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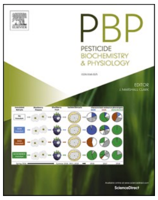

Sublethal exposure of *Trogoderma granarium* everts (Coleoptera: Dermestidae) to insecticide-treated netting alters thigmotactic arrestment and olfactory-mediated anemotaxis☆

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ABSTRACT

Long-lasting insecticide treated netting (LLIN) has a number of potential uses for the control of insect pests. Using such netting, stored products may be protected from insects including the khapra beetle (*Trogoderma granarium* Everts, Coleoptera: Dermestidae) a widespread pest of many agricultural commodities. Here we first examined whether brief exposures of larvae to LLIN, for less than 30 min, decreased the chance of eventual adult emergence compared to larvae exposed on untreated netting. Next, we observed the responses of larvae that were either not exposed to any netting, exposed to untreated netting, or exposed to LLIN for 10 min and then placed in a wind tunnel and monitored for movement toward a stimulus. The wind-tunnel assay was performed either with or without a lure containing kairomones and pheromones known to be attractive to larvae of this species. There was little effect of the LLIN on adult emergence of exposed larvae. However, there were interacting effects of untreated netting and LLIN relating to thigmotaxis and anemotaxis. Larvae not exposed to netting showed increased likelihood of walking upwind if the semiochemical lure was provided, as expected. A similar pattern was observed when the untreated netting was used, but the larvae became more likely to remain stationary in the assay after acclimating to the net. When LLIN was used, the larvae became more likely to move and there was a baseline increase in the likelihood of moving upwind. However, upwind walking was no longer related to semiochemical presentation. These observations suggest that particular care should be used in relation to the airflow patterns and semiochemical landscape of the warehouse settings in which LLIN is deployed.

1. Introduction

Sublethal effects of exposure to insecticides have been investigated in numerous contexts ranging from suppression of natural enemies such as predators and parasitoids (Desneux et al., 2007, Stara et al., 2020) to changes in fertility (Cutler and Guedes, 2017). One area where sublethal effects are particularly likely to be expressed is the disruption of specific adaptive behaviors. Many insecticides have a neuro-toxic mode of action, with severe disruption of the ion exchange pathways associated with neural signaling being a frequent target of action (Benzidane et al., 2010; El Hassani et al., 2008). Behaviors such as olfactory and gustatory responses rely heavily on such pathways, but there have been very few investigations as to how minor disruption to the sensory system from insecticides will affect behavioral responses to chemical stimuli (reviewed in Tricoire-Leignel et al., 2012).

The khapra beetle, *Trogoderma granarium* Everts, is a well-known widely distributed pest of many stored food products (Athanassiou et al., 2019). Originally of central Asian origin, it has a worldwide distribution in many nations throughout tropical and subtropical regions of the globe (Banks, 1977; Burges, 1959; Paini and Yemshanov, 2012). Populations of this insect are capable of growing so quickly in grain storage facilities that the integrity of the food supply can be significantly

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impacted if they are not controlled by vigorous enforcement of quarantine controls in multiple nations (Eliopoulos, 2013). In the mid-20th century there was a widespread establishment of *T. granarium* in the southwestern United States (Armitage, 1956a; Lindgren et al., 1955) that was ultimately eradicated successfully (Armitage, 1956b). However, continued and increasing interceptions at USA ports of entry (Myers and Hagstrum, 2012), make this a pest of utmost concern to food facilities.

Control methods have typically included a number of integrated tools. Along with visual inspection of imported products, traps baited with sex pheromones and food-related kairomones are often used to monitor for the pest in high risk areas such as receiving warehouses at ports of entry and grain production and storage facilities (Barak, 1989). Detected infestations have historically been treated with a variety of control measures to prevent establishment. These include broad spectrum insecticide applications, fumigation (Espino et al., 2014), controlled atmospheres (Vassilakos et al., 2019), or irradiation (Abdel-Kawy, 1999; Mansour, 2016). All such treatments options have advantages and disadvantages, while the most effective fumigant, methyl bromide, is being phased out of widespread use internationally because of environmental toxicity (Fields and White, 2002), with exceptions for use in quarantine situations.

Moreover, other preventative measures are being researched to control *T. granarium*, including the use of protective barriers to movement into bagged and packaged food products. Pyrethrin and pyrethroid insecticides, and the insect growth regulators methoprene and pyriproxyfen, are among products that have shown to be effective for the control of *T. granarium* (Kavallieratos et al., 2016, 2017a; Ghimire et al., 2017; Arthur et al., 2018, 2019), and other stored product pests (Arthur et al., 2020). A number of insecticides are also known to cause behavioral repellency on target species (Collins et al., 1988). For deltamethrin specifically, behavioral repellency has been shown in *Rhyzopertha dominica* (Lorini and Galley, 1998). Changes in mobility (Guedes et al., 2008) or reproductive parameters (Vélez et al., 2018) may also be sublethal effects of insecticide exposure that are important to consider in management strategies.

