11-21-2023

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Research Article

Received: 11 May 2023 Revised: 20 November 2023 Accepted article published: 21 November 2023 Published online in Wiley Online Library: 14 December 2023

(wileyonlinelibrary.com) DOI 10.1002/ps.7903

Disruption of semiochemical-mediated movement by the immature *Trogoderma variabile* Baillon and *Trogoderma inclusum* Le Conte (Coleoptera: Dermestidae) after exposure to long-lasting insecticide-incorporated netting

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Abstract

BACKGROUND: Highly mobile stored product insects may be able to readily orient in response to food cues and pheromones to attack durable commodities at each link of the postharvest supply chain. A 0.4% deltamethrin-incorporated long-lasting insecticide-incorporated netting (LLIN) is a successful novel preventative integrated pest management (IPM) tactic to intercept dispersing insects after harvest. However, it is unknown whether exposure to LLIN may affect olfaction and orientation to important semiochemicals by immature stored product dermestids, therefore the aim of this study was to assess whether exposure to LLIN disrupts the normal olfactory and chemotactic behavior of warehouse beetle, *Trogoderma variabile* Ballion (Coleoptera: Dermestidae), and the larger cabinet beetle, *T. inclusum* Le Conte (Coleoptera: Dermestidae), larval movement in the presence of important semiochemicals, including food kairomones (e.g., flour) and pheromones, e.g., (*Z*)-14-methyl-8-hexadecenal.

RESULTS: The distance moved by the larval population of *T. variabile* was reduced by 64% after 24-h exposure to LLIN compared to control netting but not immediately after exposure, while *T. inclusum* larvae movement was reduced by 50% after 24-h exposure to LLIN compared to the control netting. Generally, the olfaction and orientation of larval dermestids were affected after exposure to LLIN compared to control netting. There were species-linked differences in effects on olfaction after the insects were exposed to LLIN.

CONCLUSION: Our study suggests the use of LLIN may enhance the effectiveness of other concurrent behaviorally-based strategies such as mating disruption when used as part of a comprehensive IPM program in the postharvest environment.

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

Keywords: dermestids; movement; behavior; olfaction; integrated pest management

1 INTRODUCTION

The agricultural sector makes a significant contribution to the economy, and the postharvest setting is a crucial component of this sector. The postharvest supply chain encompasses a wide range of stages, starting from the farm where crops are harvested and extending all the way to the end consumer.[1] The postharvest supply chain involves the movement of durable agricultural commodities through on- and off-farm storage, elevators, processing facilities, warehouses, distribution centers, retail stores, and ultimately into consumer pantries. During each link, stored product pests readily attack these commodities, leading to significant damage in both the quality and quantity of these foods.[2] Every year, producers lose between 2% and 50% of their harvested

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crops due to stored product insect infestations, which leads to an economic loss of over 100 billion USD globally.[3] It is therefore vital to develop effective management strategies to prevent losses from infestations of stored product insects.

Integrated pest management (IPM) is a holistic approach of pest management which integrates various management techniques to control insects within a system, employing insecticides as a final resort.[4] Movement of insects has always been an ongoing challenge to successful IPM programs. For example, in a 2-h period *Trogoderma variabile* Ballion (Coleoptera: Dermestidae) larvae moved about 15 m on average,[5] which extrapolating to a 24-h period would be 180 m. This is easily on the same order of magnitude or greater as adult *T. variabile*, which when marked and released at a food processing plant was found to move an average of 75 m away.[6] Additionally, arrayed in the same experiment, control larval *T. variabile* walked 3.5-fold greater distances and velocity than adults.[7] Indeed, Sakka *et al.*[8] documented that in release-recapture assays, the number of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) adults found in traps (e.g., 0–20% recaptured) partially designed for dermestids were fewer than larvae (e.g., 20–70% recaptured), suggesting larval *Trogoderma* spp. are strong dispersers compared to adult stages. In addition, *T. variabile* may sometimes be the predominant species at a food facility, has been documented moving across multiple floors with mark-release-recapture, and evidence of larval *Trogoderma* spp. movement has been observed with cast larval cuticles.[9] Finally, some of these stored product insects appear capable of using alternate hosts in the landscapes outside of food facilities, including *Quercus* spp.[10] Taken together, this presents a compelling case for especially evaluating the movement of larval *Trogoderma* spp. as a potential ongoing struggle in some IPM programs.

While movement and spatial variation in pest abundance can critically affect IPM programs for food facilities,[11] one innovative method to intercept immigrating insects that has been successfully employed after harvest is long-lasting insecticide-incorporated netting (LLIN). LLIN has demonstrated both lethal[12,13] and sublethal effects on a variety of stored product pests,[7,14] making it an ideal method to cover openings such as doors, windows, vents, cracks, or crevices, intake or outtake manifolds on processing facilities, awnings on grain silos, and pallets in warehouses.[15]

LLIN incorporated with deltamethrin or alpha-cypermethrin has been highly effective against numerous important stored product insects.[12-14,16-19] For example, prior work found deltamethrin-incorporated LLIN was effective against adult red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), lesser grain borer *Rhyzopertha dominica*, rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae),[13] *Trogoderma variabile*,[20] and khapra beetle *T. granarium*.[21] Meanwhile, alpha-cypermethrin-incorporated LLIN effectively induced mortality against adult saw-tooth grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *R. dominica*, rusty grain beetle *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), *S. oryzae*, granary weevil *S. granarius* (L.) (Coleoptera: Curculionidae),[12] cigarette beetle *Lasioderma serricorne* (F.) (Coleoptera: Ptinidae), Mediterranean flour moth *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae),[17] and larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae).[18] The least susceptible species to alpha-cypermethrin-incorporated LLIN were maize weevil *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), *Tribolium castaneum*,[9] and confused flour beetle *Tribolium confusum* du Val (Coleoptera: Tenebrionidae).[18] Ranabhat *et al.*[22] found that adult phosphine-susceptible and -resistant strains of *T. castaneum* and *R. dominica* were equally susceptible to deltamethrin-incorporated netting and were also susceptible to other active ingredients such as dinotefuran, permethrin, and indoxacarb.[22]

However, there has been less work investigating the effects of netting exposure on larvae of stored product insects. Wilkins *et al.*[7] assessed life-stage specific differences in mobility of *T. castaneum* and *T. variabile* after exposure to LLIN and found that larvae were more resistant to the effects of netting than adults.[7] Nevertheless, only about half as many LLIN-exposed larvae made it to a novel food patch 25 cm away compared to a control netting-exposed group, and only a small fraction (~5%) of LLIN-exposed larvae made it to the food patch at 75 cm compared to the control group. Interestingly, control large larval and adult *T. variabile* were equally successful in dispersing to novel food patches over 48 h.[7] Importantly, this prior study did not evaluate dispersal in the presence of semiochemicals. Other work evaluated control or treated netting exposure by *T. granarium* in the presence of kairomones and pheromones in a miniature wind tunnel[21] and found interacting effects related to thigmotaxis and anemotaxis. In addition, Morrison *et al.*[23] found that *T. granarium* and *T. variable* larvae respond to their sex pheromone over and beyond a control by several fold in a variety of behavioral assays. However, to date, no work has been performed with larval *T. variabile* or *T. inclusum* on how exposure to control or insecticide-incorporated netting affects semiochemical-mediated movement. Thus, it remains unclear whether exposure to LLIN affects normal olfactory orientation and movement. This is important because the larval stage for both of these species represents the bulk of the duration of the life cycle.

Among the various stored product pests, *T. variabile* and *T. inclusum* are two significant and widespread dermestid species.[2] Both are external-infesting insects that attack various products and may be problematic in durable commodities at mills and processing plants.[24] Both species have short lifespans as an adult,[25] thus the larval stage comprises the majority of the life cycle, causing most of the damage.[26] As a result, better understanding how these species respond to novel IPM approaches is important for tailoring implementation of tactics in behaviorally compatible ways. Ranabhat and Morrison (unpublished) demonstrated how exposure to LLIN affected the movement and olfaction in the presence of important semiochemicals to different sexes of adult *T. variabile* and *T. inclusum*. Our aim was to evaluate whether immature dermestids, including larval *T. variabile* and *T. inclusum*, may behave differently compared to adults towards the same stimuli, including to conspecific-produced pheromones and food cues after exposure to LLIN by assessing their behavior in the laboratory. We hypothesized that LLIN exposure would significantly alter olfaction by one or both species through contact with deltamethrin. We did this by tracking their movement with a network camera coupled with Ethovision after brief exposure to LLIN.

2 MATERIALS AND METHODS

2.1 Sources insects

Four- to six-week-old larvae of *T. inclusum* and *T. variabile* were used for the experiment. Both strains were originally obtained from the field in north-central Kansas in 2012 and 2016, respectively. Colonies of these species were reared under controlled conditions in an environmental chamber set to a temperature of

27.5 °C, 65% relative humidity (RH), and 14 h:10 h (light:dark) photoperiod. Both species were reared on 300 g of ground dog food (SmartBlend, Lamb flavor, PurinaOne, St. Louis, MO, USA) with oats sprinkled on top and a moistened, crumpled paper towel placed on the surface in a 950-mL mason jar. Individuals were not starved and used immediately for exposures from subcultures.

2.2 Treatments

The long-lasting insecticide-incorporated polyethylene netting (2 × 2 mm mesh; D-Terrance, Vestergaard, Lausanne, Switzerland) included 0.4% deltamethrin or control netting that was identical in physical properties but without insecticide. These were used with the movement assay. We assessed the movement in the vicinity of important pheromonal and food kairomones after exposure to LLIN or control netting. Food consisted of 0.01 g of organic, unbleached flour (Heartland Mills, Marienthal, KS, USA), and pheromonal stimuli included a broad spectrum, multispecies lure (PTL lure, IL-108-10, Batch#1288200321; Insects Limited, Westfield, IN, USA), including *Trogoderma* spp. pheromone, (*Z*)-14-methyl-8-hexadecenal (Ranabhat and Morrison, unpublished), which is female-produced and cross-attractive to both species.[27] In each replicate, we used a single pellet (white color), and affixed it in place so it did not move in a Petri dish using a 1 × 1 mm square of parafilm. For each replication of testing, we used a fresh lure.

