal Health Research, USDA, Agricultural Research Service, Manhattan, KS, USA

for management of SPPs.[22–24] LLIN acts as an effective barrier to the dispersal of SPP insects as the insecticide causes direct mortality and knockdown, but can reduce mobility prior to inducing knockdown or mortality.[22,24] The efficacy of LLIN has been tested against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Trogoderma variabile* Ballion (Coleoptera: Dermestidae) using deltamethrin-incorporated netting,[22,24] and *Lasioderma serricorne* (F.) (Coleoptera: Ptinidae) and *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae) using alpha-cypermethrin coated netting.[23,25,26]

The LLIN tested here contains 0.4% deltamethrin, which acts as a neurotoxin causing erratic movement and paralysis.[27–29] Although long-term contact with LLIN was initially thought to be needed,[30–32] recent research has shown that even brief 1-min exposures to LLIN result in a multiple-fold reduction in movement, and impairment of dispersal ability to novel food resources.[14,22] Although brief exposures may not directly kill insects, impeded foraging and movement likely prevent insects from gaining further access to facilities. It is likely that encounters between dispersing insects and LLIN will be variable in length and because of effects on dispersal ability, repeated interactions with the netting seem likely.[33] Chronic exposure to insecticides can have cumulative effects even if an initial effect appears only sublethal.[34–36] Cumulative impacts of repeated short-term exposures to LLIN on SPP insects have not been previously investigated.

A variety of biotic and abiotic factors are known to influence the efficacy of insecticides and could also modulate the efficacy of LLIN. For example, insects given food post insecticide exposure show significantly greater recovery compared with those without food.[32,37–40] Recent feeding and mating status can influence tolerance to insecticides[41] as well as movement away from an area after exposure to insecticides.[42] Temperature also plays a significant role in the response to insecticides, with warmer temperatures generally increasing toxicity for many compound classes.[43,44] However, little information is available on how biotic or abiotic factors affect recovery after exposure to LLIN. Because LLIN has a unique design allowing insects to pass through while being exposed to insecticide, testing biotic and abiotic factors in response to LLIN exposure is necessary to understand its overall effectiveness.

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a cosmopolitan SPP that can infest a wide range of foods, persist on small amounts of commodities,[3,45] is an effective colonizer, can move long distances,[6,45,46] and reproduces in high numbers.[47,48] *Tribolium castaneum* show significant but low mortality as adults after short exposure to LLIN, but sublethal effects on movement and behavior after exposure to LLIN have been observed.[22] Here, we tested the effects of multiple exposures to deltamethrin-based LLIN over a 5-day period compared with a single longer exposure to LLIN to evaluate cumulative effects over time. We also assessed biotic (e.g. beetle age during exposure, presence of food during recovery) and abiotic (e.g. time of day during exposure, environmental conditions during recovery) factors on the effectiveness of LLIN. These results provide crucial information on factors that can impact efficacy of LLIN on *T. castaneum* and how multiple exposures cumulatively impact insect mortality and movement.

2 MATERIALS AND METHODS

2.1 Insect rearing and population

A laboratory population of *T. castaneum* was used in experiments. This population had been kept at the USDA Center for Grain and Animal Health Research for over 30 years free of exposure to insecticide and maintained by subculturing ~ 50 adult individuals every month and allowing them to mate and lay eggs on 95% organic, unbleached flour conditioned with 5% brewer's yeast. Populations were kept in 0.95-L glass jars with filter paper lids for air flow in an environmental chamber set at a temperature of 28 °C, relative humidity (RH) of 65%, and a 16:8 h light/dark photoperiod. Insects used in assays below were adults of mixed sex and age but were typically < 30 days old unless otherwise specified.

2.2 Bioassay

D-Terrence netting (Vestergaard Inc.) with mesh size of 2×2 mm, incorporated with 0.4% deltamethrin was the LLIN used for assays. For all assays, netting was cut into circles and used to line bottoms of 100×100 mm diameter Petri dishes. For all conditions listed below, a total of ten replicates of ten *T. castaneum* adults were tested on netting with or without (e.g. control) insecticide and the number of affected beetles was recorded. Untreated netting was physically identical to treated netting but lacked insecticide.

2.3 Multiple day exposure to netting

Exposure over multiple days was conducted with cohorts of ten adult *T. castaneum* collected from continuous cultures and placed on netting for 10 min before being removed and placed on 0.41 $\pm$ 0.03 g (mean $\pm$ SE) of flour for recovery. The number of affected beetles was assessed 6 and 24 h after exposure. After 24-h recovery, the same beetles were exposed again for 10 min by sieving them from the flour (sieve #25, 0.71 mm^2 mesh size, Dual Mfg. Co.) and placing them back on netting for another 10-min exposure period. Beetles were then placed on new flour and their condition assessed 6 and 24 h later. These exposures were repeated every 24 h for a total of 5 days (cumulative exposure time 50 min); after five exposures, beetles were assessed at 6 h and again after 72 h. A single, 30-min continuous exposure period treatment was also performed using same methodology and evaluated after 6 and 24 h of recovery time. Beetles were recorded as unaffected if they were active and behaving normally with coordinated walking and leg movements or affected if they had uncoordinated walking, were on their backs with uncoordinated leg movement, or there was no movement.[22] Three blocks of ten Petri dishes containing ten adult beetles were assayed. All assays were performed between 8:00 a.m. and 9:00 a.m., and beetles recovered under laboratory conditions.

Data were analyzed using *proc glimmix* in SAS (version 9.4, SAS Institute) with proportion affected as the dependent variable and exposure frequency (10-min exposure on day 1 to day 5 or a single 30-min exposure) and recovery time (6 or 24 h) as the main explanatory variables as well as the interaction between the two. Replicate and block were random effects. LS-means were calculated for the interaction effect with Tukey adjustment ($\alpha = 0.05$).

2.4 Differential exposure and recovery conditions

Four biotic and abiotic factors associated with a 30-min exposure to LLIN were assayed: age of beetle at exposure, time of day at exposure, food presence during recovery, and temperature and RH during recovery. Beetles were exposed to LLIN in groups of ten individuals but then placed singly into wells of a 24-well plate with 0.05 ± 0.01 g of flour, except for the no-food treatment. Three blocks consisting of 100 beetles each were performed per treatment combination. Unless otherwise described, beetles were exposed to netting at 8:00 a.m. Condition of beetles was assessed as affected or unaffected after 6 ± 0.5 h post exposure (referred to as 6 h, hereafter) and again at 24 ± 0.5 h (24 h, hereafter).

Each of the four assays was independently conducted. First, age of beetle at exposure was controlled by collecting pupae from populations in culture and collecting individuals daily at adult emergence. Adults aged 1–6 days (young, hereafter) or 14–20 days (old, hereafter) were placed in 0.24-L glass jars with standard media before assay. Second, exposure at a specific time of day was assessed by exposing beetles at 8:00 a.m., 11.00 a.m., or 6.00 p.m. Third, to assess the effects of food on recovery, beetles were either given flour during recovery or were not provided food. Fourth, the effects of temperature and RH during recovery were assessed by placing beetles into an incubator set at 28 °C and 65% RH or keeping beetles on the laboratory bench at an average temperature of 23 ± 0.1 °C and RH between 40 and 46%, depending on the replicate. Temperature and RH were recorded at a minimum of 3, 6, and 24 h after exposure (HOBO Temp/RH Logger, Onset; Fig. S1).