Furthermore, the delivery of insecticide treatments to stored product pests provides the potential for options that might afford protection without direct application to the commodity. Applications may target surfaces near commodities (Arthur, 1997) or barriers such as netting (Msango and Longwe, 2013) or packaging (Kim et al., 2013). The closely related, *Trogoderma variabile* can be effectively knocked down by deltamethrin impregnated bags (Kavallieratos et al., 2017b). Long-lasting insecticide treated netting, (LLIN) similar to that used to protect humans from disease-vectoring mosquitos (Alonso et al., 1991) is one option worthy of consideration. The use of such netting to cover openings or gaps in storage facilities or transportation vessels could reduce the populations of several stored product pests. One would assume that many insects encountering such a barrier might spend a brief amount of time exposed to the netting before finding a way through or retreating. While it has been determined that very brief exposure to such materials may strongly negatively affect targeted insets such as *Tribolium castaneum* and *Rhyzopertha dominica* (Morrison et al., 2018), it is not well understood how such brief sub-lethal exposures to the material will impact the behavioral attributes of the insect. For example, it has been shown that brief exposures to deltamethrin-treated netting versus untreated netting decreases general movement rate of larvae and adult warehouse beetle, *Trogoderma variabile* Ballion, and interferes with host orientation (Wilkins et al., 2020).

Trogoderma granarium larvae have a propensity to enter small cracks and crevices in the buildings where infestations occur. Thus, another factor to consider is that the physical texture of netting itself, which provides a micro-heterogeneous environment that might encourage thigmotactic arrestment if used to cover gaps or openings in storage warehouses. Furthermore, *T. granarium* has a tendency to easily enter into a facultative diapause stage when experiencing unfavorable conditions (Burges, 1962; Hadaway, 1955; Hinton, 1945; Wilches et al., 2016). The diapause can last for months, meaning cryptic larvae are a risk for re-infestation of products. It is likely that for *T. granarium* thigmotaxis and diapause are related, as sheltering is known to reduce desiccation in other species (Gilbert, 2014). Larval behavioral interactions with netting are very critical to understand before deploying this technology.

In this study we examined hypotheses regarding the use of LLIN and its impact on olfactory behavior in *T. granarium* larvae. First, we determined whether short-term exposures to untreated versus LLIN, ranging from 1 to 30 min, affected survival of larvae through emergence as adults. Next, we examined whether such short-term exposures to LLIN or untreated netting affected upwind orientation toward semiochemicals normally attractive to *T. granarium* larvae. These experiments provided insight into how sublethal insecticide exposure affected movement in relation to the physical and chemical environment. For *T. granarium*, manipulating these interactions may be helpful in developing better management practices.

2. Materials and methods

2.1. Insects

This research study was conducted at the United States Department of Agriculture Animal and Plant Health Inspection Service, Science and Technology, Otis Laboratory, Buzzards Bay, MA, USA. The *T. granarium* larvae used in this experiment were from colonies originating from a field strain collected from Pakistan in 2011. *T. granarium* colonies were reared on a combination diet of 160 g ground dog food (Purina Dog Chow Complete, Nestlé Purina PetCare Company, St. Louis, MO, USA) mixed with 20 g of wheat germ (The Mennel Milling Company, Fostoria, OH, USA), and sprinkled with 20 g of rolled oats (Heartland Mill, Marienthal, KS, USA) on the surface in a 0.95 L glass jar. *T. granarium* colonies were maintained in an environmental growth chamber (Percival Scientific, Boone Iowa) at 30 °C in continuous darkness. For all experiments large, 4–5 mm larvae were used. All experiments were conducted in the Otis Laboratory containment facility under direct observation.

2.2. Long-lasting insecticide treated netting (LLIN) material

The deltamethrin treated long-lasting insecticide treated netting (LLIN) material used in this study was obtained from a commercial manufacturer (Vestergaard, Lausanne, Switzerland). The LLIN had a load rate of 0.4% (4000 ppm) deltamethrin technical grade, mesh size of 32 holes/cm^2, and a base polymer of polyethylene. The untreated control netting used in this study was constructed with the same polyethylene material and mesh size, but did not contain any insecticide.

2.3. Adult emergence after short-term LLIN exposure of larvae

Distinct arenas for exposure to untreated netting or LLIN, and others for post-exposure observation were created as follows. First, a 9 cm diameter circle of either treated or untreated netting was cut from the source material (Fig. 1A). The netting discs were placed inside the bottom portions of Petri dishes which each had a corresponding 9 cm diameter (internal area of 62 cm^2, height 20 mm). Post-exposure arenas were also created to provide a neutral environment for housing the exposed insects with a typical diet (Fig. 1B). Filter paper disks (8.5 cm diameter, Whatman No. 1, GE Healthcare) were placed on the bottom of the plastic Petri dishes. The filter paper was then secured along the edges using adhesive caulking (DAP Kwik Seal, DAP Products Inc., Baltimore, MD) and the internal vertical edges of the dish coated with Fluon® (polytetrafluoroethylene, Sigma-Aldrich Co., St. Louis, MO) to prevent larvae from crawling out of the arena and escaping. After construction of these post-exposure arenas, they were allowed to dry at ambient conditions for at least 24 h.