2.3 Movement assay

The movement of larvae after exposure to the 0.4% deltamethrin LLIN or a control netting in response to food cues (using 0.01 g of flour) or with conspecific sex pheromones (using a single bead from a disaggregated PTL lure held in place with a small square of parafilm), was tracked in six individual arenas (100 × 15 mm depth × height) with a piece of filter paper (85 mm depth; Ahlstrom-Munksjö, Helsinki, Finland) lining the bottom. Movement was tracked for 30 min using a network camera (GigE, Basler AG, Ahrenburg, Germany) affixed 76 cm above and centered over the dishes. The Petri dishes were backlit using a LED light box (42 × 30 cm^2 width × length, L LPB3; Litup, Shenzhen, China) to increase contrast and affixed in place with white foam board. The video was streamed to a computer and processed in Ethovision (v.14.5; Noldus Inc., Leesburg, VA, USA). Prior to use in the movement assay, larvae of *T. variabile* or *T. inclusum* were exposed to the 0.4% deltamethrin LLIN or a control netting for 1 min in a 21 × 21 cm^2 square Petri dish, then their movement was tracked individually after a postexposure holding duration of 1 min or 24 h to assess immediate versus delayed impacts. The position of treatments was randomized between each replicate to control for positional bias. A small 1.1-cm hidden stimulus zone encircled each stimulus, midway and centered on each half of the arena wherein movement was tracked separately from each half of the arena (control *vs* treatment). The small stimulus zone was used to evaluate interactions with the semiochemical cues. The total distance moved (cm), instantaneous velocity (cm/s), frequency of entering each half of the Petri dish and stimulus zone, cumulative duration spent in each zone (s), and latency of entering each zone (s) over a 30-min trial period was logged after exposure to a given treatment. The control side of the arena remained empty. A total of $n = 16$ replicates were run per treatment combination for both species. Thus, in total, there were 384 individual larvae tested and 11 520 min of video was recorded.

2.4 No-choice release-recapture assay

A release-recapture experiment was conducted for the larvae of both *T. variabile* and *T. inclusum* where larvae were exposed to the 0.4%

Table 1. Summary of statistical model results for the distance moved by larval dermestids after exposure to 0.4% deltamethrin incorporated long-lasting insecticide netting in response to important semiochemicals (e.g., negative ctrl, food, or *Trogoderma* spp. sex pheromone) over a 30-min period at the Center for Grain and Animal Health Research in Manhattan, KS

	ANOVA		
Variable	df	*F*	*P*
Larval *Trogoderma variabile*			
Netting	1	0.61	0.44
Treatment	2	3.35	0.04
Postexposure	1	0.06	0.81
Netting: treatment	2	3.88	0.02
Netting: postexposure	1	4.45	0.04
Treatment: postexposure	2	1.66	0.19
Netting: treatment: postexposure	2	4.77	0.01
Residuals	159		
Larval *Trogoderma inclusum*			
Treatment	2	4.10	0.02
Netting	1	11.8	0.001
Postexposure	1	5.27	0.02
Treatment: netting	2	3.19	0.04
Treatment: postexposure	2	4.27	0.02
Netting: postexposure	1	11.8	0.001
Treatment: netting: postexposure	2	2.77	0.07
Residuals	155		

Table 2. Summary of statistical model results for the instantaneous velocity moved by larval dermestids after exposure to 0.4% deltamethrin incorporated long-lasting insecticide netting in response to important semiochemicals (e.g., negative ctrl, food, or *Trogoderma* spp. sex pheromone) over a 30-min period at the Center for Grain and Animal Health Research in Manhattan, KS

	ANOVA		
Variable	df	*F*	*P*
Larval *Trogoderma variabile*			
Netting	1	0.85	0.36
Treatment	2	3.47	0.03
Postexposure	1	0.33	0.56
Netting: treatment	2	4.50	0.01
Netting: postexposure	1	5.02	0.03
Treatment: postexposure	2	1.44	0.24
Netting: treatment: postexposure	2	4.80	0.01
Residuals	159		
Larval *Trogoderma inclusum*			
Treatment	2	0.06	0.95
Netting	1	1.28	0.26
Postexposure	1	0.004	0.95
Treatment: netting	2	0.37	0.69
Treatment: postexposure	2	0.13	0.88
Netting: postexposure	1	0.95	0.33
Treatment: netting: postexposure	2	0.07	0.93
Residuals	155		

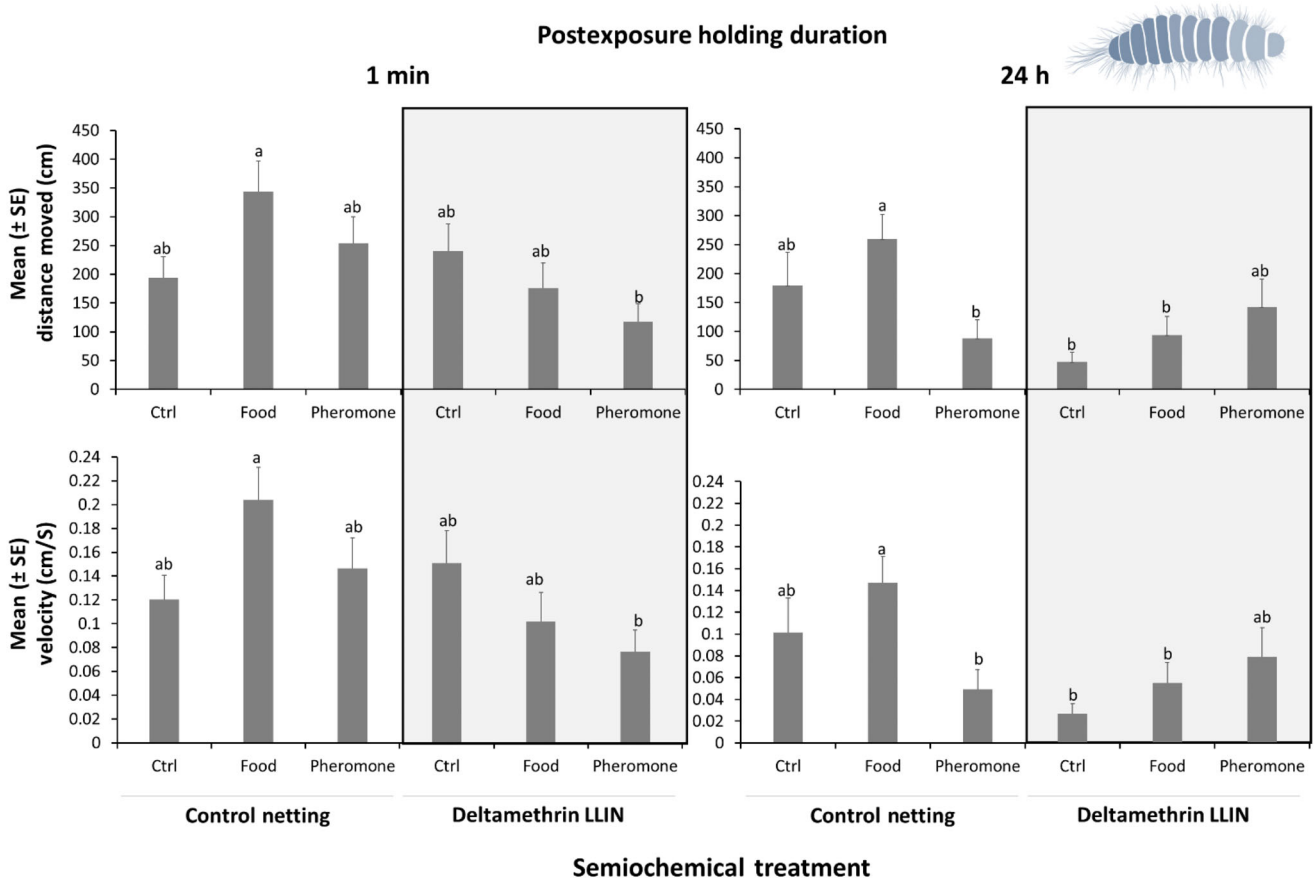

Figure 1. Distance moved (±SE, cm) (top panels) or instantaneous velocity (±SE, cm/s) (bottom panels) by larval *Trogoderma variabile* after 1 min of exposure to control netting without insecticide or to 0.4% deltamethrin-incorporated long-lasting insecticide netting (LLIN) in the presence of food (0.01 g of wheat flour), *Trogoderma* spp. pheromone (e.g., (*Z*)-14-methyl-8-hexadecenal) or a negative control without stimuli during a 30-min movement assay immediately after exposure (left column) or 24 h later (right column). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). The shaded gray area represents LLIN-exposed treatments. Ctrl, unbaited control.

deltamethrin LLIN and control netting for 1 min. After exposure, treated insects were released at one corner of the sanded plastic bin (60 × 41.6 × 16.5 cm^3 length × width ×height). A commercial pitfall trap (Dome Trap™, Trécé, Inc., Adair, OK, USA) that contained a PTL lure (using only a single white bead per trap as above) or 0.01 g flour or no stimuli (unbaited for control) was deployed in the opposite corner, diagonally across from the release point in the bin. The bins were located in a large (4.8 × 2.1 × 6 m length × width × height) walk-in environmental chamber (Percival Instruments, Dallas County, IA, USA) set at constant conditions (27.5 °C, 60% RH, and 14 h:10 h light:dark). A total of 10 larvae were released in each bin during each replicate. Treated larvae were given 24 h to disperse to the semiochemicals in each trap, and then the number of insects captured inside the trap, found on the bottom of the trap, on the stimulus half of container, or on the nonstimulus half of the container was recorded. A total of $n = 12$ replicates were performed per treatment combination for the larvae of each species.