For each assay, data were analyzed using *proc logistic* in SAS with affected individuals scored as '1' and unaffected individuals scored as '0'. Each assay was analyzed individually with number of affected individuals as the dependent variable and recovery time (6 and 24 h), the biotic and abiotic conditions tested, and the interaction of recovery time and these conditions as main effects. The odds ratio was conditions tested. LS-means were calculated using a Tukey adjustment for interaction effect.

2.5 Time of day and age effects on beetle movement on netting

To quantify how time of day and age affect beetle movement and subsequently LLIN effectiveness, movement parameters of individuals while on LLIN and untreated netting were assessed. Two separate experiments were conducted, the first to compare movement of old versus young beetles and the second to compare movement at 8:00 a.m., 11:00 a.m., and 6:00 p.m. Individual beetles were video-recorded for 2 min using a digital video camera (HDR-XR520V; Sony Corporation) and videos were analyzed for movement using Ethovision XT (Version 8.0, Noldus Information Technology). Information extracted using Ethovision included total distance moved (cm), velocity (cm s^{-1}), time moving (s; where moving is defined as a start velocity of 2.00 cm s^{-1} and stop velocity of 1.75 cm s^{-1}) or not moving (s; where not moving is start velocity of < 2.00 cm s^{-1} and stop velocity is < 1.75 cm s^{-1}), and frequency and duration (s) of movement classified as highly mobile (60% of the beetle's body changing position between frames at ten frames per second), mobile (20% body change), or immobile (0% body change). Three blocks of ten individuals for each netting and at each time or age were tested. Age data and time of day data were analyzed separately, using *proc glimmix* in SAS, with age of beetle or time of day and netting type (control versus LLIN), as well as their interaction, as main fixed effects. Block was used as a random effect. LS-means were calculated for age of the beetle with Tukey adjustment ($\alpha = 0.05$).

3 RESULTS

Beetles exposed to untreated netting had fewer than 4% affected individuals for any conditions tested (Table S1). The low percent of affected individuals on untreated netting is similar to other studies (Morrison *et al.*, 2018) and further analyses were not conducted.

3.1 Multiple day exposure to netting

Over the course of 5 days of repeated 10-min exposures to LLIN, proportion affected steadily increased, with the highest proportion after 5 days and the lowest at 1 day of exposure (Fig. 1). The cumulative impacts of shorter exposures were of the same magnitude as a single 30-min exposure after four 10-min exposures at 6 h post treatment and after five 10-min exposures at 24 h post treatment. The interaction of exposure frequency (multiple 10-min or a single 30-min exposure) and recovery time was significant ($F_{5,319} = 2.49$, $P = 0.031$), as was the main effect of exposure frequency ($F_{5,319} = 63.85$, $P < 0.0001$) but not recovery time ($F_{1,319} = 1.70$, $P = 0.19$). A higher proportion of individuals recovered 24 h post treatment compared with 6 h post treatment after a single exposure, but after more than two or more exposures the number of affected beetles did not differ between 6 and 24 h post treatment. In addition, after five exposures, beetles remained affected up to 72 h after exposure (Fig. 1).

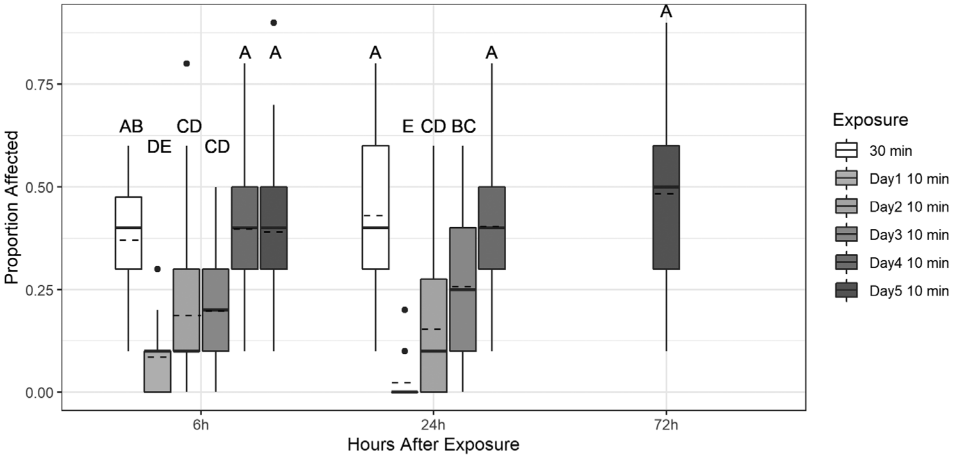

Figure 1. Proportion of beetles affected after multiple 10-min exposures (gray bars) or a single 30-min exposure (white bar). The proportion of beetles affected is out of ten beetles per Petri dish in the laboratory. Beetles were assessed for affected status 6 or 24 h after exposure, except on day 5 (not assessed until 72 h recovery time). Edges of boxes represent 25% and 75% range of data (IQR), the solid line within the box is the median, the dashed line is the mean, and whiskers are 1.5 × IQR. Outliers fall outside this range. Bars with shared letters are not significantly different from each other (Tukey adjusted significant differences, $\alpha = 0.05$).

Table 1. Type III analysis of effects for various recovery and exposure conditions

Effect	DF	Wald χ^2	*P*-value
Abiotic conditions			
Environment	1	20.64	<0.0001
Recovery time	1	0.063	0.80
Environment × Recovery time	1	0.48	0.49
Beetle age			
Age	1	15.82	<0.0001
Recovery time	1	22.71	<0.0001
Age × Recovery time	1	5.34	0.021
Time of day			
Time tested	2	5.48	0.064
Recovery time	1	4.64	0.031
Time tested × Recovery time	2	4.03	0.13
Presence of food			
Diet	1	9.37	0.0022
Recovery time	1	0.85	0.36
Diet × Recovery time	1	0.10	0.75

3.2 Differential exposure and recovery conditions

Temperature, presence of food, time of day, and beetle age significantly influenced recovery with a significant interaction of age and recovery time after exposure to LLIN (Table 1; Fig. 2). The presence of food during recovery led to 10% fewer affected adults compared with recovery without food. Recovery in the incubator, where the temperature was ~ 4–5 °C warmer and RH was 20% higher than on the laboratory bench, resulted in > 10% fewer affected individuals compared with the bench top. Almost 20% more young beetles were classified as affected at 24 h compared with old beetles at 6 h ($Z = 6.24$, $P < 0.0001$) and old beetles at 24 h ($Z = 4.69$, $P < 0.0001$; Fig. 2). Young beetles also showed an almost 20% increase in the number affected from 6 to 24 h post treatment ($Z = -5.16$, $P < 0.0001$). Further, the number of affected beetles significantly varied by the time of day during which exposure occurred (Table 1) with 5–6% more beetles affected when they were exposed at 6:00 p.m. compared with exposure at 8:00 a.m. ($Z = -2.22$, $P = 0.068$). Percentage recovery was similar, as there were 7–8% fewer beetles affected after 24 h compared with after 6 h at both 8:00 a.m. and 6:00 p.m. exposure times ($Z = 2.15$, $P < 0.05$).