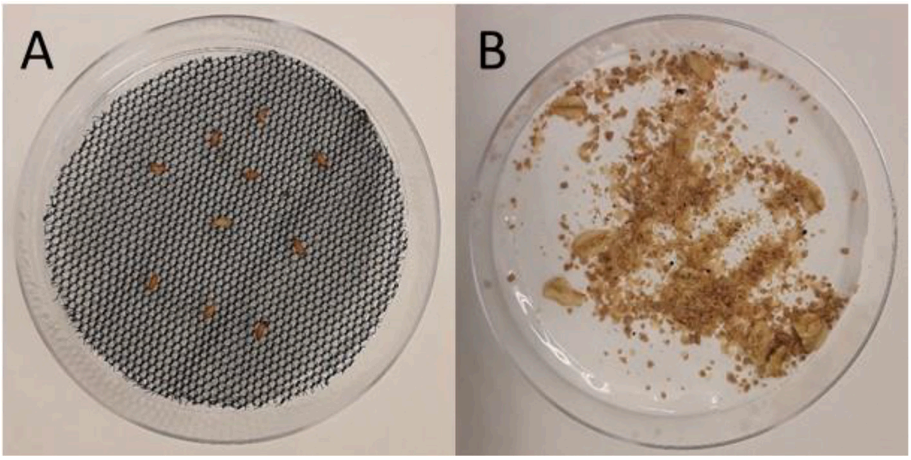

Fig. 1. Arenas for assessing short term exposure effects of LLIN. A petri dish containing untreated netting or LLIN (A) is used to expose ten larvae for the allotted time, which are then placed on a petri dish arena with diet (B) and observed.

The effect of short-term exposure of *T. granarium* larvae on LLIN was assessed as follows. Five sets of 10 larvae, were placed on either the untreated netting or the LLIN and held for time periods of 1, 5, 10, 15, or 30 min at ambient conditions on a laboratory benchtop. After each exposure period larvae were removed from the netting material and placed into a post-exposure arena, along with ~1 g of diet, and then placed into an environmental chamber set at 30 °C in continuous darkness. Larvae were observed after 7 d and 1 month, for the number of adult beetles that emerged. This procedure was repeated three times for a total of 15 replicates per time period and material type (LLIN or untreated netting). The percent adult emergence was recorded for each material type (LLIN or untreated) and length of exposure.

2.4. *Effect of LLIN and odor stimulation on movement*

The effects of the LLIN and untreated netting on the movement of *T. granarium* larvae toward olfactory stimuli were explored with a miniature wind tunnel assay. All individuals assayed were removed from the colony in the morning and assayed within 8 h of their removal. *T. granarium* larvae were placed into the bottom portion of a plastic Petri dish lined with filter paper, as described previously, to allow natural movement of the insects. Approximately 200 larvae were removed, and those observed to be moving were selected for the experimental manipulations described below.

The wind tunnel consisted of a 12 × 12 × 3 cm L:H:W electric fan that pushed ambient air through a charcoal filter, straightened the air flow through a metal grate and finally compressed the air flow to 12 × 5 cm over a 26.5 cm distance within a steel encasement at an air speed of 1.18 m/s (Morrison et al., 2020). The miniature wind tunnel was arranged as depicted in Fig. 2, laid out on top of a 0.5 m by 1 m piece of bench top liner paper. The front edge of the testing arena (10.5 × 14 cm paper) was placed 30.5 cm downwind of the wind tunnel. The stimulus (air and lure vs. air alone) was placed upwind from the leading edge of the arena, approximately 7.5 cm directly in front of the wind tunnel. A single larva was placed in the center of the testing arena, 38 cm from the air source. The entire apparatus was placed within a biological safety cabinet with an air exchange of 0.4 linear m/s to prevent the accumulation of extraneous odors. The testing arenas were replaced each time there was a change in stimulus treatment.

Six treatments in a 2 × 3 factorial design were deployed. One factor was the inclusion of an olfactory stimulus in the wind tunnel. The stimulus presented was 0.13 g of Pantry Patrol gel (Insects Limited, Inc., Westfield, IN), referred to throughout as “lure”, which contains a mixture of several known sex pheromones and kairomone attractants that affect a wide array of stored product pests. This commercially available product was determined to be the most consistently effective attractant in previous bioassays with *T. granarium* larvae, including the same wind tunnel bioassay (Morrison et al., 2020). The control treatment for this factor, was the absence of an olfactory stimulus, and is referred to simply as “air.”