2.5 Statistical analysis

In the movement assay, prior to data analysis, the track viewer was used to evaluate all samples and eliminate any where there was false accumulation of distance due to cursor bounce or other artifacts of Ethovision. Afterwards, the distance moved and instantaneous velocity were analyzed with a linear mixed model, using the run date as a random variable. The frequency, cumulative duration spent in, and latency to entering control/stimulus zones and control/treatment half zone were analyzed with a multivariate analysis of variance (MANOVA) followed by sequential ANOVAs for each response variable. In each case, the semiochemical treatment, exposure to netting, and postexposure holding duration were fixed, explanatory variables, along with their two-way and three-way interactions. Assumptions of normality and homogeneity in variances were checked by inspecting residuals and diagnostic plots, and log transformation was used when appropriate, which corrected any issues. Tukey HSD was used for multiple comparisons on a significant result from the overall model. For the release-recapture experiment, we used a generalized linear model based on a quasipoisson distribution (e.g., because over dispersion was a problem), with the same model structure as above. All analyses were run using R software,[28] with $\alpha = 0.05$ and Tukey HSD for multiple comparisons. Finally, we used Student's *t*-test for select *post hoc* comparisons between treatment and control zones in the movement assay, denoted in figures with asterisks.

3 RESULTS

3.1 Trogoderma variabile movement

None of the main effects (e.g., exposure to LLIN, semiochemical presence, or postexposure holding duration) significantly affected

Table 3. Summary of statistical model results for the frequency of entering each zone by larval dermestids after exposure to 0.4% deltamethrin incorporated long-lasting insecticide netting in the presence of important semiochemicals, including a negative control, food, and *Trogoderma* spp. sex pheromone, over a 30-min period at the Center for Grain and Animal Health Research in Manhattan, KS

				Sequential ANOVAs							
	MANOVA			Frequency - stimulus treatment zone		Frequency - stimulus control zone		Frequency of half - treatment zone		Frequency of half - control zone	
Variable	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Immature *Trogoderma variabile*											
Netting	1	5.87	0.001	8.23	0.01	17.6	0.0001	1.92	0.17	2.57	0.11
Treatment	2	5.50	0.001	1.94	0.15	9.44	0.0001	0.04	0.96	0.45	0.64
Postexposure	1	6.71	0.0001	10.0	0.01	8.50	0.0041	15.7	0.001	10.0	0.01
Netting: treatment	2	1.36	0.25	0.52	0.60	0.15	0.86	2.01	0.14	1.74	0.18
Netting: postexposure	1	1.86	0.12	0.72	0.40	1.83	0.18	6.79	0.01	2.59	0.11
Treatment: postexposure	2	3.10	0.02	1.00	0.37	4.79	0.01	0.72	0.49	0.57	0.57
Netting: treatment: postexposure	2	2.10	0.08	1.42	0.25	3.03	0.05	0.23	0.79	0.12	0.89
Residuals	159										
Immature *Trogoderma inclusum*											
Treatment	2	5.64	0.001	4.22	0.02	1.57	0.21	3.31	0.04	2.49	0.09
Netting	1	0.71	0.58	0.05	0.82	2.28	0.13	0.07	0.79	0.49	0.49
Postexposure	1	11.7	0.0001	3.13	0.08	9.94	0.01	32.7	0.0001	19.1	0.0001
Treatment: netting	2	1.90	0.11	1.16	0.32	1.97	0.14	0.81	0.45	2.10	0.13
Treatment: postexposure	2	1.91	0.11	3.21	0.04	0.07	0.93	0.28	0.76	0.33	0.72
Netting: postexposure	1	0.59	0.67	0.23	0.63	2.08	0.15	0.01	0.90	0.01	0.96
Treatment: netting: postexposure	2	3.30	0.01	0.65	0.53	5.02	0.01	3.27	0.04	0.61	0.55
Residuals	155										

the distance moved or velocity of larval *T. variabile* (Tables 1 and 2). The two-way interactions of netting with the treatment or postexposure holding duration and three-way interaction significantly affected the distance and velocity moved by immature *T. variabile* (Tables 1 and 2). For example, 24 h after exposure to LLIN, the distance moved in response to the food stimuli was reduced by 2.8-fold compared to the control netting (Tukey HSD; Fig. 1). Similarly, the velocity was reduced by 62% in the presence of food 24 h after exposure to LLN compared to the control netting (Fig. 1).

3.2 Behavior of immatures *T. variabile*

3.2.1 *Frequency of entering each zone*

Semiochemical treatment, exposure to netting, and postexposure holding duration all had a significant effect on the frequency of entries into the stimulus zones and the half zones by larvae of *T. variabile* (MANOVA; Table 3). Overall there were 1.3–2.4-fold more frequent entries in the treatment stimulus zone by larvae of *T. variabile* after exposure to control netting than exposure to LLIN (Table 3 and Fig. 2). Furthermore, postexposure holding duration significantly affected the frequency of entering both the stimulus zone and the control half zone, with 1.6–1.7-fold more entries immediately after exposure compared to 24 h later (Table 3). After 24 h, there were twice the number of entries in the control stimulus zone than the treatment stimulus zone containing food after exposure by larval *T. variabile* to control netting, but entries equalized after exposure to LLIN (Fig. 2). The two-way interaction between semiochemical treatment and postexposure holding duration resulted in 1.8–3.1-fold more entries into the treatment half of the arena immediately after exposure compared to 24 h later (Fig. 3).

3.2.2 *Cumulative duration spent in each zone*

The cumulative duration spent in each zone by larval *T. variabile* was significantly affected by all the factors in the model, including semiochemical treatment, exposure to netting, and postexposure holding duration (Table 4). The cumulative duration spent in each zone was also significantly impacted by each two-way interaction among those variables (Table 4). Exposure to LLIN resulted in 2.2-fold more time spent in the treatment stimulus zone and an 85% reduction in time spent in the control stimulus zones by larval *T. variabile* (Table 4). The semiochemical treatment also resulted in 1.5-fold more time spent by larvae of *T. variabile* in the control stimulus zone in arenas with pheromones compared to when no stimuli were present (Fig. 2). However, the cumulative duration spent in the treatment stimulus zone baited with pheromone was reduced by 3-fold after exposure to the LLIN compared to control netting, whereas the cumulative duration spent in the control stimulus zone when unbaited was increased by 2.5-fold after exposure to the LLIN compared to control netting. After a 24-h postexposure holding duration to LLIN, the cumulative duration spent in the stimulus treatment zone baited with food was increased by 2.6-fold compared to exposure to the control netting. Furthermore, the cumulative duration spent in the control stimulus zone in arenas with pheromonal stimuli was reduced significantly by 15-fold 24 h after exposure to LLIN compared to control netting (Fig. 2). In addition, the cumulative duration spent by immature *T. variabile* on the control half of the arena 24 h after exposure to LLIN was 1.4-fold higher in unbaited arenas than in

arenas with food cues (Fig. 3). The cumulative duration spent in the treatment half zone baited with pheromone was also increased by 1.4-fold 24 h after exposure to LLIN compared to the control netting (Fig. 3).

3.2.3 *Latency to each zone*

For immature *T. variabile*, the latency to finding each zone was significantly affected by the semiochemical treatment, exposure to netting, and postexposure holding duration (Table 5). There was a 1.5-fold increase and an 18% reduction in the time it took *T. variabile* larvae to find the treatment and control stimulus zones, respectively, at 24 h compared to 1 min (Fig. 3). The latency to first entry of the control side of the arena was 9-fold greater in the presence of food by *T. variabile* larvae after exposure to control netting compared to the LLIN (*t*-test: $t = 4.85$, df = 26, $P < 0.0001$; Fig. 3). We observed that after exposure to control netting, the latency to the treatment zone half of the arena in the presence of the pheromone treatment was increased by 3.1-fold compared to the unbaited control, while the latency to the control half in the presence of the pheromone was reduced by 42% compared to the unbaited control, but this equalized after exposure to LLIN (Fig. 3). In