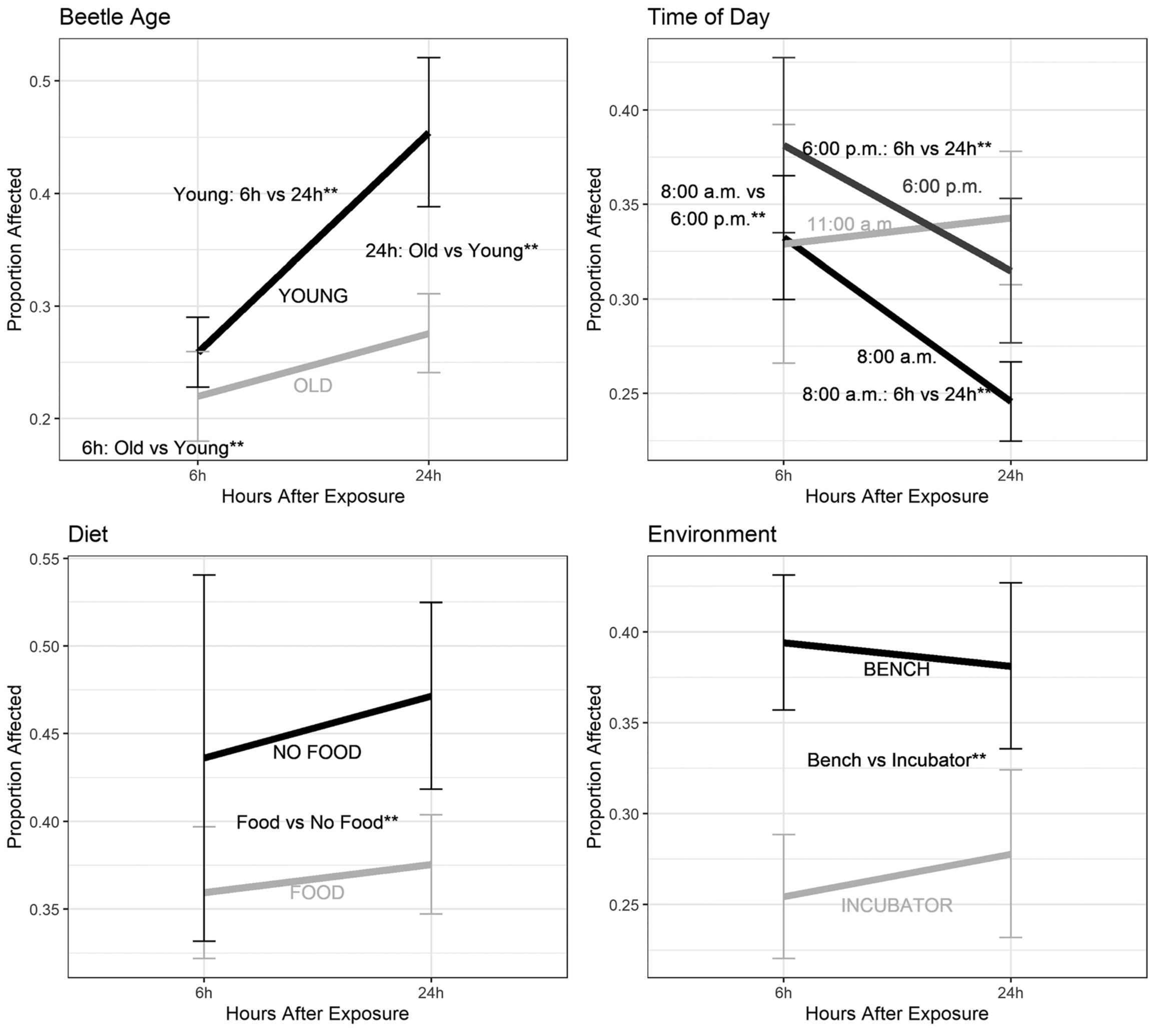

Figure 2. Proportion of beetles affected under various conditions. Beetles were assessed as single individuals and analyzed as such; proportion affected is represented in the graph as a visual tool. Each condition is represented by a line and panel. The shade of each line is solely for ease of visual interpretation of corresponding factor levels. Error bars represent the mean ± SE of the data based on the three blocks of data collection. Beetle age and time of day were conditions at time of LLIN exposure, whereas diet and environment are recovery conditions. **Significant (Tukey $\alpha < 0.05$) differences in the number of affected beetles between treatments.

Table 2. Type III tests for movement on netting for old versus young beetles and Tukey-adjusted LS-means ± standard error (estimate) for the age of the beetle

Variable	Effect	*F*	*P*-value	Estimate: Old	Estimate: Young
Distance moved (cm)	Age of beetle	**14.49**	**0.0002**	67.30 ± 3.36	53.23 ± 3.12
	Netting treatment	0.02	0.88		
	Age-by-Netting	0.17	0.68		
Velocity (cm s^{-1})	Age of beetle	**14.81**	**0.0002**	0.56 ± 0.028	0.45 ± 0.026
	Netting treatment	0.01	0.92		
	Age-by-Netting	0.08	0.78		
Duration moving	Age of beetle	**6.66**	**0.011**	7.55 ± 0.44	6.21 ± 0.41
	Netting treatment	0.09	0.77		
	Age-by-Netting	0.36	0.55		
Duration not moving	Age of beetle	3.13	0.080	112.07 ± 0.51$^{\mathrm{NS}}$	113.34 ± 0.51$^{\mathrm{NS}}$
	Netting treatment	0	0.97		
	Age-by-Netting	0.72	0.40		
Frequency highly mobile	Age of beetle	**6.02**	**0.016**	219.25 ± 14.60	183.0 ± 13.53
	Netting treatment	0.8	0.37		
	Age-by-Netting	0.3	0.59		
Duration highly mobile	Age of beetle	**5.1**	**0.026**	9.17 ± 0.58	7.73 ± 0.53
	Netting treatment	0.68	0.41		
	Age-by-Netting	0.41	0.52		
Frequency mobile	Age of beetle	**20.05**	**<0.0001**	203.29 ± 10.20	139.22 ± 10.03
	Netting treatment	0.19	0.66		
	Age-by-Netting	0	0.96		
Duration mobile	Age of beetle	**19.14**	**<0.0001**	8.62 ± 0.46	5.79 ± 0.45
	Netting treatment	0.15	0.70		
	Age-by-Netting	0.08	0.78		
Frequency immobile	Age of beetle	**19.73**	**<0.0001**	372.74 ± 17.01	283.02 ± 15.91
	Netting treatment	0.61	0.44		
	Age-by-Netting	0	0.99		
Duration immobile	Age of beetle	**11.24**	**0.0011**	101.94 ± 0.86	106.03 ± 0.86
	Netting treatment	0.09	0.77		
	Age-by-Netting	0.33	0.57		

Tukey group is within variables only. For Type III tests, denominator df = 112, numerator df = 1. Netting treatment is treated or untreated netting. NS = not significantly different at $P < 0.05$. All other comparisons are significant for differences in age of beetle at $P < 0.05$ and are given in bold.

3.3 Time of day and age effects of beetle movement on netting

Netting type (control or treated) did not significantly affect any movement variables and the interaction of beetle age and netting type was not significant (Table 2). For beetle age, older beetles were less affected by LLIN compared with younger beetles, and movement on netting over the course of our 2-min assays showed that older beetles moved significantly quicker and over longer distances than younger beetles (Table 2; Fig. 3). Distance moved, velocity, total time moving, frequency and duration of high mobility, frequency and duration of moderate mobility, and frequency and duration of immobility all significantly varied with age of beetle, with older beetles systematically exhibiting more activity than younger beetles (Fig. 3). For example, younger beetles spent an average of 5 s more time immobile over the course of the 120-s assay (as measured by changes in body position) than older beetles (Fig. 3f) and older beetles moved > 14 cm more over 2 min (Fig. 3a) and spent > 1 s more time moving (Fig. 3c). There was no significant difference between old and young beetles in total time spent not moving (as measured by threshold velocity) (Fig. 3d; Table 2).