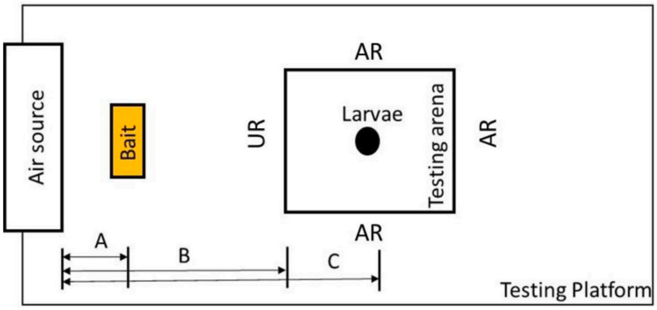

Fig. 2. Diagram of the miniature wind tunnel set-up for observing *T. granarium* larval movement after 10 min of exposure to LLIN, untreated netting, or filter paper (no netting). The laminar flow air source is located on the far left. In front of the air source, approximately 7.5 cm (A), was the placement of the stimulus. The front edge of the testing arena (10.5 × 14 cm) was approximately 30.5 cm (B) from the air source. A single *T. granarium* larvae was placed at approximately 38 cm (C) from the air source, in the middle of the testing arena (10.5 × 14 cm). Upwind responses (UR) were toward the air source, while avoidant responses (AR) were toward the other arena edges.

The other factor, which consisted of three levels, included no exposure to any netting (control), exposure to untreated netting, or exposure to LLIN. The administration of these three treatments was rotated while using one of the two stimulus treatments (air and lure vs. air alone). Netting exposure involved placing the larvae into a Petri dish (internal area of 62 cm^2, height 20 mm) containing the untreated netting or LLIN cut to fit inside the arena, as described in Section 2.2. Larvae were held on the netting for 10 min. This time was selected because it became clear in the first experiment that there was little effect of any of the exposures in the range of 1–30 min on adult emergence. Thus, we could be certain that the exposure was sublethal. The exposures were staggered across two different Petri dishes, one with an untreated net and the other with the LLIN. This allowed at least one assay to be performed every 5 min. Individuals not exposed to netting were also assayed while the same stimulus treatment was available, either before, between, or after trials using individuals timed as if they were exposed to netting.

After administering the netting treatment, a single *T. granarium* larva was placed in the center of the testing arena (Fig. 2). The larva was given

2 min to move more than a single body length from the initial position, and if it did not move, it was classified as a "non-mover" (NM). If the larva began to move the stopwatch was reset and it was then allowed up to three-minutes to move within the testing arena and make a decision as to direction of movement. It was determined that a decision was made, and the larvae had left the arena, if it had translocated more than half of its body length over the arena's edge. The specific edge exited and the time to make this decision after beginning to move was noted. The average tangential velocity toward the edge exited was calculated by dividing the distance needed to leave the front or back edge (7 cm) or the side edges (5.25 cm). Larvae that exited the edge of the arena nearest to the odor source (stimulus) were classified as a positive upwind response (UR) (Fig. 2). Larvae that exited from the arena on any other edge, were considered avoidant responses (AR) (Fig. 2). Larvae that moved within the constraints of the arena, but did not exit the arena were considered to have no response (NR). A total of 100 individual replications were performed for each of the six treatment combinations. From each of the six treatments, 10 to 20 of these reps were performed in a given day.

2.5. *Statistical analyses*

All analyses were performed using SAS (SAS Institute, version 9.4, 2012). We examined the proportion adult emergences using the LOGISTIC procedure in SAS. Model effects for adult emergence were netting treatment and time of exposure. The aggregate effect of the individual Petri dish effects was also included in the model.

Similarly, the effects of the netting treatments and semiochemical presentation upon the proportion of individuals exhibiting particular behaviors in the wind tunnel were also analyzed using a logistic model. For one of the behavioral analysis performed, the proportion of the individuals not moving (NM) was considered as the response variable. We also performed another logistic regression considering the proportion of those moving upwind (UR), given that they moved in the assay. The effects of the netting and stimulus treatments were computed with their interaction. Contrasts were constructed to explore comparisons within the main effects and interactions, using Ward's X^2 when they were determined to be significant at $\alpha = 0.05$.

The effects of netting on the velocity of *T. granarium* larvae leaving the arena (only UR and AR) were analyzed using the General Linear Model (GLM) for ANOVA procedure. These data were log transformed before analysis to allow assumptions of normality (Kolmogorov-Smirnov, $p = 0.09$), and then back-transformed to the original scale for presentation of means and standard error. The model used behavioral choice (AR vs UR), netting treatment (no netting, untreated netting, LLIN), and stimulus (air vs. lure) and the interaction between netting and stimulus as main effects. Individual comparisons within main effects or interaction effects were evaluated with a Tukey adjustment at $\alpha = 0.05$.