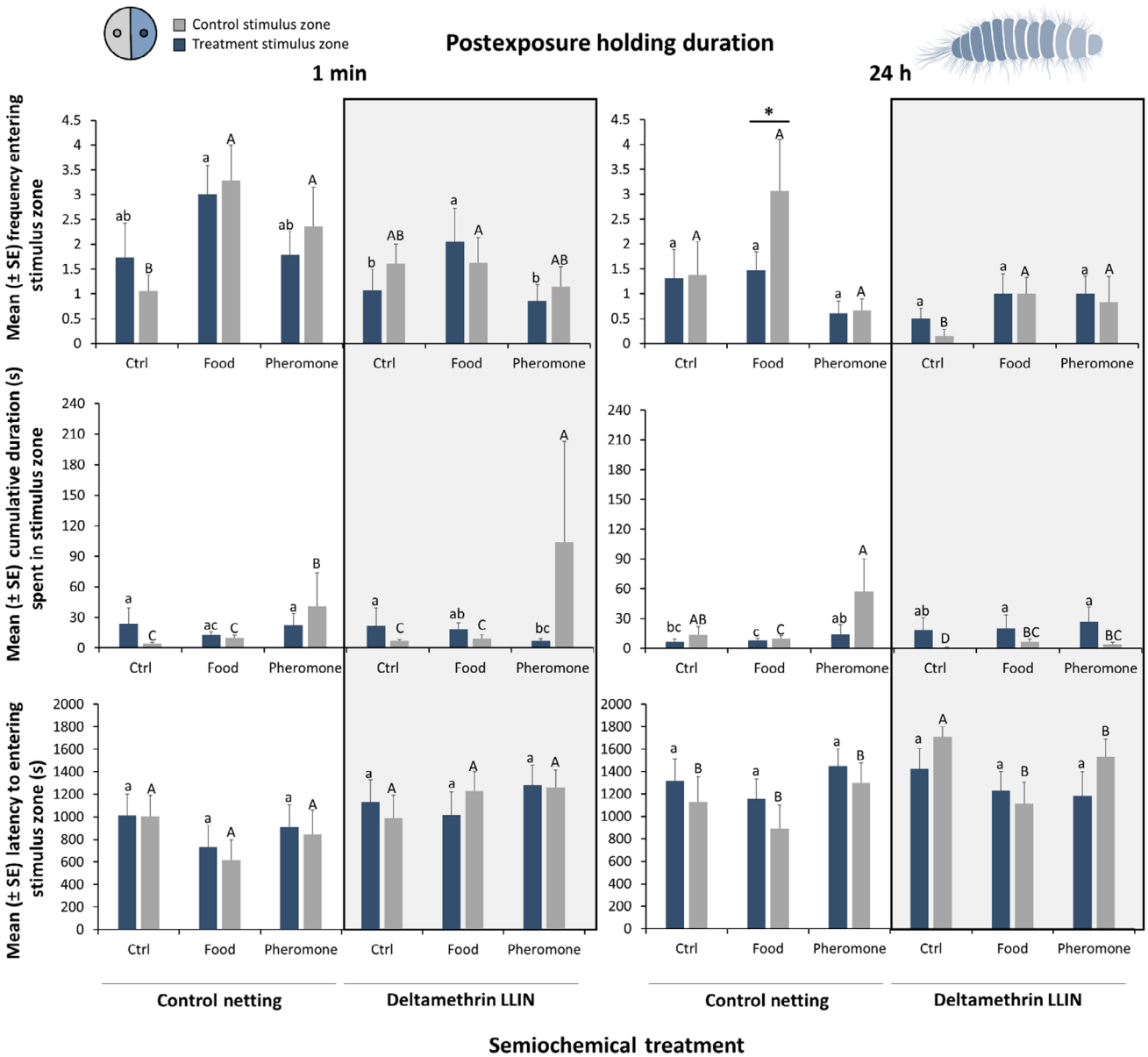

Figure 2. Frequency of entering (±SE) (top panels), cumulative duration spent in (±SE) (middle panels) or latency to finding (bottom panels) the treatment stimulus zone (blue bars) or control stimulus zone (gray bars) by larval *Trogoderma variabile* after 1 min of exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting insecticide netting (LLIN) in the presence of important semiochemicals including food (e.g., 0.01 g of wheat flour), *Trogoderma* spp. pheromone (e.g., (*Z*)-14-methyl-8-hexadecenal) or a negative control without stimuli during a 30-min movement assay immediately after exposure (left column) or 24 h later (right column). Capitalized letters represent multiple comparisons among treatments within the control stimulus zones, while lower case letters represent multiple comparisons among treatments in the treatment stimulus zones within a given period for a specific response variable. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). *Post hoc* comparisons have been added between treatment and control stimulus zones where there was a significant difference in response with asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$, Student's *t* test). The shaded gray area represents LLIN-exposed treatments. Ctrl, unbaited control.

addition, the latency to finding the treatment half of the arena baited with pheromone was reduced by 4-fold, and the latency to finding the control half of the arena in the presence of pheromone was increased by 3-fold by *T. variabile* larvae 24 h after exposure to LLIN compared to control netting, but this response also equilibrated between the zones after exposure to LLIN (Fig. 3).

3.3 Release and recapture of immatures *T. variabile*

A total of 2–7.5% larval *T. variabile* were recaptured in traps, indicating that interpretation of the data is possible. There was 1.2–2.4-fold more *T. variabile* larvae in traps with pheromones than in those left unbaited or with food only, respectively (MANOVA; Table 6 and Fig. 4). Exposure to LLIN significantly altered the pattern of captures of larval *T. variabile* compared to exposure to control netting, decreasing efficacy of food as a bait (Fig. S1). There were 1.5–1.9-fold more larvae underneath the trap in the control and pheromone treatment compared to the traps with food only (Fig. 4). In addition, there was a qualitative interaction between netting and semiochemical, wherein semiochemical did not greatly affect the number of larvae underneath a trap after exposure to control netting, but there

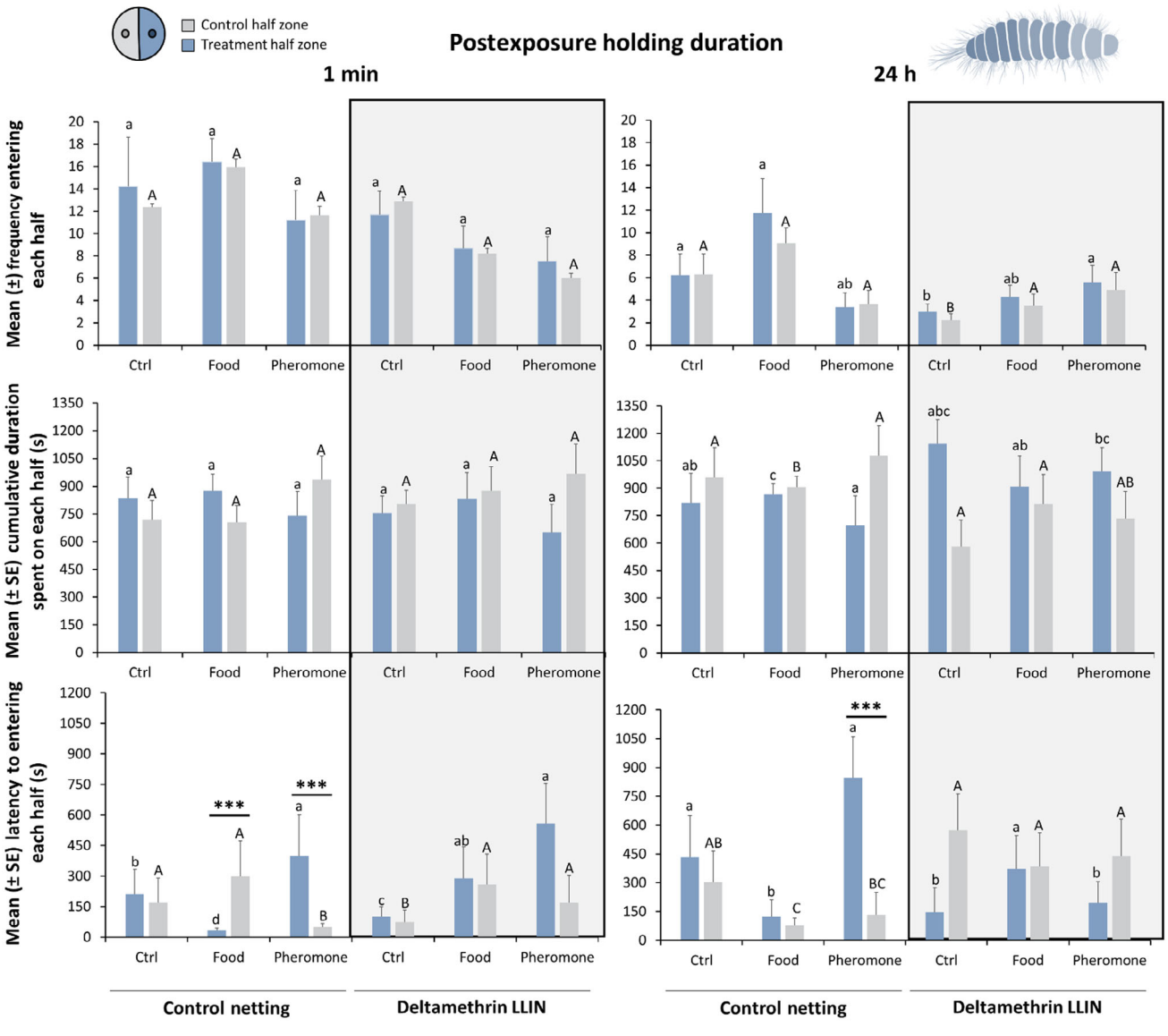

Figure 3. Frequency of entering (±SE) (top panels), cumulative duration spent in (±SE) (middle panels) or latency to finding (bottom panels) the treatment half (light blue bars) or control half (light gray bars) of the Petri dish by larval *Trogoderma variabile* after 1 min of exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting insecticide netting (LLIN) in the presence of important semiochemicals including food (e.g., 0.01 g of wheat flour), *Trogoderma* spp. pheromone (e.g., (*Z*)-14-methyl-8-hexadecenal) or a negative control without stimuli during a 30-min movement assay immediately after exposure (left column) or 24 h later (right column). Capitalized letters represent multiple comparisons among treatments within the control half of the arena, while lower case letters represent multiple comparisons among treatments in the treatment half of the arena within a given period for a specific response variable. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). *Post hoc* comparisons have been added between treatment and control halves of the arenas where there was a significant difference in response with asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$, Student's *t* test). The shaded gray area represents LLIN-exposed treatments. Ctrl, unbaited control.