When time of day at exposure was varied, there was a significant main effect of netting type and a significant interaction between time of day and netting type (Table 3). For reference, the number of affected beetles tended to be lower with exposure at 8:00 a.m. than at 6:00 p.m. Overall, beetles exposed to treated netting at 8:00 a.m. tended to spend more time not moving or immobile compared with beetles exposed at 6:00 p.m. (Fig. 4d,f), although overall velocity and distance moved did not differ (Fig. 4a,b). Beetles exposed to treated netting at 11:00 a.m. also tended to have a higher total distance moved compared with beetles exposed at 6:00 p.m. and spent more time immobile compared with beetles exposed at 6:00 p.m. (Fig. 4a,f). Time spent in high mobility and total time spent moving did not differ among tested times of day (Table 3). There were significant differences in time (s) moving and duration of high mobility between the types of netting (Fig. 4c,d), with beetles exposed to treated netting having significantly more movement than those exposed to control netting.

4 DISCUSSION

Age and physiological status can greatly influence metabolism and detoxification of insecticides[49–51] and thus, quantifying recovery or efficacy of insecticides on beetles in different physiological states can aid in assessing the full implications of deploying LLIN as a tool in IPM. Our data confirm that multiple

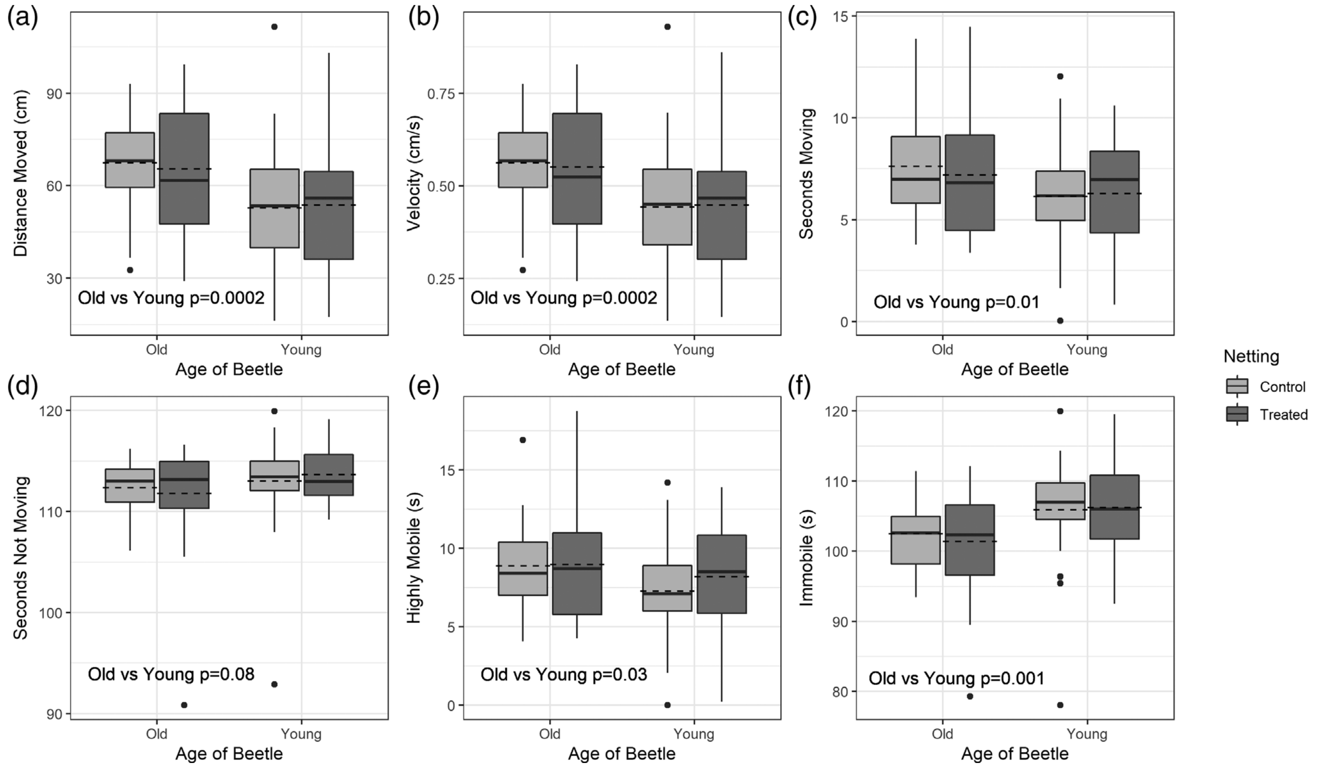

Figure 3. Mobility of young and old beetles exposed to either control netting (gray bars) or long-lasting insecticide treated netting (LLIN; black bars). All comparisons between young and old beetles are significantly different unless noted. Mobility was characterized by 2-min assays: (a) distance moved (cm); (b) velocity (cm s^{-1}); (c) time moving (s), measured by high velocity (>1.75 cm s^{-1}); (d) time spent not moving (s), not significantly different between ages (velocity of <1.75 cm s^{-1}); (e) time spent highly mobile (s), defined by 60% of the beetle's body changing position between frames at ten frames per second; and (f) time spent immobile (s), defined as 0% of the beetle's body changing position between frames at ten frames per second. Edges of boxes represent the 25% and 75% range of data (IQR), the solid line within the box is the median, the dashed line is the mean, and whiskers are 1.5 × IQR. Outliers fall outside this range.

exposures to LLIN have compounding effects and beetles cannot overcome the effects of previous exposure by metabolizing or detoxifying the insecticide during our experimental time frame. In the short term, when comparing the number of affected beetles exposed to LLIN after 6 and 24 h, beetles show some recovery after LLIN exposure, especially after a single 10-min exposure. Over time, however, the most-affected beetles were found at 72 h after a fifth exposure, suggesting the effects of multiple exposures are long-lasting. Interestingly, one 30-min exposure had the same effect on beetles as four or five repeated 10-min exposures. In the field, beetles may be exposed to LLIN for only a short time before falling off; knowing that multiple exposures can result in increased numbers of affected adults is important to understand the scope of application of LLIN. Low recovery was also demonstrated as delayed mortality in *L. serricorne* and *E. elutella* after exposure to alpha-cypermethrin netting[23] and is especially relevant for controlling beetles immigrating into food facilities or netting deployed as a protective layer on a commodity.[25] Even if beetles are knocked down by a short exposure to netting and then recover, they may encounter netting again as they move around. This also supports use of LLIN in an attract-and-kill application, where beetles may contact LLIN multiple times as a result of attractive semiochemicals deployed near netting.[52] Indeed, attract-and-kill based interception traps at commercial and pilot-scale food facilities appear a promising approach as Wilkins *et al.*[33] found strong response to attractants and impaired progeny production in traps with LLIN.

Variation in the proportion of affected beetles tended to increase as the number of exposures increased, suggesting that individual beetles have different levels of susceptibility that could affect the efficacy of LLIN within a population. For example, after a single 10-min exposure, the mean proportion of beetles affected ranged from 0 to 0.3 (SD 0.05–0.09), whereas after four or five exposures, proportion affected ranged from 0.1 to 0.9 (SD 0.17–0.20). Because there is a wide range of proportion affected as the number of exposures increases, there may be differences in cumulative uptake of insecticides in beetles and a variety of mechanisms to create resistance to LLIN. For example, in other insects, mechanisms for resistance to deltamethrin have been identified as enhanced detoxification and oxidase levels in *Triatoma infestans* Klug (Hemiptera: Reduviidae)[53] and *Cydia pomonella* L. (Lepidoptera: Tortricidae),[54,55] which can lead to population-level changes in response to insecticide treatments. Accounting for multiple exposures and the rate at which resistance may develop over time in a population can provide a baseline for mitigation of resistance prior to LLIN deployment in the field.