In addition to using the logistic models described above, which allow for determining significant differences in behavioral outcomes between treatments, the categorical wind tunnel behavioral choices were also analyzed using correspondence analysis. The additional analysis allowed interpretation of correlations between the treatments and behavioral outcomes. The CORRESP procedure in SAS was thus used to examine associations between the four behavioral outcomes and the six netting and stimulus treatments. The analysis determines two dimensions that best explain deviations from expected values, and graphically depicts the contributions of each of the behavioral outcomes and experimental treatments to these dimensions.

3. Results

3.1. *Effect of short-term exposure of T. granarium larvae on adult emergence*

For all combinations of netting material and time of exposure in this study there were generally high levels of adult emergence after the exposure of larvae. Adult emergence for larvae exposed on the untreated netting material ranged from 84 to 99%, while for larvae exposed on the LLIN it ranged from 81 to 91% (Fig. 3). None of the effects considered in the logistical regression model were significant as evaluated using Wald X^2. The effects tested included LLIN versus untreated netting ($X^2 = 0.59$, df. = 1, $P = 0.44$), time ($X^2 = 8.03$, df = 4, $P = 0.09$), interaction of netting and time ($X^2 = 7.25$, df = 4, $P = 0.12$).

3.2. *Effect of LLIN on attraction to a stimulus*

The velocity by which larvae exited the arena was significantly affected by whether the stimulus edge (UR) or the other edges (AR) were chosen (Type III MSE = 3.81, $F = 13.53$, df = 1, $P = 0.0003$), with an exit of the stimulus edge being faster (Fig. 4A). The effect of netting on velocity was marginal (Type III MSE = 0.82, $F = 2.91$, df = 2, $P = 0.06$), and the effect of using a lure as a stimulus were not significant (Type III MSE = 0.59, $F = 2.09$, df = 1, $P = 0.15$), but the interaction of the two factors was significant (Type III MSE = 1.02, $F = 3.62$, $P = 0.03$). Larvae not exposed to netting moved significantly faster than those exposed to the untreated netting (Fig. 4B), but not significantly different from those larvae exposed to the LLIN. The LLIN had no immediate effect, positive or negative, on the velocity of *T. granarium* larvae after a 10 min exposure, compared with all other netting and stimulus combinations. Among all of the combinations of netting and stimulus presentation, the treatment consisting of no netting and clean air had the highest larval velocity, but in direct comparison only larvae that were exposed to untreated netting and the semiochemical lure were significantly slower (Fig. 4B).

Observing the breakdown of *T. granarium* larval choices (Fig. 5), larvae exposed to the LLIN or untreated netting had a higher percentage of non-movers compared to larvae not exposed to netting (no net). Logistic regression with respect to the proportion of individuals not moving during the analysis indicated a strongly significant effect of the netting treatment (Table 1). However, there was not a significant effect of the stimulus provided or the interaction between the factors. Generally, about 21–27% of individuals not exposed to netting or exposed to the LLIN did not move, but >40% remained stationary for the untreated netting exposures (Fig. 5). The proportion of non-movers in this untreated netting exposure group was significantly greater than that in the

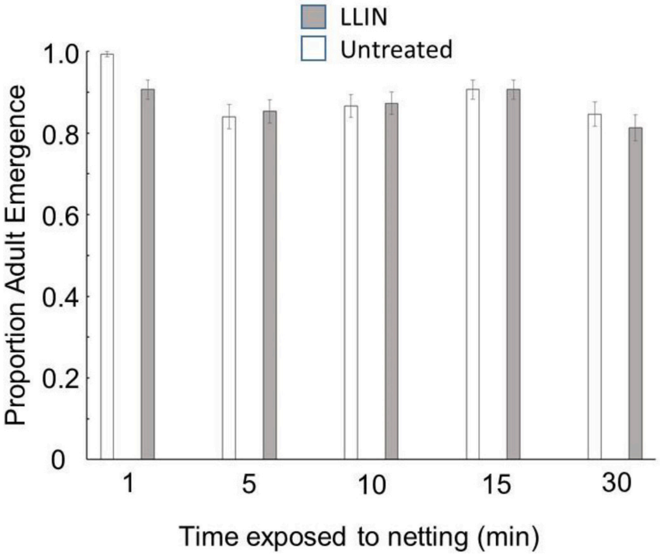

Fig. 3. Adult emergence (mean ± SE) of *T. granarium* larvae exposed to the LLIN or untreated netting for 1, 5, 10, 15, or 30 min, removed to untreated arenas, and observed for adult emergence. White bars represent untreated adult emergence and grey bars represent treatment (LLIN) adult emergences.