Table 4. Summary of statistical model results for the cumulative duration spent in each zone by larval dermestids after exposure to 0.4% deltamethrin incorporated long-lasting insecticide netting in the presence of important semiochemicals, including a negative control, food, and *Trogoderma* spp. sex pheromone, over a 30-min period at the Center for Grain and Animal Health Research in Manhattan, KS

				Sequential ANOVAs							
		MANOVA		Cumulative duration - stimulus treatment zone		Cumulative duration - stimulus control zone		Cumulative duration in half - treatment zone		Cumulative duration in half - control zone	
Variable	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Larval *Trogoderma variabile*											
Netting	1	3.73	0.01	7.51	0.01	5.82	0.02	1.98	0.16	2.57	0.11
Treatment	2	70.6	0.0001	2.23	0.11	141.2	0.0001	4.70	0.01	4.39	0.01
Postexposure	1	5.78	0.001	0.03	0.87	18.73	0.0001	3.24	0.07	1.76	0.19
Netting: treatment	2	3.12	0.02	3.79	0.02	3.22	0.04	4.13	0.02	1.56	0.21
Netting: postexposure	1	2.85	0.03	0.71	0.40	8.20	0.01	1.89	0.17	0.14	0.71
Treatment: postexposure	2	6.33	0.0001	2.58	0.08	6.54	0.01	1.87	0.16	2.45	0.09
Netting: treatment: postexposure	2	2.01	0.10	0.68	0.51	2.60	0.08	2.43	0.09	1.70	0.19
Residuals	159										
Larval *Trogoderma inclusum*											
Treatment	2	2.64	0.04	1.58	0.21	4.14	0.02	1.83	0.16	2.22	0.11
Netting	1	26.9	0.0001	0.31	0.58	104	0.0001	13.0	0.001	12.4	0.0001
Postexposure	1	8.01	0.0001	9.48	0.01	19.42	0.0001	12.3	0.001	12.7	0.001
Treatment: netting	2	2.46	0.05	0.73	0.48	0.93	0.40	3.15	0.05	4.23	0.02
Treatment: postexposure	2	0.95	0.44	0.06	0.94	1.79	0.17	0.42	0.66	0.35	0.71
Netting: postexposure	1	0.92	0.46	1.05	0.31	1.14	0.29	0.97	0.33	0.72	0.40
Treatment: netting: postexposure	2	1.57	0.18	0.49	0.62	1.84	0.16	2.54	0.08	2.80	0.06
Residuals	155										

Table 5. Summary of statistical model results for the latency to finding each zone by larval dermestids after exposure to 0.4% deltamethrin incorporated long-lasting insecticide netting in the presence of important semiochemicals, including a negative control, food, and *Trogoderma* spp. sex pheromone, over a 30-min period at the Center for Grain and Animal Health Research in Manhattan, KS

				Sequential ANOVAs							
		MANOVA		Latency to the stimulus treatment zone		Latency to half the treatment zone		Latency to the stimulus control zone		Latency to half the control zone	
Variable	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Larval *Trogoderma variabile*											
Netting	1	11.8	0.0001	0.07	0.79	2.02	0.16	2.49	0.12	42.7	0.0001
Treatment	2	10.9	0.0001	0.56	0.57	16.6	0.0001	2.27	0.11	3.76	0.03
Postexposure	1	10.6	0.0001	4.37	0.03	10.1	0.002	0.41	0.52	26.1	0.0001
Netting: treatment	2	1.66	0.16	0.57	0.57	2.56	0.08	1.02	0.36	0.84	0.43
Netting: postexposure	1	2.04	0.09	1.98	0.16	0.11	0.74	3.75	0.06	3.64	0.06
Treatment: postexposure	2	2.42	0.05	0.01	0.99	3.37	0.04	1.19	0.31	1.52	0.22
Netting: treatment: postexposure	2	1.88	0.12	1.10	0.33	0.04	0.96	0.35	0.71	2.21	0.11
Residuals	159										
Larval *Trogoderma inclusum*											
Treatment	2	9.50	0.0001	2.04	0.13	12.0	0.0001	4.16	0.02	8.53	0.001
Netting	1	9.78	0.0001	1.20	0.27	17.0	0.0001	0.73	0.40	22.4	0.0001
Postexposure	1	16.7	0.0001	1.14	0.29	23.9	0.0001	4.93	0.03	40.2	0.0001
Treatment: netting	2	1.09	0.36	0.52	0.59	1.22	0.30	0.41	0.66	0.96	0.39
Treatment: postexposure	2	6.00	0.001	2.41	0.09	8.48	0.001	0.65	0.53	1.92	0.15
Netting: postexposure	1	1.55	0.19	0.01	0.91	5.62	0.02	0.39	0.53	0.17	0.68
Treatment: netting: postexposure	2	2.04	0.09	0.57	0.56	3.71	0.03	0.09	0.92	0.50	0.61
Residuals	155										

Table 6. Summary of statistical model results for the release-recapture assay using larval dermestids after exposure to 0.4% deltamethrin incorporated long-lasting insecticide netting in the presence of important semiochemicals, including a negative control, food, and *Trogoderma* spp. sex pheromone, over a 24-h period under constant conditions at the Center for Grain and Animal Health Research in Manhattan, KS

		MANOVA			Sequential ANOVAs							
					Stimulus half		Underneath trap		Captured in trap		Nonstimulus half	
Variable	df	Denominator degrees of freedom (den) df	Approximate *F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Trogoderma variabile												
Semiochemical	2	64	3.81	0.01	0.84	0.44	3.59	0.03	3.7	0.03	1.2	0.30
Netting	1	63	1.16	0.34	0.90	0.35	2.81	0.10	0.5	0.47	0.9	0.35
Semiochemical: netting	2	64	3.55	0.02	0.67	0.52	6.25	0.01	0.9	0.42	2	0.15
Residuals	66											
Trogoderma inclusum												
Semiochemical	2	64	3.10	0.02	3.65	0.03	2.27	0.11	0.8	0.47	3.70	0.03
Netting	1	63	42.1	0.0001	15.6	0.001	53.6	0.0001	18	0.0001	168	0.0001
Semiochemical: netting	2	64	1.91	0.12	1.15	0.32	2.78	0.07	0.9	0.42	0.3	0.71
Residuals	66											

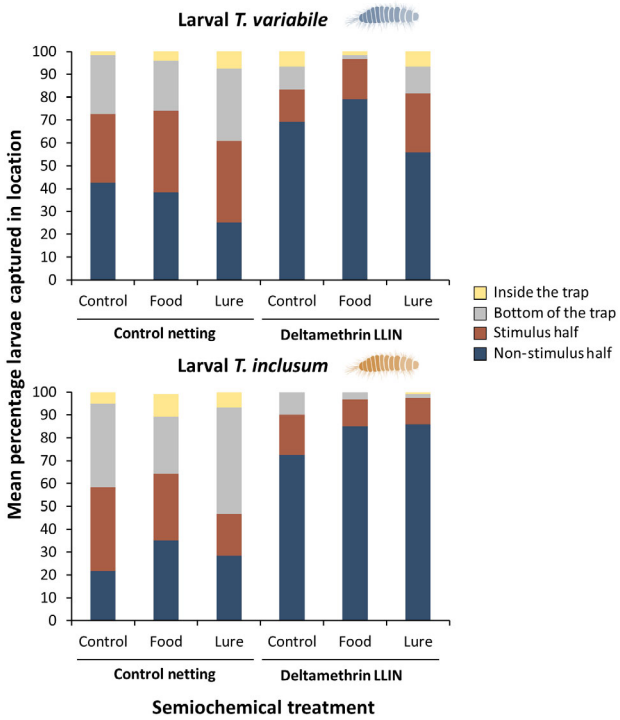

Figure 4. Mean percentage of larval *Trogoderma variabile* (top panel) and larval *Trogoderma inclusum* (bottom panel) recaptured inside the trap (yellow), underneath the trap (gray), on the stimulus half of the container (red), or the nonstimulus half of the container (blue) after 1 min of exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting insecticide netting (LLIN) in a laboratory release-recapture assay under constant conditions in the presence of important semiochemicals including food (e.g., 0.01 g of wheat flour), *Trogoderma* spp. pheromone (e.g., (*Z*)-14-methyl-8-hexadecenal) or a negative control without stimuli after 24 h.

were 6–7-fold more larvae underneath in the control and pheromone treatment, respectively, compared to the traps with food after exposure to LLIN (Fig. 4).

3.4 Trogoderma inclusum movement

The main effects of semiochemical treatment, exposure to netting, and postexposure holding duration significantly affected the distance moved by immature *T. inclusum* (Table 1). The two-way interactions among the main effects also significantly affected the distance moved by *T. inclusum* larvae. The distance moved by immature *T. inclusum* was significantly reduced by 2-fold after 24-h exposure to LLIN compared to the control netting (Fig. 5). We found similar results to the velocity moved by *T. inclusum*.

3.5 Behavior of *T. inclusum*

3.5.1 Frequency of entering each zone

For *T. inclusum* larvae, the frequency of entering each zone was significantly affected by the semiochemical present and postexposure holding duration (Table 3). In addition, the three-way interaction among all the main factors, including semiochemical treatment, exposure to netting, and postexposure holding duration, significantly affected the frequency of entering each zone (Table 3). The frequency of entering the treatment stimulus zone and each treatment half of the arena was increased by 1.4-fold and reduced by 26%, respectively, compared to the control without stimuli. The postexposure holding duration resulted in 1.6–2.6-fold more entries in the control stimulus zone and control half of the arena, respectively, by *T. inclusum* larvae at 1 min compared to 24 h (Figs 6 and 7). However, at 24 h after exposure to LLIN, there was a 57% decrease in the frequency of entries by *T. inclusum* larvae into the treatment stimulus zone in the presence of pheromone compared to the unbaited control, while after exposure to control netting, the frequency of entries increased by 2.9-fold in the presence of pheromone compared to the unbaited control (Fig. 6). One minute after exposure to control netting, the frequency of entering the treatment half of the arena by *T. inclusum* larvae was reduced by 48–54% in the presence of the pheromone and food, respectively, compared