We also found that younger beetles were affected significantly more than older beetles and the number affected also increased from 6 to 24 h post exposure, although this was less pronounced for older beetles, indicating a delayed effect of LLIN contact. Effects of age on insecticide susceptibility have been noted in other studies. For example, in tsetse flies, older females had lower mortality than younger females when exposed to a range of insecticides[56]

Table 3. Type III tests for movement on netting for time of day beetles were assayed and Tukey adjusted LS-means ± standard error (estimate) for the time of day

Variable	Effect	*F*	*P*-value	Estimate: Treated	Estimate: Control
Distance moved (cm)	Time of day	2.10	0.13	8:00 a.m.: 85.11 ± 5.82	8:00 a.m.: 61.47 ± 5.82
	Netting treatment	**8.91**	**0.0033**	11:00: 90.54 ± 5.82	11:00 a.m.: 73.16 ± 5.82
	Time-by-Netting	**5.70**	**0.0040**	6:00 p.m.: 70.39 ± 5.82	6:00 p.m.: 76.93 ± 5.82
Velocity (cm s^{-1})	Time of day	1.44	0.24	8:00 a.m.: 0.76 ± 0.052	8:00 a.m.: 0.48 ± 0.052
	Netting treatment	**16.00**	**<0.0001**	11:00 a.m.: 0.78 ± 0.052	11:00 a.m.: 0.61 ± 0.052
	Time-by-Netting	**3.72**	**0.026**	6:00 p.m.: 0.68 ± 0.052	6:00 p.m.: 0.66 ± 0.052
Duration moving	Time of day	0.27	0.76		
	Netting treatment	**3.64**	**0.058**	$7.35 \pm 0.43^{\mathrm{NS}}$	$6.51 \pm 0.43^{\mathrm{NS}}$
	Time-by-Netting	1.77	0.17		
Duration not moving	Time of day	**8.92**	**0.0002**	8:00 a.m.: 116.86 ± 2.54 11:00 a.m.: 111.64 ± 2.54 6:00 p.m.: 106.18 ± 2.54	
	Netting treatment	**15.43**	**0.0001**	107.50 ± 2.32	115.62 ± 2.32
	Time-by-Netting	1.42	0.2437		
Frequency highly mobile	Time of day	0.78	0.46		
	Netting treatment	**4.87**	**0.029**	185.66 ± 9.79	159.93 ± 9.79
	Time-by-Netting	0.33	0.72		
Duration highly mobile	Time of day	1.11	0.33		
	Netting treatment	**5.40**	**0.021**	9.00 ± 0.48	7.58 ± 0.48
	Time-by-Netting	0.13	0.88		
Frequency mobile	Time of day	**3.64**	**0.028**	8:00 a.m.: 239.27 ± 22.55 11:00 a.m.: 263.48 ± 22.55 6:00 p.m.: 299.70 ± 22.55	
	Netting treatment	3.18	0.076		
	Time-by-Netting	0.98	0.38		
Duration mobile	Time of day	**3.56**	**0.031**	8:00 a.m.: 11.29 ± 2.20 11:00 a.m.: 12.47 ± 2.20 6:00 p.m.: 14.74 ± 2.20	
	Netting treatment	**7.71**	**0.0061**	14.32 ± 2.14	11.34 ± 2.14
	Time-by-Netting	0.56	0.57		
Frequency immobile	Time of day	0.11	0.90		
	Netting treatment	**7.44**	**0.0071**	377.54 ± 27.89	326.63 ± 27.89
	Time-by-Netting	1.03	0.36		
Duration immobile	Time of day	**9.74**	**<0.0001**	8:00 a.m.: 104.09 ± 2.36 11:00 a.m.: 98.53 ± 2.36 6:00 p.m.: 89.48 ± 2.36	
	Netting treatment	**18.25**	**<0.0001**	91.54 ± 1.93	103.20 ± 1.93
	Time-by-Netting	1.29	0.28		

Numerator df = 2 for time of day, 1 for netting treatment, and 2 for interaction. Denominator df = 172. NS = not significantly different at $P < 0.05$. Significant comparisons are given in bold; estimates for significant time of day main effect are for control and treated netting combined. Durations are given in seconds (s).

and younger *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) nymphs were also more susceptible than older nymphs to imidacloprid.[57] Time of day at exposure also had an impact on the proportion of affected beetles, with beetles exposed at 8:00 a.m. being less affected than beetles exposed at 6:00 p.m. Beetles exposed at both 8:00 a.m. and 6:00 p.m. showed 7–8% declines in proportion affected from 6 to 24 h recovery, showing the magnitude of recovery is similar for 8:00 a.m. and 6:00 p.m. time points but the initial impact of the insecticide is different.

Movement assays on older and younger beetles indicated that older beetles move more quickly over longer distances during the 2-min assays compared with younger beetles, which spent more total time immobile. Increased time immobile for younger beetles increases direct and sustained exposure to LLIN, whereas quicker, older beetles reduce time spent in direct contact with LLIN. Prior work demonstrated species-specific differences in movement that may contribute to differences in insecticide efficacy and changes in sublethal movement over time.[58] By contrast, beetles at 8:00 a.m. and 11:00 a.m. moved less compared with beetles at 6:00 p.m. yet were less affected by LLIN, which may be attributable to daily patterns in *T. castaneum* movement or flight activity[59,60] or circadian rhythms of metabolism and detoxification. For example, *Anopheles gambiae* Giles (Diptera: Culicidae) mosquitos show a diel pattern in knockdown time after exposure to deltamethrin.[61] Other factors associated with age and response to insecticides may be cuticle hardness and