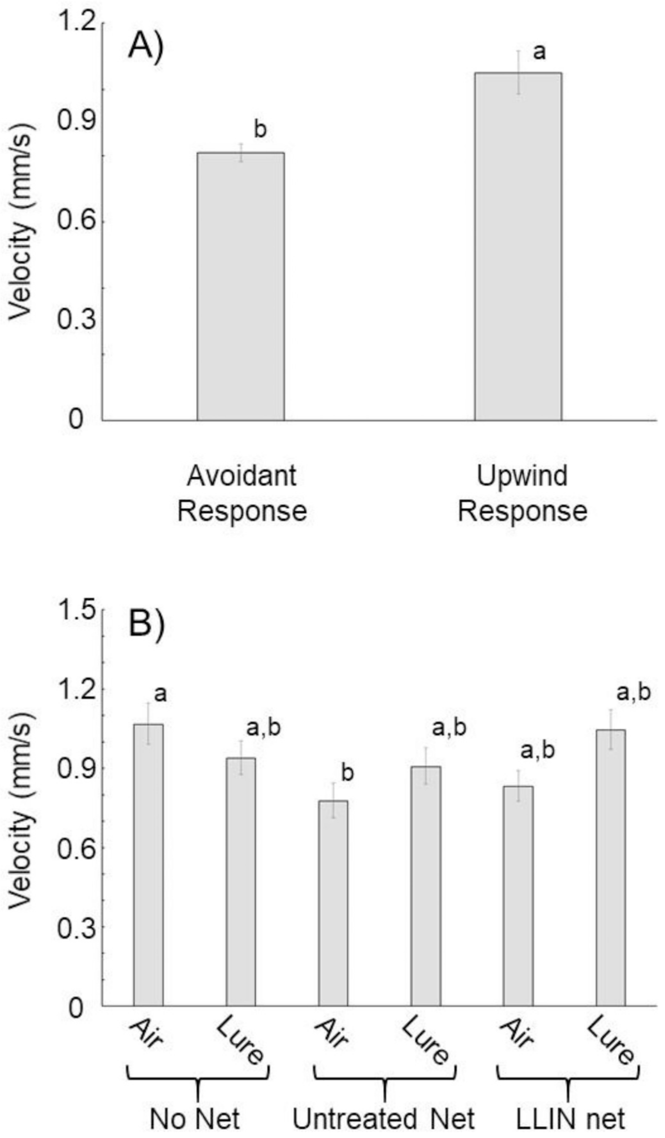

Fig. 4. Mean (± SE) tangential velocity of larvae exiting the arena. Velocities are pooled for the two types of exiting responses (A), followed by the netting treatments (B). Different lowercase letters in both graphs indicate significant differences (α = 0.05) after Tukey's adjustment for treatments.

no netting or treated netting groups (Table 1).

Among the individuals that did move in the assay (NM), there were significant effects of netting, or stimulus presentation, and their interaction upon the likelihood that they would move upwind to the stimulus. (Table 1). When exploring the interaction effects in more detail (Table 2), it was apparent that the LLIN altered the effect of the stimulus on upwind movement. Among trials where no stimulus was presented (air only) the upwind movement was significantly higher after exposure to the LLIN in comparison to either no netting or untreated netting. However, when a stimulus was provided, the upwind movement diminished slightly and was significantly less than when there was no net exposure. Moreover, when there was a lack of exposure to netting, there was a clear significant four-fold increase in the effect of the lure stimulus, which did not occur in either of the netting treatments (Fig. 5, Table 2).

The principle inertia values of the two dimensions (0.366 and 0.351) identified by correspondence analysis indicated similar, but relatively low contributions toward explaining the variation in the data (X^2 = 877.8, 17. 9% and X^2 = 841.7, 17.2%, respectively). The graphical representation of the dimensions (Fig. 6) indicates that the lure stimulation with no netting exposure and the upwind response are correlated positively with both dimensions. Negative values for dimension one, and positive values for dimension two are indicative of non-moving behavior and the untreated netting material, regardless of odor stimulus. The other treatments and behavioral categories tend to have values near zero for dimension one, but a range of values for the second dimension from near zero for the LLIN to close to negative one for the no netting treatment with clean air stimulus. The avoidant response and no response categories were intermediate on this axis with dimension 1 being near zero, and dimension 2 being near −0.5 in value.

4. Discussion

The results indicate that exposure to untreated netting was associated with a decreased tendency for the larvae to subsequently move. The frequency of events where the larvae did not move at all increased when untreated netting was used. The correspondence analysis also supports the relationship, with the not moving response having similar dimensional coordinates as both of the untreated netting treatments, with or without the odor lure added. Indicating a similar trend, the velocity of responses decreased in the untreated netting responses. These results indicate that exposure to netting itself, without the insecticide, may be triggering the well-known tendency of larvae to hide in crevices, which is often noted in extension publications (United States Department of Agriculture, 2012). Thigmotaxis is a widely observed phenomenon in insects, but poorly described. Variation in thigmotaxis within a population, is influenced by certain stimuli (Mosquera and Lorenzo, 2020) and may in turn affect other behaviors (Doriaa et al., 2019; Steinbauer, 2009). For *T. granarium* it seems as if a thigmotactic arrestment induced by exposure to the untreated netting was manifested as a tendency to remain stationary once larvae were transferred to the open wind tunnel assay. Otherwise the relative tendencies to perform other behaviors within the arena was similar to those of larvae not exposed to netting, after some movement commenced (Fig. 5).