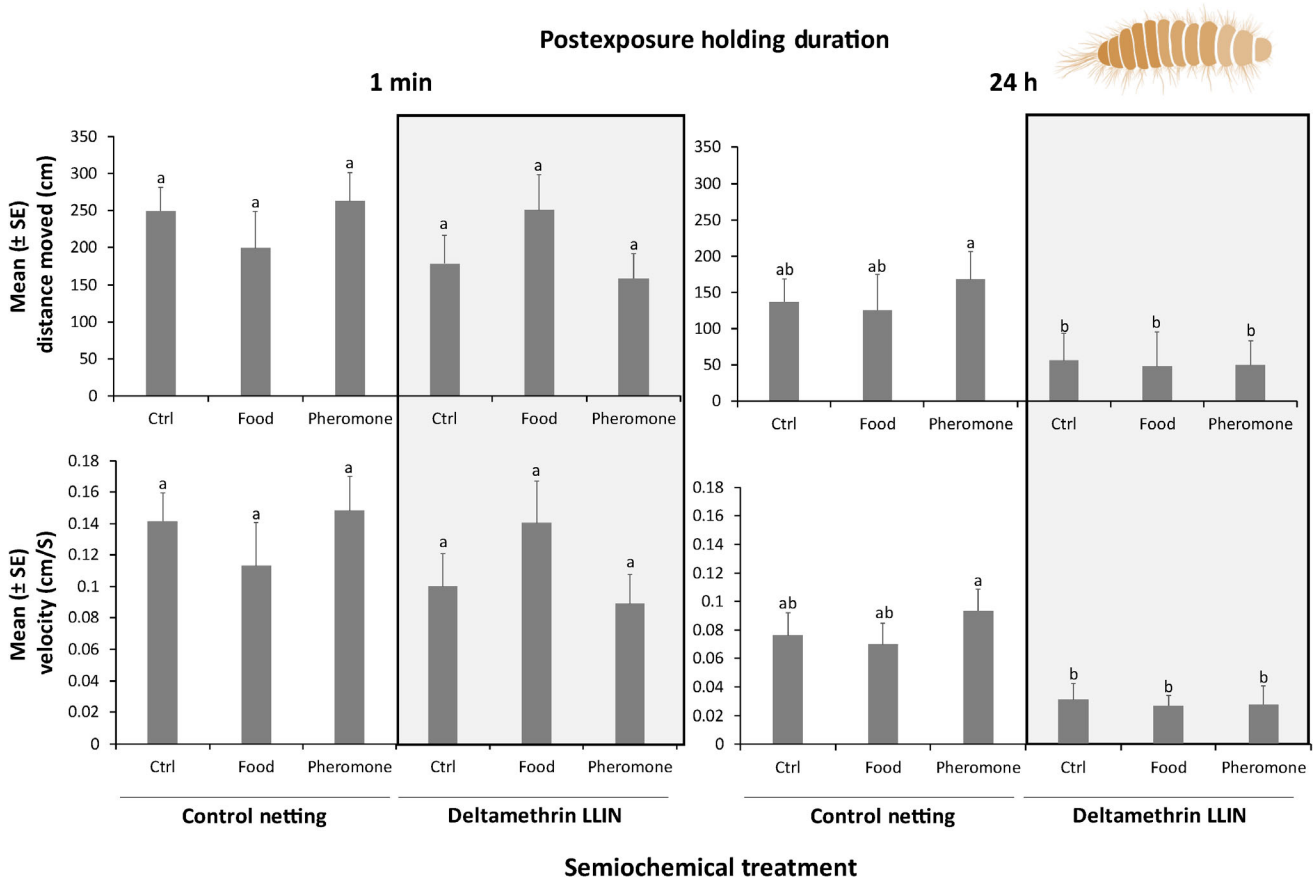

Figure 5. Distance moved (±SE, cm) (top panels) or instantaneous velocity (±SE, cm/s) (bottom panels) by larval *Trogoderma inclusum* after 1 min of exposure to control netting without insecticide or to 0.4% deltamethrin-incorporated long-lasting insecticide netting (LLIN) in the presence of food (0.01 g of wheat flour), *Trogoderma* spp. pheromone (e.g., (*Z*)-14-methyl-8-hexadecenal) or a negative control without stimuli during a 30-min movement assay immediately after exposure (left column) or 24 h later (right column). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). The shaded gray area represents LLIN-exposed treatments. Ctrl, unbaited control.

to the unbaited control, whereas larvae exposed to LLIN increased their frequency of entering the treatment half of the arena by 1.3-fold when exposed to food cues or reduced their frequency of entries by 22% in the presence of pheromone compared to the unbaited control (Fig. 7).

3.5.2 *Cumulative duration spent in each zone*

For *T. inclusum* larvae, the cumulative duration spent in the stimulus zone or half zone was significantly affected by all main effects in the model (MANOVA; Table 4). However, none of the interactions except treatment by netting significantly affected the cumulative duration spent in each zone (Table 4). Larval *T. inclusum* spent 1.4–1.5-fold more time in the control stimulus zone when the arena had food or pheromone, respectively, compared to when left unbaited (Fig. 6). Furthermore, exposure to the netting affected the cumulative duration spent in the control and treatment halves of the arena as well as the control stimulus zone. There was an increase by 4-fold in the cumulative duration spent in the control stimulus zone with pheromone immediately after exposure to LLIN compared to control netting (Fig. 6). After 24 h, there was an increase in the cumulative duration spent in the control stimulus zones in the absence of any stimuli after exposure to LLIN compared to control netting (Fig. 6). Furthermore, for *T. inclusum* larvae, 24 h after exposure to LLIN, the cumulative duration spent in the treatment stimulus zone containing food stimuli was decreased by 88% while the cumulative duration spent in the control stimulus zone containing food stimuli was increased by 5-fold, when exposed to LLIN compared to the control netting (Fig. 6). Finally, the cumulative duration spent by larval *T. inclusum* in the control halves of the arenas after exposure to LLIN was 1.7–3.5-fold greater than the treatment halves of the arenas, whereas there was no significant difference in the control (Fig. 7).

3.5.3 *Latency treatment, control stimulus, and half zone*

All the main effects, including semiochemical treatment, exposure to netting, and postexposure exposure holding duration, significantly affected the latency to finding each zone by larval populations of *T. inclusum* (Table 5). Furthermore, the two-way interaction between semiochemical treatment and postexposure holding duration affected the latency to finding each zone by *T. inclusum* larvae. At 1 min after control netting exposure, there was a 27-fold greater latency to the treatment half of the arena compared to the control half of the arena by larval *T. inclusum* when food was present (Fig. 7), but this was no longer statistically significant after exposure to LLIN. Furthermore, after 24 h, *T. inclusum* larvae experienced increased latency by 9-fold to finding the stimulus zone when it had pheromone after exposure to LLIN compared to control stimulus zones (Fig. 7).

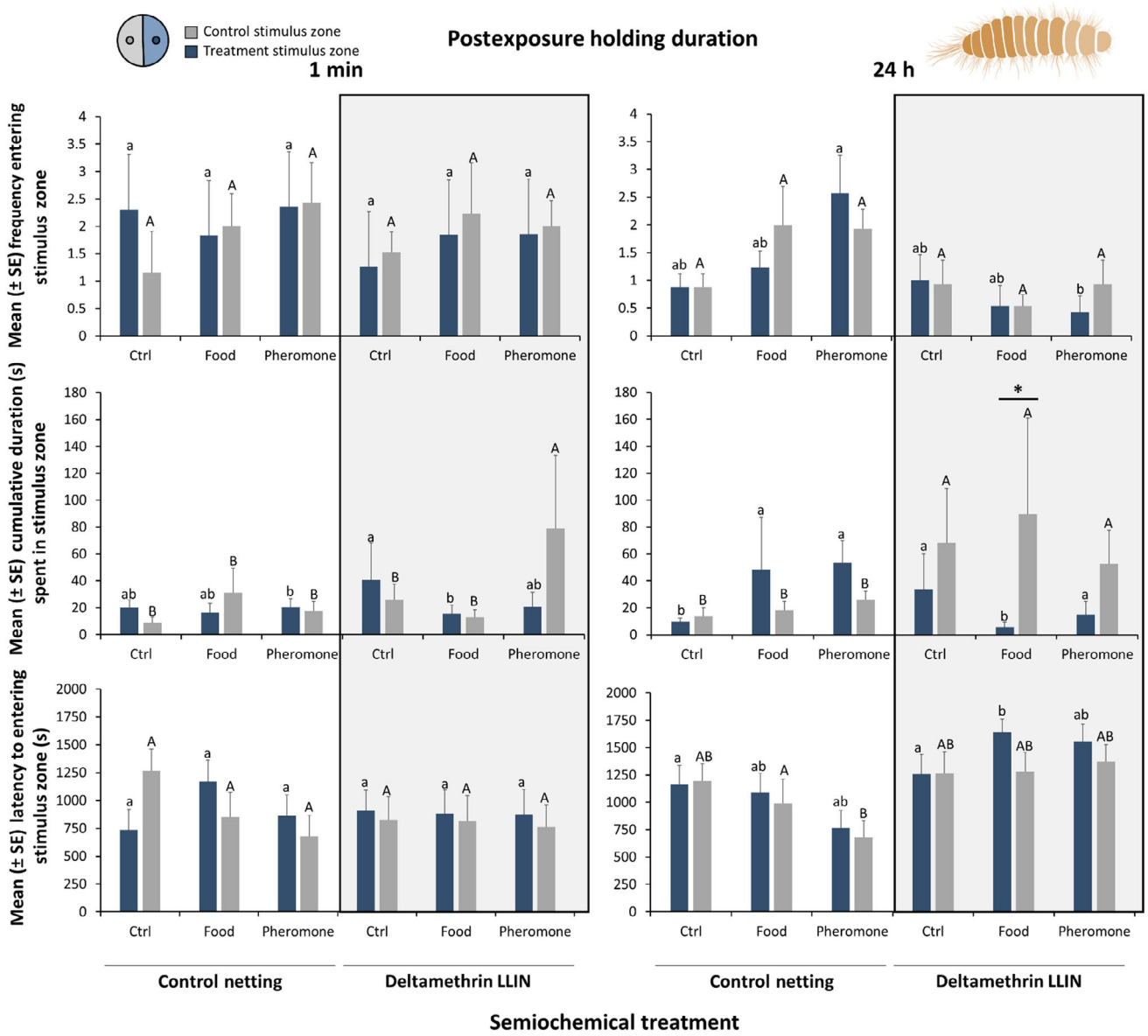

Figure 6. Frequency of entering (±SE) (top panels), cumulative duration spent in (±SE) (middle panels) or latency to finding (bottom panels) the treatment stimulus zone (blue bars) or control stimulus zone (gray bars) by larval *Trogoderma inclusum* after 1 min of exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting insecticide netting (LLIN) in the presence of important semiochemicals including food (e.g., 0.01 g of wheat flour), *Trogoderma* spp. pheromone (e.g., (*Z*)-14-methyl-8-hexadecenal) or a negative control without stimuli during a 30-min movement assay immediately after exposure (left column) or 24 h later (right column). Capitalized letters represent multiple comparisons among treatments within the control stimulus zones, while lower case letters represent multiple comparisons among treatments in the treatment stimulus zones within a given period for a specific response variable. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). *Post hoc* comparisons have been added between treatment and control stimulus zones where there was a significant difference in response with asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$, Student's *t* test). The shaded gray area represents LLIN-exposed treatments. Ctrl, unbaited control.