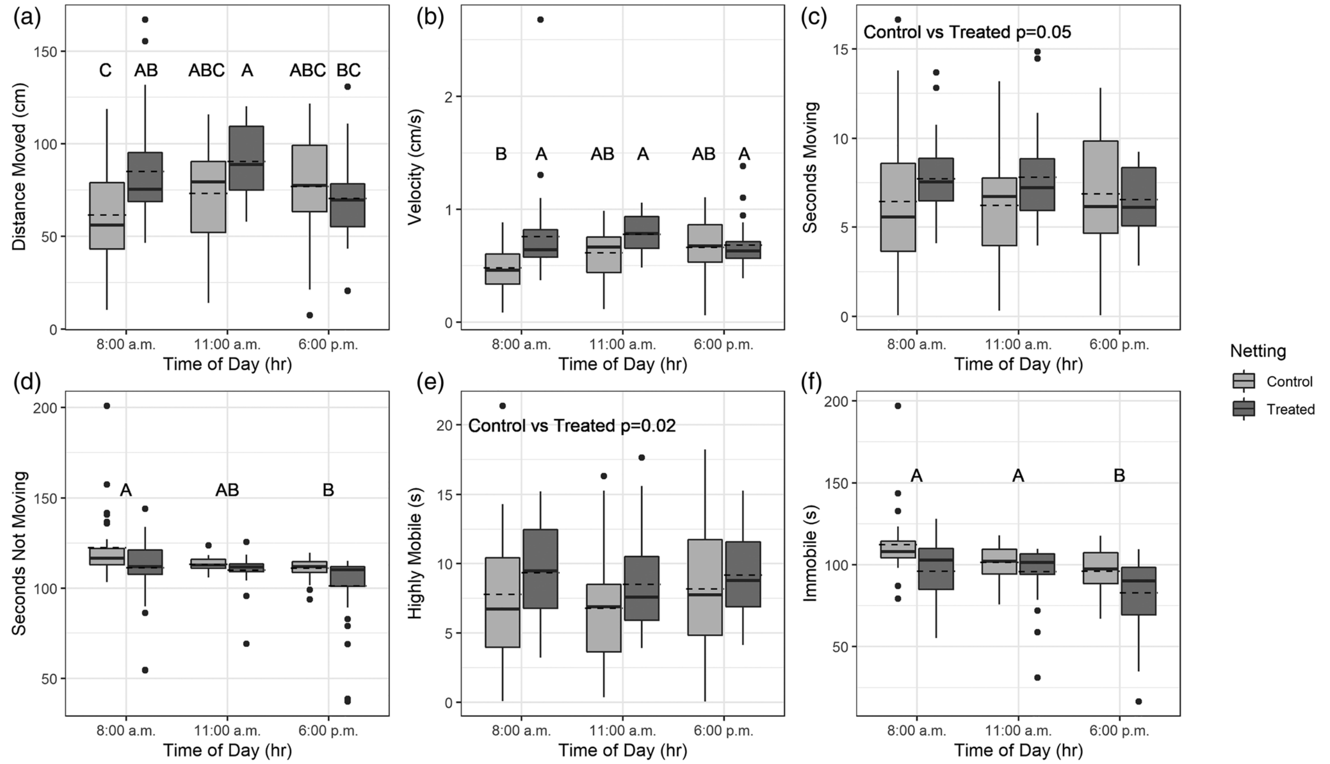

Figure 4. Mobility assay by time of day. Edges of boxes represent the 25% and 75% range of data (IQR), the solid line within the box is the median, the dashed line is the mean, and whiskers are 1.5 × IQR. Outliers fall outside this range. Bars with shared letters within a panel are not significantly different from each other (Tukey, $\alpha < 0.05$). Panels lacking letters indicate no significant differences among the treatments.

thickness, which may increase as beetles age, resulting in lower penetration of insecticides.[62,63] Interestingly, we observed an effect of time of day on movement of beetles on treated and untreated netting in experiments where time of day was varied, but not in behavior assays where beetle age was varied, which were performed at approximately the same times. An increase in overall movement on treated compared with untreated netting suggests that sensitivity to insecticide may vary throughout the day, and insects may have increased sensitivity at earlier times in the day (8:00 a.m. and 11:00 a.m.), which is when behavioral differences between movement on treated and untreated netting are most apparent. Further examination of diel effects is critically necessary to understand the effects of insect movement, metabolism, and overall effectiveness of LLIN.

Two conditions also tested during the recovery period were the presence of food and differences in abiotic factors (temperature and RH). The presence of food after insecticide exposure was associated with reductions in the numbers of affected individuals, consistent with previous research showing that food decreases the efficacy of contact insecticides.[37–40] Mechanisms for reduced efficacy of insecticides in the presence of food are not understood although it has been hypothesized as being due to increased metabolism and detoxification in the presence of food[64] or increased moisture and humidity associated with food,[65] drawing insecticide away from the cuticle. In any case, sanitation to remove residual food where LLIN will be deployed should enhance efficacy.

Fewer beetles were affected after recovery at higher humidity and temperature compared with ambient conditions with no temperature or humidity control. Although we cannot tease apart the relationship with temperature and humidity in this experiment, temperature only varied by ~ 4 °C from incubator to bench and was well within the upper and lower limits of temperature tolerance for *T. castaneum*.[66] Humidity varied by ~ 20% (Fig. S1), suggesting that humidity may be the main driver of these recovery conditions. Both temperature and humidity can influence the efficacy of insecticides based on the mode of action and species of insect assayed. For example, higher humidity has also been suggested to increase survival after insecticide exposure in some lice species[67] but decrease survivorship of *Oryzaephilus surinamensis* (L.).[68] Lower temperatures have also been shown to reduce the efficacy of insecticides against *O. surinamensis*,[68] *T. castaneum*, and *Sitophilus granarius*[69] but were associated with improved efficacy against house flies, *Musca domestica* L.[70] However, temperature has been found to have variable impacts on efficacy of different insecticides against *T. castaneum*,[71,72] reducing mortality after exposure to cyfluthrin at higher temperatures,[73] increasing mortality at higher temperatures after exposure to thiamethoxam,[74] and increasing mortality and reducing progeny production at higher temperatures after exposure to chlorphenayr,[75] suggesting that mode of action plays a large role in how temperature affects response to insecticides.

5 CONCLUSION

The assays described here provide a baseline for the assessment of behavioral and physiological differences in response to exposure to LLIN. Minimizing direct insecticide contact on commodities is increasingly important as we seek alternative methods for protecting food supplies from insect damage. Using insecticide-treated packaging or netting is an effective way to impede movement, and reproduction, and reduce survival of different life stages of a variety of SPPs.[12,14,22–24] We have shown that multiple exposures have cumulative effects on insects, which is important because some species require extended duration of exposure to LLIN for complete knockdown.[25] We have shown that variation in age, time of day, availability of food, and temperature and humidity can affect the efficacy of netting in ways relevant for its use in food facilities. Seemingly minor differences can have significant impacts on the proportion of affected beetles,[42,61,69,76] although these differences would probably not impact the overall efficacy of netting in the field. These results suggest a substantial amount of variation in the number of affected beetles and recovery over time, and controlling these factors or broadening the range of factors tested when assessing the efficacy of this netting and other contact insecticides will provide facility managers with broader efficacy estimates and implementation plans and practices when considering LLIN in sanitation and IPM plans.

ACKNOWLEDGEMENTS

We thank Rich Hammel for maintaining laboratory populations and Shelby Abts, Adriane Fifield, Valerie Nguyen, Josie Pierce, and Nicholas Van Pelt for helping with data collection. This work was funded, in part, by a USDA NIFA CPPM Grant #2017-70006-27262. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Hagstrum DW and Phillips TW, Evolution of stored-product entomology: protecting the world food supply. *Annu Rev Entomol* **62**: 379–397 (2017).

2 Potter C, The biology and distribution of *Rhyzopertha dominica* (Fab). *T Roy Ent Soc London* **83**:449–482 (1935).

3 Sokoloff A, *The biology of Tribolium*, Vol. **1**. Clarendon Press, Oxford (1972).

4 Mohandass S, Arthur F, Zhu K and Throne JE, Biology and management of *Plodia interpunctella* (Lepidoptera: Pyralidae) in stored products. *J Stored Prod Res* **43**:302–311 (2007).

5 Edde PA, A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. *J Stored Prod Res* **48**:1–18 (2012).

6 Campbell J and Hagstrum D, Patch exploitation by *Tribolium castaneum*: movement patterns, distribution, and oviposition. *J Stored Prod Res* **38**:55–68 (2002).

7 Campbell J and Runnion C, Patch exploitation by female red flour beetles, *Tribolium castaneum*. *J Insect Sci* **3** (2003). Available: http://insectscience.org/3.20

8 Rees D, Subramanyam B and Hagstrum D, *Integrated Management of Insects in Stored Products*. Marcel Dekker, New York (1996).

9 Heaps J, *Insect Management for Food Storage and Processing*. AAAC International, St. Paul, MN (2006).

10 Hagstrum D and Subramanyam B, *Fundamentals of Stored-Product Entomology*. AACC International, St. Paul, MN (2006).

11 Morrison WR III, Bruce A, Wilkins RV, Albin CE and Arthur FH, Sanitation improves stored product insect pest management. *Insects* **10**:77 (2019).