While the physical texture of netting seems to negatively impact movement, the insecticide seems to negate this effect and encourage more movement, with a reduction in the number of insects being characterized as non-movers (Fig. 5). For bedbugs, it has been suggested that thigmotaxis may encourage uptake of insecticides from surfaces (Kells and Hymel, 2016). Conversely, these results for *T. granarium* indicate that thigmotaxis in turn can be affected by LLIN contact. Insecticide exposure more often seems to lead to reduced movement by insects, with the presumption that it is adaptive to reduce further exposure (Sparks et al., 1989). For the closely related species *T. variabile*, a more typically expected reduction of movement after insecticide exposure was noted (Wilkins et al., 2020).

However, interaction with other behavioral stimulants can mitigate the tendency of insecticide exposure to reduce movement (Alzogaray and Zerba, 2001; Reynoso et al., 2018). In this study on *T. granarium* larvae, wind exposure appeared to be the factor that induced this activity. For LLIN exposed larvae, there was significantly more frequent walking upwind to clean air than there was for the control larvae, but significantly fewer walked up toward the lure (Table 2). Thus, while the insects not exposed to netting demonstrated the expected response to move upwind to semiochemicals (Morrison et al., 2020), the LLIN-exposed insects seemed to move more indiscriminately, often upwind, but not orienting to semiochemicals. Exposure to wind may be interacting with insecticide exposure to cause the observed increase in mobility. It would be of interest to further explore the degree to which these negative effects on thigmotactic arrestment and semiochemical orientation are related. It has been noted in cockroaches that thigmotaxis is mediated by antennal perception (Okada and Toh, 2006). It has not yet been confirmed if either of these two behaviors are linked to antennal physiology in *T. granarium* larvae, or if other sensilla elsewhere on the body are involved.

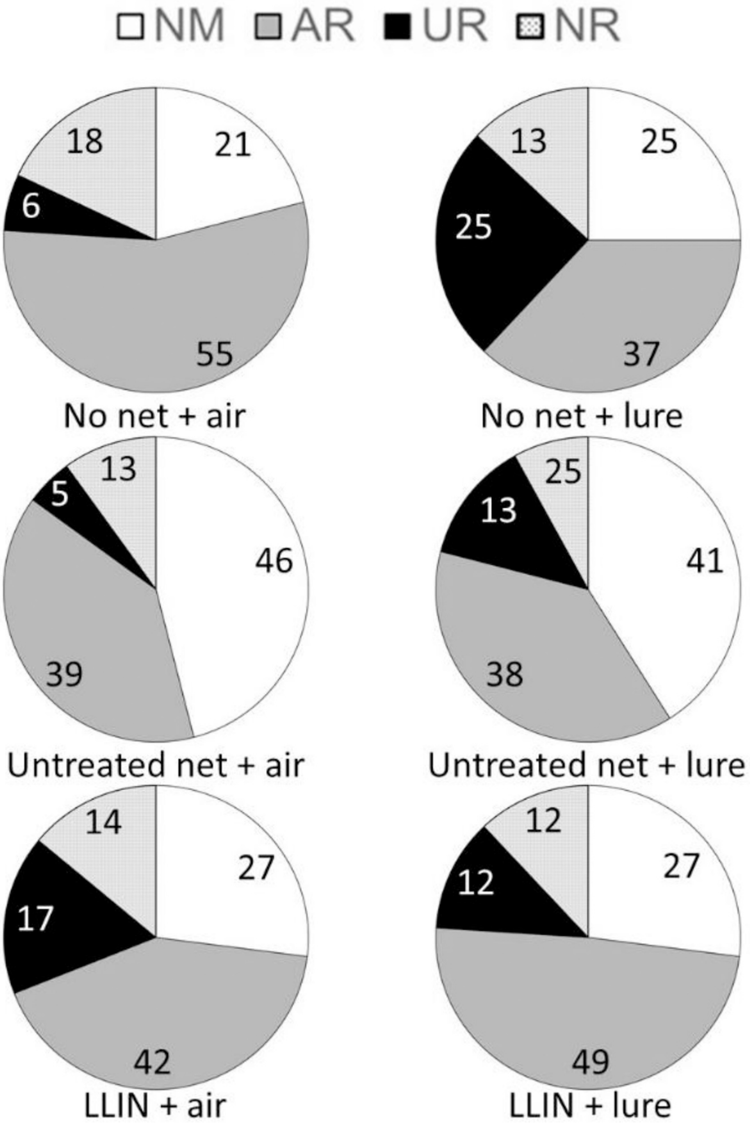

Fig. 5. Pie charts indicating the proportions of larvae exhibiting no movement (NM), avoidant response (AR), upwind response (UR), or no exiting response (NR) for each of the experimental treatments. Numbers within charts indicate percentage from a hundred responses per treatment.