3.6 Release and recapture of immatures *T. inclusum*

A total of 3.8% larval *T. inclusum* were recaptured in traps, indicating that reasonable interpretation of the data is possible. There were 2-fold more *T. inclusum* larvae captured in traps with food compared to those left unbaited (MANOVA; Table 6 and Fig. 4). There was a 96% decrease in the capture of *T. inclusum* larvae in traps after exposure to LLIN compared to control netting (Fig. 4). Exposure to LLIN led to decreased movement and significantly reduced capture of larval *T. inclusum* by all traps compared to control netting (Fig. S1). There were 2.9-fold more *T. inclusum* larvae on the nonstimulus half of the arenas after exposure to LLIN compared to control netting, while there was a 51% reduction of larvae on the stimulus half of the release arena (Fig. 4). There were 7.2-fold more larvae underneath the trap after exposure to control netting compared to after exposure to LLIN.

4 DISCUSSION

In this study, we have built on prior work dealing with how exposure to LLIN affects olfaction by dermestids. Given the complex study design and large number of tests, we have included a synthesis figure to help guide the reader (Fig. 8). In particular, here

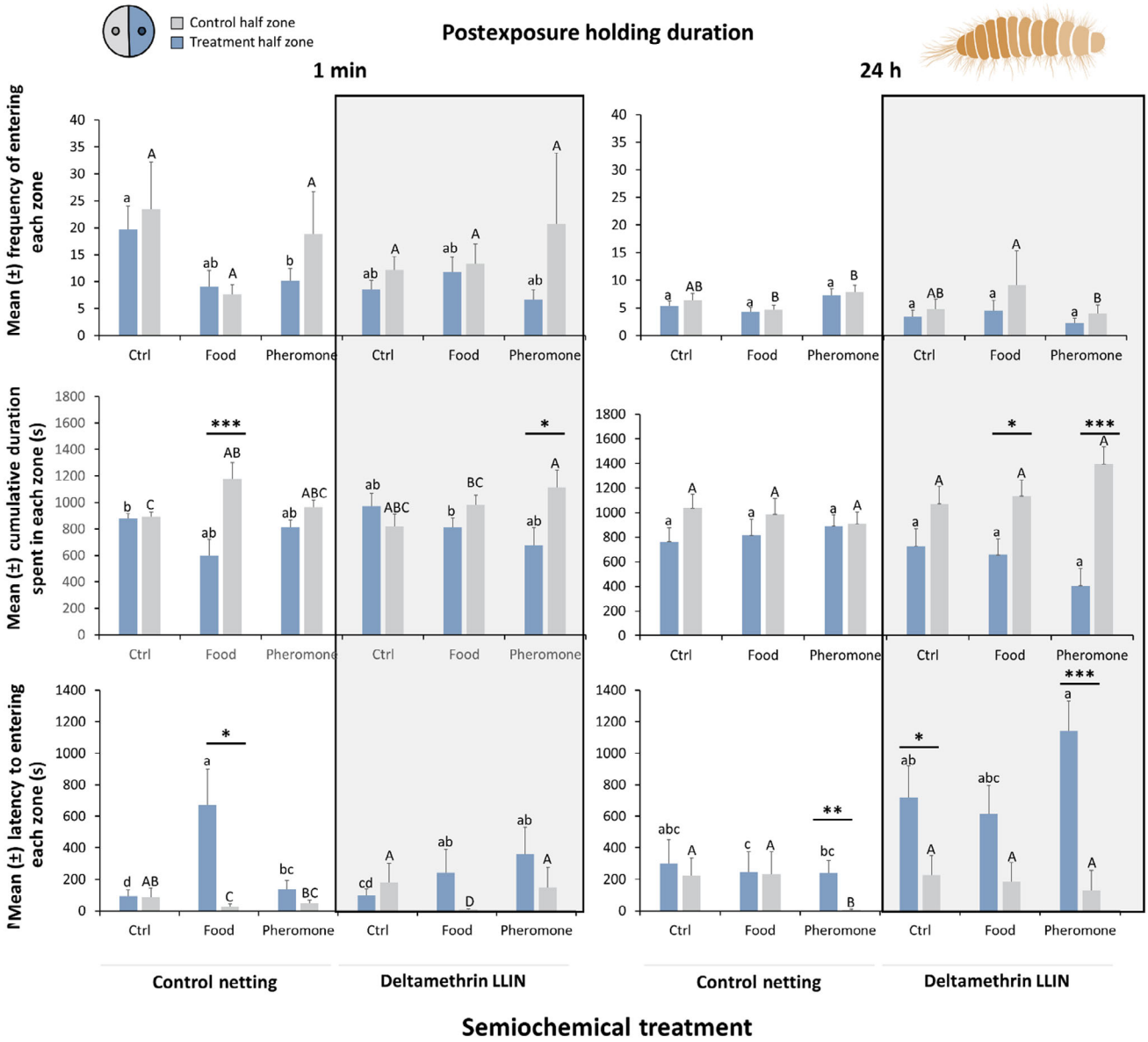

Figure 7. Frequency of entering (±SE) (top panels), cumulative duration spent in (±SE) (middle panels) or latency to finding (bottom panels) the treatment half (light blue bars) or control half (light gray bars) of the Petri dish by larval *Trogoderma inclusum* after 1 min of exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting insecticide netting (LLIN) in the presence of important semiochemicals including food (e.g., 0.01 g of wheat flour), *Trogoderma* spp. pheromone (e.g., (*Z*)-14-methyl-8-hexadecenal) or a negative control without stimuli during a 30-min movement assay immediately after exposure (left column) or 24 h later (right column). Capitalized letters represent multiple comparisons among treatments within the control half of the arena, while lower case letters represent multiple comparisons among treatments in the treatment half of the arena within a given period for a specific response variable. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). *Post hoc* comparisons have been added between treatment and control halves of the arenas where there was a significant difference in response with asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$, Student's *t* test). The shaded gray area represents LLIN-exposed treatments. Ctrl, unbaited control.

we have evaluated the response of dermestid larvae to LLIN for the first time in the presence of semiochemical stimuli such as food or pheromones. We found a delayed effect of brief exposures to LLIN on *T. variabile* and *T. inclusum* larvae, but *T. inclusum* was relatively less affected. This is despite the fact that control larval *T. variabile* and *T. inclusum* moved 175–250 cm in just 30 min periods, translating to 84–120 m in a 24-h period if the same rate holds. Thus, regardless of the apparent importance of crawling to translocate for larval *Trogoderma* spp. in this study, *T. variabile* and to a lesser extent *T. inclusum* were affected by LLIN. Prior work with residual deltamethrin on concrete in the laboratory at rates of 8–24 mg deltamethrin per m^2 when provisioned with food were found to be more effective at inducing mortality against adults of *T. variabile* and *T. inclusum* than larvae.[24] Likewise, other work has generally found dermestid larvae, including *T. variabile*, were much more resistant to changes in mobility after brief exposures of deltamethrin-incorporated LLIN compared to adults, resulting in decreased efficacy,[7] but up to this point, little research has evaluated how exposure may affect movement in response to olfactory cues present in the environment.

In the current study, larval *T. variabile* moved the least in the presence of pheromones after 1 min of LLIN exposure but

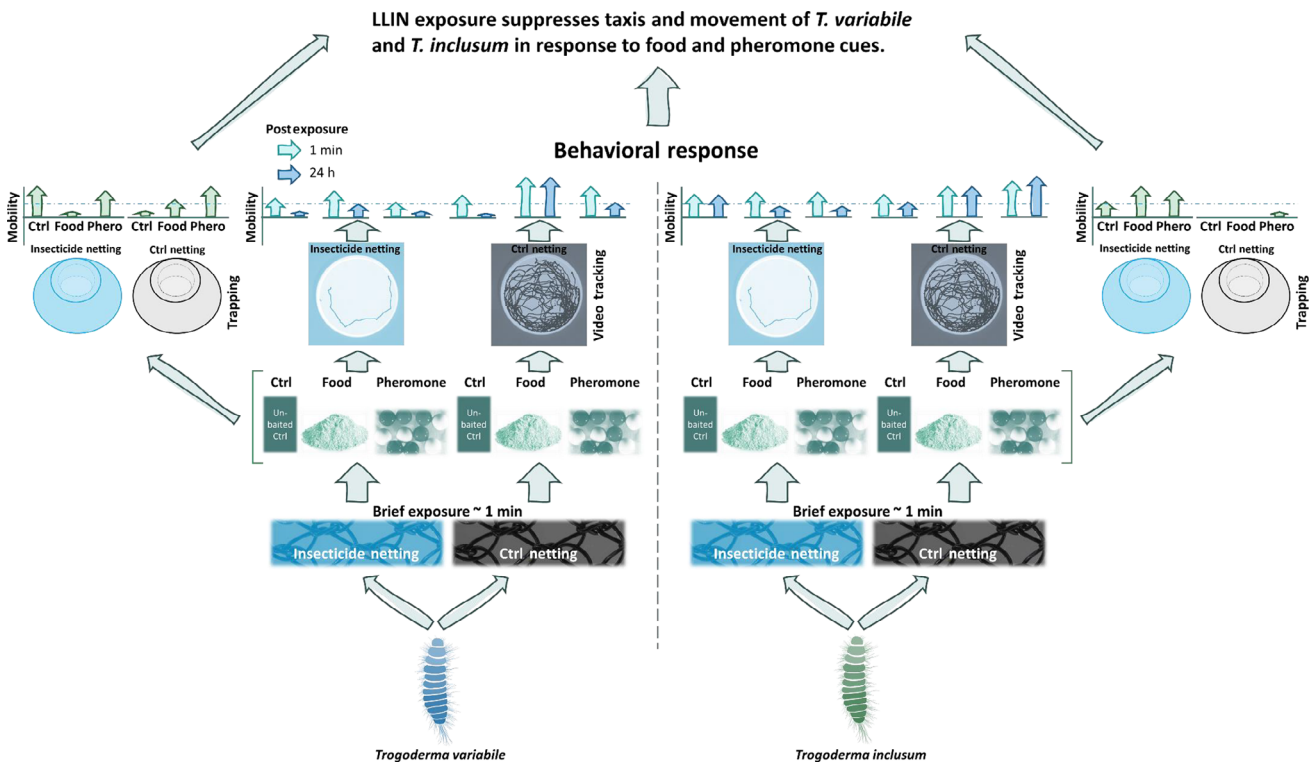

Figure 8. Synthesis schematic of experimental design, materials and methods, main results, and take-home conclusions from manuscript testing larval *Trogoderma variabile* and *Trogoderma inclusum* after exposure to control netting or long-lasting insecticide netting for 1 min then assessing movement using Ethovision or recapture in commercial pitfall traps in the presence of no stimuli, food cues, or pheromones for *Trogoderma* spp. Ctrl, unbaited control.