12 Scheff DS, Subramanyam B and Arthur FH, Effect of methoprene treated polymer packaging on fecundity, egg hatchability, and egg-to-adult emergence of *Tribolium castaneum* and *Trogoderma variabile*. *J Stored Prod Res* **69**:227–234 (2016).

13 Kavallieratos NG, Athanassiou CG and Arthur FH, Effectiveness of insecticide-incorporated bags to control stored-product beetles. *J Stored Prod Res* **70**:18–24 (2017).

14 Scheff DS and Arthur FH, Fecundity of *Tribolium castaneum* and *Tribolium confusum* adults after exposure to deltamethrin packaging. *J Pest Sci* **91**:717–725 (2018).

15 Phillips TW and Throne JE, Biorational approaches to managing stored-product insects. *Annu Rev Entomol* **55**:375–397 (2010).

16 Lengeler C, Insecticide treated bednets and curtains for malaria control. *Cochrane Database Syst Rev* **2**:CD000363 (2000).

17 Calvete C, Estrada R, Miranda M, Del Rio R, Borrás D, Beldron F *et al*., Protection of livestock against bluetongue virus vector *Culicoides imicola* using insecticide-treated netting in open areas. *Med Vet Entomol* **24**:169–175 (2010) 2010.

18 Dáder B, Legarrea S, Moreno A, Plaza M, Carmo-Sousa M, Amor F *et al*., Control of insect vectors and plant viruses in protected crops by novel pyrethroid-treated nets. *Pest Manag Sci* **71**:1397–1406 (2015).

19 Kuhar T, Short B, Krawczyk G and Leskey T, Deltamethrin-incorporated nets as an integrated pest management tool for the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae). *J Econ Entomol* **110**: 543–545 (2017).

20 Giuseppino SP, Paolo BP, Roberta N, Leonardo M and Federico RP, Efficacy of long lasting insecticide nets in killing *Halyomorpha halys* in pear orchards. *Outlooks Pest Manag* **29**:70–74 (2018).

21 Marianelli L, Paoli F, Sabbatini Peverieri G, Benvenuti C, Barzanti GP, Bosio G *et al*., Long-lasting insecticide-treated nets: a new integrated pest management approach for *Popillia japonica* (Coleoptera: Scarabaeidae). *Integr Environ Assess* **15**:259–265 (2019).

22 Morrison WR III, Wilkins RV, Gerken AR, Scheff DS, Zhu KY, Arthur FH *et al*., Mobility of adult *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) after exposure to long-lasting insecticide-incorporated netting. *J Econ Entomol* **111**:2443–2453 (2018).

23 Rumbos C, Sakka M, Schaffert S, Sterz T, Austin J, Bozoglou C *et al*., Evaluation of Carifend®, an alpha-cypermethrin-coated polyester net, for the control of *Lasioderma serricorne* and *Ephestia elutella* in stored tobacco. *J Pest Sci* **91**:751–759 (2018).

24 Wilkins RV, Zhu KY, Campbell JF and Morrison WR III, Mobility and dispersal of two cosmopolitan stored product insects are adversely affected by long-lasting insecticide netting in a life stage-dependent manner. *J Econ Entomol* **113**:1768–1779 (2020).

25 Athanassiou CG, Rumbos CI, Stephou VK, Sakka M, Schaffert S, Sterz T *et al*., Field evaluation of Carifend net for the protection of stored tobacco from storage insect pests. *J Stored Prod Res* **81**:46–52 (2019).

26 Paloukas YZ, Agrafioti P, Rumbos CI, Schaffert S, Sterz T, Bozoglou C *et al*., Evaluation of Carifend for the control of stored product beetles. *J Stored Prod Res* **85**:101534 (2020).

27 Chalmers AE, Miller TA and Olsen RW, Deltamethrin: a neurophysiological study of the sites of action. *Pest Biochem Phys* **27**:36–41 (1987).

28 de la Cerda E, Navarro-Polanco RA and Sánchez-Chapula JA, Modulation of cardiac action potential and underlying ionic currents by the pyrethroid insecticide deltamethrin. *Arch Med Res* **33**:448–454 (2002).

29 Shafer T, Rijal S and Gross G, Complete inhibition of spontaneous activity in neuronal networks in vitro by deltamethrin and permethrin. *Neurotoxicology* **29**:203–212 (2008).

30 Preisler HK and Robertson JL, Analysis of time–dose–mortality data. *J Econ Entomol* **82**:1534–1542 (1989).

31 Mansoor-ul-Hasan SM, Ullah A, Wakil W and Javed A, Response of *Trogoderma granarium* (Everts) to different doses of *Haloxylon recurvum* and deltamethrin. *Pak Entomol* **28**:25–30 (2006).

32 Sehgal B, Subramanyam B, Arthur FH and Gill BS, Variation in susceptibility of laboratory and field strains of three stored-grain insect species to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin applied to concrete surfaces. *Pest Manag Sci* **70**:576–587 (2014).

33 Wilkins RV, Campbell JF, Zhu KY, Starkus L, McKay T and Morrison WR III, The use of long-lasting insecticide-incorporated netting and interception traps at pilot-scale warehouses and commercial facilities to prevent infestation by stored product beetles. *Agric Ecosyst Environ* (2020).

34 Kavallieratos NG, Athanassiou CG, Vayias BJ and Betsi PCC, Insecticidal efficacy of fipronil against four stored-product insect pests: influence of commodity, dose, exposure interval, relative humidity and temperature. *Pest Manag Sci* **66**:640–649 (2010).

35 Maloney EM, Morrissey CA, Headley JV, Peru KM and Liber K, Cumulative toxicity of neonicotinoid insecticide mixtures to *Chironomus dilutus* under acute exposure scenarios. *Environ Toxicol Chem* **36**: 3091–3101 (2017).

36 Maloney EM, Morrissey CA, Headley JV, Peru KM and Liber K, Can chronic exposure to imidacloprid, clothianidin, and thiamethoxam mixtures exert greater than additive toxicity in *Chironomus dilutus? Ecotoxicol Environ Safe* **156**:354–365 (2018).

37 Arthur FH, Effects of a food source on red flour beetle (Coleoptera: Tenebrionidae) survival after exposure on concrete treated with cyfluthrin. *J Econ Entomol* **91**:773–778 (1998).

38 Arthur FH, Impact of accumulated food on survival of *Tribolium castaneum* on concrete treated with cyfluthrin wettable powder. *J Stored Prod Res* **36**:15–23 (2000).

39 Arthur FH, Impact of food source on survival of red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae) exposed to diatomaceous earth. *J Econ Entomol* **93**:1347–1356 (2000).

40 Toews MD, Campbell JF and Arthur FH, The presence of flour affects the efficacy of aerosolized insecticides used to treat the red flour beetle *Tribolium castaneum*. *J Insect Sci* **10**:1–14 (2010).

41 Kramer RD, Koehler PG, Patterson RS and Slansky F, Nutritional status and insecticide tolerance in German cockroaches (Orthoptera: Blattellidae). *J Econ Entomol* **83**:1912–1917 (1990).

42 Chareonviriyaphap T, Kongmee M, Bangs MJ, Sathantriphop S, Meunworn V, Parbaripai A *et al*., Influence of nutritional and physiological status on behavioral responses of *Aedes aegypti* (Diptera: Culicidae) to deltamethrin and cypermethrin. *J Vector Ecol* **31**:13 (2005).