Table 1
Main effects and interaction effects of experimental treatments on proportion of *T. granarium* larvae not moving in the behavioral assay (NM) or walking upwind to the stimulus (UR) given that it does move using a logistic model.

		No Movement Response (NM)		Upwind Response (UR)	
Effect	df	Ward X^2	*P*	Ward X^2	*P*
netting	2	15.3	<0.001	8.43	0.015
None vs Untreated	1	13.49	<0.001	0.12	0.733
None vs Treated	1	0.98	0.322	6.64	0.010
Treated vs Untreated	1	7.65	0.006	4.00	0.046
stimulus	1	0.45	0.502	13.6	<0.001
netting*stimulus	2	0.95	0.622	12.6	0.002

For proportion not moving $n = 100$ for all treatments. Sample sizes for upwind response exclude non-movers and are provided in Table 2 with contrasts involving netting*stimulus interaction.

Table 2
The proportion of *T. granarium* larvae walking upwind (UR) for each netting and stimulus combination.

	No Net		Untreated Netting		**LLIN**	
	Air	Lure	Air	Lure	Air	Lure
Proportion moving upwind*	0.076	0.333	0.093	0.220	0.233	0.164
Netting effect on air stimulus	a	–	a	–	b	–
Netting effect on lure stimulus	–	a	–	a,b	–	b
Stimulus effect on no netting	a	b	–	–	–	–
Stimulus effect on untreated netting	–	–	a	a	–	–
Stimulus effect on LLIN	–	–	–	–	a	a
Total number of trials	79	75	54	59	73	73

[*] Different letters indicate significant differences between treatment combinations evaluated by Ward X^2 at $\alpha = 0.05$.

The effect of the netting appears to be primarily linked to the propensity for movement and the types of anemotaxic and thigmotaxic behaviors occurring after exposure. Netting treatment appeared to have less of an effect on the walking velocity of the insects compared to

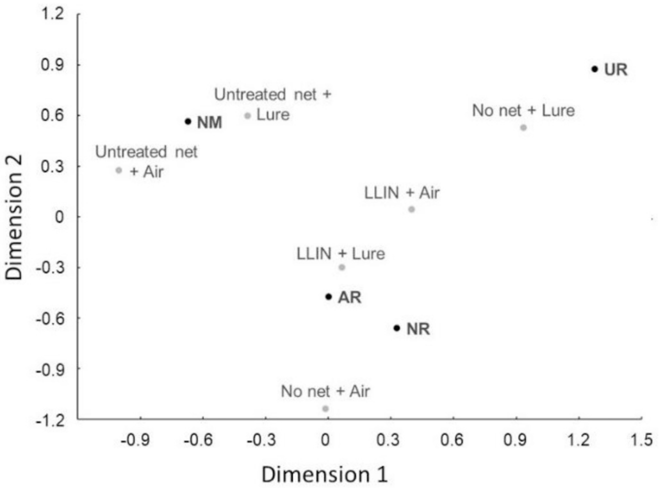

Fig. 6. Graph summarizing the column coordinates for correspondence analysis involving the behavioral categories (bold) vs, the experimental treatments. The behavioral candidates include no movement (NM), avoidant response (AR), upwind response (UR), or no exiting response (NR).

untreated controls. Only the clean air response between no netting exposed larvae and those exposed to untreated netting differed in velocity. This pattern is consistent with the overall decreased movement caused by the untreated netting. Otherwise, it was clear that upwind responses tended to have a greater velocity than avoidant response, but the experimental procedure of releasing the larvae facing upwind may explain this result, which is clearly not linked to netting treatment.

The management implications of these observations are also important to consider. The currently used *T. granarium* wall traps use a highly textured insert that encourages the crevice seeking behavior of larvae (Barak, 1989). The results of this study emphasize how untreated textured structures and netting may be helpful to incorporate in trapping designs. The physical barrier of the netting itself could reduce the larval movement after exposure thus limiting the spread of *T. granarium* from location to location. However, LLIN may encourage movement, and may not necessarily affect the ability of *T. granarium* to locate traps. Furthermore, deployment of LLIN netting may require consideration of airflow patterns. Minimizing airflow around places where LLIN is deployed may be useful if active walking behavior by larvae is triggered by LLIN in field conditions. Based on these lab observations, air flow may stimulate movement away from the net and place commodities at risk, perhaps particularly if the commodities are located upwind of the netting material. Deploying traps just upwind of LLIN material may also be helpful for managing the insect.

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