the most with pheromones after 24 h compared to the respective controls (e.g., no stimuli), but in both periods, after exposure to control netting movement was greatest when food was present. Likewise, the efficacy of lures in traps was altered after exposure to LLIN by larval *T. variabile*, with a substantial decrease in the efficacy of the lure when food was used as a bait compared to unbaited traps, which were more effective. Overall, this suggests that the normal olfactory processes are changing after exposure to LLIN. Based on the patterns with distance moved by larval *T. variabile*, it is possible different underlying molecular mechanisms are taking place for (1) 1-min and 24-h control netting exposure, (2) 1-min LLIN exposure, and (3) 24-h LLIN exposure. These individuals each show qualitatively different patterns of response. For example, Ming *et al.* (unpublished) showed that a combination of nutrition and physical absorption allowed *T. castaneum* to detoxify the deltamethrin in LLIN. Other work found non-netting exposed *T. variabile* larvae also had sometimes fluctuating behavioral responses to pheromone and food cues in two-choice tests and arrestment studies.[23] Thus, exposure to LLIN appears to interfere with normal olfactory processes by *T. variabile*.

By contrast, altering the olfaction of larval *T. inclusum* after LLIN exposure manifested differently than that of *T. variabile*. Here, exposure to LLIN resulted in less time spent with food and pheromones, and a longer latency to finding those cues that may be indicative of resources (e.g., mates, food, habitat) by *T. inclusum* larvae compared to controls. In addition, effects seemed to be significantly delayed, and only appear at 24 h, not at 1 min after exposure. Together, this suggests that larval *T. inclusum* is more robust to brief exposures to LLIN than *T. variabile*, although its olfactory processes do eventually become altered, albeit in more nuanced ways. Unlike the diagnostic mobility patterns found in *T. variabile*, we observed a general suppression in the distance moved, but only after 24 h for LLIN-exposed *T. inclusum* larvae. In a companion study with adult *T. variabile* and *T. inclusum*, Ranabhat *et al.* (unpublished) found that the olfaction of adult *T. inclusum* was more affected than that of *T. variabile*. Here, we seem to have documented the reverse pattern with larvae, with *T. variabile* larvae more affected than *T. inclusum* when orienting to semiochemicals after exposure to LLIN.

In addition, the frequency of entries into the stimulus zones when pheromone was present by larval *T. inclusum* after exposure to LLIN was significantly reduced compared to control netting. While part of this definitely may be from reduced movement, it also suggests that exposure to LLIN interferes with normal olfactory orientation by larval *T. inclusum*. Prior work has found that under normal circumstances *Trogoderma* spp. are more likely to move upwind when there is an odor source that contains both sex pheromone and food kairomones.[21] In addition, exposure to cuticular extracts from conspecifics resulted in aversion by *T. inclusum*, likely due to the presence of oleic acid.[29] Presence of oleic acid likely was not an issue in the present study because it is usually a marker of death, and we used only alive or affected individuals. While it is possible other abiotic factors affected responsiveness to pheromonal and food cues, all treatments were represented equally between 9:00 and 19:00 under constant conditions (27.5 ± 0.03 °C, 65 ± 3% RH), which corresponds to peak activity for *T. inclusum*. In addition, all larvae were kept under similar prior holding conditions, then exposed uniformly. The fact that there were a lower number of entries into the stimulus zones with pheromones after exposure to LLIN implies that *T. inclusum* larvae cannot easily locate pheromone sources after exposure to LLIN, a fact born out by the results of the latency to finding the treatment zone. This may yield many

benefits to food facilities trying to protect their commodities and exclude insects from immigrating to new areas.

In the trapping assay, larval *T. inclusum* were not able to locate traps effectively after LLIN exposure. While some *T. inclusum* showed movement away from the release point after 24 h, few made it to a trap, and even fewer made it inside a trap. This suggests that normal olfactory processes may be interrupted. This was despite using the commercially available and widespread Dome® trap, which was shown to be the most sensitive trap among competing traps for stored product insects in Greece.[30] Nonetheless, the control captures are in line with prior work on trapping *T. variabile* and other dermestids using a variety of trap designs and lures, even in the laboratory and in confined spaces.[31] In that study, they found between 0% and 40% recapture in traps. It is likely there was relatively low capture in our controls as a result of disaggregating one company's lure and using with another's trap. Generally, companies design their traps as comprehensive trapping systems that are more effective when used as designed.[30] Prior work has found that *Trogoderma* can be the predominate species in a food facility, with larval stages showing movement and present in high traffic areas such as on conveyors of a processing line.[6] In sampling at 15 Spanish food facilities over 2 years, a prior study captured 4418 *T. inclusum*, including a few larvae in traps.[31] In the USA, out of seven dried distillers grains facilities, heavy *T. variabile* infestations were found at all of them and heavy infestations of *T. inclusum* were found at five.[32] These studies suggest that this work on evaluating trapping is relevant given the food security risk *T. inclusum* poses.

We found that exposure to deltamethrin-based LLIN disrupted response to food and sex pheromone. Deltamethrin is among the chemical class of type II pyrethroids, which are a newer and less studied chemistry in stored product insects.[33] Deltamethrin is a contact insecticide that generally interferes with the production and propagation of nerve signals because it prevents the proper functioning of the activation gate for the sodium ion channel.[34] As most chemosensation happens through nerve-mediated signals from the antennae in insects, and most larval dermestidae, including *Trogoderma* spp. have antennae and sensoria,[35] it makes sense that response to these semiochemicals would be suppressed after contact with deltamethrin LLIN. Ultimately, this may make LLIN more effective because it signifies that after contact, stored product insects are not likely to be able to find food sources or mates after contact at food facilities. This will be especially true if movements are less coordinated, which has generally been documented after exposure to deltamethrin LLIN.[7,15] Interestingly, even though an insecticide was employed that can interfere with nerve signals, there was still generally robust movement by larvae, hovering around 47–250 cm in a half an hour period by *T. variabile* and *T. inculsum*. This was likely facilitated by some morphological features of the larvae that included dense and abundant setae over much of their body that may allow for minimal contact with LLIN. Future work should investigate how stored product insects may detoxify deltamethrin after contact with LLIN.

While we only tested food and the sex pheromone in this assay, it is possible the inclusion of other types of cues could have resulted in similar or different outcomes in behavior by these two species of *Trogoderma*. For example, necromones are another class of signal, which includes oleic acid. Oleic acid was shown to be repellent to *T. inclusum* and *T. variabile* at high concentrations, but it was attractive at low concentrations to *T. variabile*.[29] While necromones did not reduce attraction to traps with conspecifics, they did not hinder attraction and may enhance it in some cases by *T. variabile* and *T. inclusum*.[36] In other situations, necromones may have little effect on the stability of the volatile emissions from traps.[37] It is unknown whether LLIN exposure would affect the behavioral response of *Trogoderma* spp. to these necromones. Other cues include microbial volatiles (reviewed in Ponce *et al.*),[38] to which stored product insects may have a conserved response,[39] and interspecific insect cues (e.g., Athanassiou *et al.*[40] Quellhorst *et al.*[41]). It would be interesting and useful to evaluate how exposure to LLIN affects orientation to these other cues.

There is an increasing acknowledgement that understanding the behavioral and community ecology is important for implementing IPM tactics.[42] Indeed, Quellhorst *et al.*[41] found that a more realistic behavioral test was required to help provide a comprehensive picture of efficacy of a novel formulation of an insecticide compared to an older formulation. There has not been a lot of research at the intersection of insecticide efficacy and chemical ecology, especially after harvest with stored product insects. Our study contributes to this intersection and supports the role for LLIN exposure changing basic olfactory and movement processes in these two species of dermestids. This has implications for food facilities that include likely delayed ability to reach and find commodities, and potential conspecifics. This may increase commodity protection but may have a side effect of interrupting monitoring programs. However, the effect on monitoring programs may be minimal if individual insects eventually succumb to LLIN exposure. Overall, this study increases our understanding of how effective LLIN is as a tool after harvest, and brings a greater appreciation of how it may affect key underlying fundamental biological processes apart from mortality. Follow-up work should understand whether these findings translate to pilot-scale warehouses and commercial food facilities.

ACKNOWLEDGEMENTS

We would like to acknowledge the support of the Morrison Lab (USDA). This work was funded by USDA NIFA Crop Protection and Pest Management Grant #2020-70006-33000. The use of trade names is for the purposes of providing scientific information only and does not constitute an endorsement by the United States Department of Agriculture, Kansas State University (KSU), and University of Nebraska-Lincoln (UNL). The USDA, KSU, and UNL are equal-opportunity employers. This manuscript is contribution No. 23-281-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS, USA.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Ag Data Commons at https://doi.org/10.15482/USDA.ADC/1529176, reference number 1529176.

SUPPORTING INFORMATION

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

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