43 Musser FR and Shelton AM, The influence of post-exposure temperature on the toxicity of insecticides to *Ostrinia nubilalis* (Lepidoptera: Crambidae). *Pest Manag Sci* **61**:508–510 (2005).

44 Raj Boina D, Onagbola EO, Salyani M and Stelinski LL, Influence of post-treatment temperature on the toxicity of insecticides against *Diaphorina citri* (Hemiptera: Psyllidae). *J Econ Entomol* **102**:685–691 (2009).

45 Ziegler JR, Evolution of the migration response: emigration by *Tribolium* and the influence of age. *Evolution* **30**:579–592 (1976).

46 Semeao AA, Campbell JF, Whitworth RJ and Sloderbeck PE, Movement of *Tribolium castaneum* within a flour mill. *J Stored Prod Res* **54**:17–22 (2013).

47 Leslie P and Park T, The intrinsic rate of natural increase of *Tribolium castaneum* Herbst. *Ecology* **30**:469–477 (1949).

48 Howe R, The effect of temperature and humidity on the rate of development and mortality of *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). *Ann Appl Biol* **44**:356–368 (1956).

49 Yu S, Age variation in insecticide susceptibility and detoxification capacity of fall armyworm (Lepidoptera: Noctuidae) larva. *J Econ Entomol* **76**:219–222 (1983).

50 Kim Y, Lee J, Kang S and Han S, Age variation in insecticide susceptibility and biochemical changes of beet armyworm, *Spodoptera exigua* (Hüubner). *J Asia Pac Entomol* **1**:109–113 (1998).

51 Rinkevich FD, Margotta JW, Pittman JM, Danka RG, Tarver MR, Ottea JA *et al*., Genetics, synergists, and age affect insecticide sensitivity of the honey bee, *Apis mellifera*. *PLoS One* **10**:e0139841 (2015).

52 Morrison WR III, Poling B and Leskey TC, The consequences of sublethal exposure to insecticide on the survivorship and mobility of *Halyomorpha halys* (Hemiptera: Pentatomidae). *Pest Manag Sci* **73**: 389–396 (2017).

53 González Audino P, Vassena C, Barrios S, Zerba E and Picollo MI, Role of enhanced detoxication in a deltamethrin-resistant population of *Triatoma infestans* (Hemiptera, Reduviidae) from Argentina. *MemInst Oswaldo Cruz* **99**:335–339 (2004).

54 Sauphanor B, Cuany A, Bouvier J, Brosse V, Amichot M and Bergé J, Mechanism of resistance to deltamethrin in *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). *Pest Biochem Phys* **58**:109–117 (1997).

55 Bouvier JC, Cuany A, Monier C, Brosse V and Sauphanor B, Enzymatic diagnosis of resistance to deltamethrin in diapausing larvae of the codling moth, *Cydia pomonella* (L.). *Arch Insect Biochem* **39**:55–64 (1998).

56 Burnett G, The susceptibility of tsetse flies to topical applications of insecticides. III. The effects of age and pregnancy on the susceptibility of adults of *Glossina morsitans* Westw. *Bull Entomol Res* **53**: 337–345 (1962).

57 Nauen R, Bielza P, Denholm I and Gorman K, Age-specific expression of resistance to a neonicotinoid insecticide in the whitefly *Bemisia tabaci*. *Pest Manag Sci* **64**:1106–1110 (2008).

58 Arthur FH, Athanassiou CG and Morrison WR III, Mobility of stored product beetles after exposure to a combination insecticide containing deltamethrin, methoprene, and a piperonyl butoxide synergist depends on species, concentration, and exposure time. *Insects* **11**:151–164 (2020).

59 Giles P, Observations in Kenya on the flight activity of stored products insects, particularly *Sitophilus zeamais* Motsch. *J Stored Prod Res* **4**: 317–329 (1969).

60 Boon K and Ho S, Factors influencing the post-fumigation reinfestation of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in a rice warehouse. *J Stored Prod Res* **24**:87–90 (1988).

61 Balmert NJ, Rund SS, Ghazi JP, Zhou P and Duffield GE, Time-of-day specific changes in metabolic detoxification and insecticide resistance in the malaria mosquito *Anopheles gambiae*. *J Insect Physiol* **64**:30–39 (2014).

62 Grodowitz MJ, Center TD and Freedman JE, A physiological age-grading system for *Neochetina eichhorniae* (Warner) (Coleoptera: Curculionidae), a biological control agent of water hyacinth, *Eichhornia crassipes* (Mart.) Solms. *Biol Control* **9**:89–105 (1997).

63 Perez-Mendoza J, Throne J and Baker J, Ovarian physiology and age-grading in the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae). *J Stored Prod Res* **40**:179–196 (2004).

64 Kharel K, Arthur FH, Zhu KY, Campbell JF and Subramanyam B, Susceptibility of different life stages of *Tribolium confusum* to pyrethrin aerosol: effects of a flour food source on insecticidal efficacy. *J Pest Sci* **87**:295–300 (2014).

65 Fraenkel G and Blewett M, The utilisation of metabolic water in insects. *Bull Entomol Res* **35**:127–139 (1944).

66 Scharf I, Galkin N and Halle S, Disentangling the consequences of growth temperature and adult acclimation temperature on starvation and thermal tolerance in the red flour beetle. *Evol Biol* **42**: 54–62 (2015).

67 Gallardo A, Cueto GM and Picollo MI, *Pediculus humanus capitis* (head lice) and *Pediculus humanus humanus* (body lice): response to laboratory temperature and humidity and susceptibility to monoterpenoids. *Parasitol Res* **105**:163–167 (2009).

68 Barson G, The effects of temperature and humidity on the toxicity of three organophosphorus insecticides to adult *Oryzaephilus surinamensis* (L.). *Pest Sci* **14**:145–152 (1983).

69 Tyler P and Binns T, The influence of temperature on the susceptibility to eight organophosphorus insecticides of susceptible and resistant strains of *Tribolium castaneum*, *Oryzaephilus surinamensis* and *Sitophilus granarius*. *J Stored Prod Res* **18**:13–19 (1982).

70 Devries DH and Georghiou GP, Influence of temperature on the toxicity of insecticides to susceptible and resistant house flies. *J Econ Entomol* **72**:48–50 (1979).

71 Arthur FH, Toxicity of diatomaceous earth to red flour beetles and confused flour beetles (Coleoptera: Tenebrionidae): effects of temperature and relative humidity. *J Econ Entomol* **93**:526–532 (2000).

72 Arthur FH, Immediate and delayed mortality of *Oryzaephilus surinamensis* (L.) exposed on wheat treated with diatomaceous earth: effects of temperature, relative humidity, and exposure interval. *J Stored Prod Res* **37**:13–21 (2000).

73 Arthur FH, Effect of temperature on residual toxicity of cyfluthrin wettable powder. *J Econ Entomol* **92**:695–699 (1999).

74 Arthur FH, Yue B and Wilde GE, Susceptibility of stored-product beetles on wheat and maize treated with thiamethoxam: effects of concentration, exposure interval, and temperature. *J Stored Prod Res* **40**: 527–546 (2004).

75 Arthur FH, Dosage rate, temperature, and food source provisioning affect susceptibility of *Tribolium castaneum* and *Tribolium confusum* to chlorfenapyr. *J Pest Sci* **86**:507–513 (2013).

76 Banken JA and Stark JD, Stage and age influence on the susceptibility of *Coccinella septempunctata* (Coleoptera: Coccinellidae) after direct exposure to Neemix, a neem insecticide. *J Econ Entomol* **90**: 1102–1105 (1997).