

Integrating a novel tactic, long-lasting insecticide-incorporated netting into integrated pest management programs for food facilities

by

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Abstract

Stored products play a significant role in both the economy and global food security. However, as stored products proceed through the post-harvest supply chain, stored product insects readily attack them, resulting in reduced quality and quantity of food. Pest management after harvest has relied heavily on fumigation to combat infestations. However, fumigant options have become limited due to increasing insect resistance to phosphine, and regulatory restrictions on the use of methyl bromide in most settings due to its harmful impact on the ozone layer. Further, there is increasing consumer demand for products with minimal or no insecticide usage throughout the supply chain after harvest. Thus, there has been a push to diversify integrated pest management programs (IPM) after harvest. One way to intercept insects from the landscape is by using long-lasting insecticide-incorporated netting (LLIN). Prior work has found that LLIN causes lethal and sublethal effects in multiple stored product insects. Nonetheless, there is a need by stakeholders to understand whether LLIN can support phosphine resistance management programs. In addition, it is important to understand how to integrate LLIN into comprehensive IPM programs with existing tactics at food facilities, such as fumigation and residual insecticide usage. Further, it is crucial to know whether LLIN deployment can affect the long-term dynamics of stored product insects at food facilities. My project aims to expand the use of LLIN in the post-harvest supply chain by addressing these concerns directly. Using video-tracking and Ethovision software, I have found exposure to deltamethrin LLIN for 0.5, 2, or 60 min resulted in substantial and equally adverse mobility reductions for phosphine-resistant and phosphine-susceptible strains of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae). In addition, when assessing deltamethrin, permethrin, indoxacarb, and dinotefuran on surrogate netting at 1%, most active ingredients (a.i.)

were found to suitably induce mortality in both strains of *T. castaneum* and *R. dominica*. I also assessed the movement of larval and adult sexed dermestids (*Trogoderma variabile* Baillon [Coleoptera: Dermestidae] and *T. inclusum* Le Conte) in the presence of food kairomones and pheromones ((Z)-14-methyl-8-hexadecenal) after exposure to LLIN or control netting. Generally, the olfaction and orientation of larval dermestids were affected after exposure to LLIN compared to control netting. Overall, out of 40 possible olfactory and taxis patterns which could be disrupted, exposure to LLIN interrupted 68–73% of these in adult *T. inclusum* and *T. variabile*, respectively, compared to control netting-exposed individuals. Our study suggests the use of LLIN may enhance the effectiveness of other concurrent behaviorally-based strategies. Further, in a field bulk storage experiment over two years, data demonstrated that LLIN can reduce the number of fumigations required by 68–91% while reducing insect dispersal and progeny production by 83–99% and 89–99%, respectively. This consistently shows that LLIN use can enhance fumigation by reducing reliance on it and making the remaining fumigations more effective. Further, our data showed there is a marginal boost of combining LLIN with two common residual insecticides, CentanylTM EC (a.i. deltamethrin), and Evergreen[®] (a.i. natural pyrethrins), translating to 10–20% fewer *T. castaneum* progeny produced. Finally, I modeled the stage-structured dynamics of *T. castaneum* at food facilities with and without two types of LLIN and showed that deploying LLIN may contribute to the local extirpation of *T. castaneum* within as few as 15 generations. Therefore, LLIN (whether deltamethrin or alpha-cypermethrin) shows promise as a new way to combat stored product pests in and around food facilities, while diversifying pest management programs, and is compatible with existing tactics. My research should help to speed the adoption of LLIN by stakeholders at food facilities while helping increase the sustainability of IPM after harvest.

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Dedication

To my mother and father

Shanta Ranabhat & Hari Charan Ranabhat

Chapter 1 - Efficacy of long-lasting insecticide-incorporated netting in controlling preharvest and postharvest pest insects: a meta-analysis study

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Abstract

Long-lasting insecticide-incorporated netting (LLIN) is a newer tactic to intercept insect movement and reduce the damage caused by insect species in agriculture. LLIN can be integrated with other diverse management tactics in pre- and postharvest settings, but no study has yet looked at the combined patterns across all undertaken studies to determine efficacy. Thus, we have carried out a meta-analysis on the direct and sublethal efficacy of LLIN, using literature published between 1990–2024 in agriculture. Additional criteria for inclusion included that the netting must contain an active ingredient and proper control. This left us with a total of 44 peer-reviewed publications with 285 data points on the efficacy of LLIN in agriculture. LLIN exposure was 8.6-fold and 257-fold more effective in causing direct mortality and sublethal effects in the laboratory, respectively, compared to the field. However, broad generalization is limited because of the low sample size for field studies. Direct mortality was equally observed after exposure in preharvest and postharvest systems, but sublethal effects were 4.9-fold in postharvest environments. Deltamethrin and alpha-cypermethrin-incorporated netting inflict similar direct mortality across taxa in both pre- and postharvest settings. LLIN exposure has shown direct efficacy against 19 out of 23 tested species and has induced sublethal effects in 10 out of 16 tested species. Overall, our study synthesizes the current knowledge on a promising new technology for agriculture, we highlight knowledge gaps, including a lack of field-relevant data, and finally suggest areas for further research in this field considering the entire production chain.

Keywords: LLIN, stored products, vegetables, horticulture, integrated pest management

Introduction

Pest infestation is a significant threat to global food security, with global preharvest losses of up to 35% (Pimentel and Pimentel 2019). This simply includes the period from when crops are planted, through growth, and until harvest. In addition, postharvest losses resulting from infestations by insect species in stored cereals are generally estimated to be around 20–30% worldwide (Tadesse 2020). However, these losses can exceed 50% in developing countries, as loss depends on the crop, climate of the area, storage techniques, and control strategies employed (Boxall 2001; Abass et al. 2014; Hagstrum & Phillips 2017; Bezabih et al. 2022; Berhe et al. 2023). There are various management tactics such as physical control (packaging, silos and other structures, sanitation), cultural control (cover crops and genetically resistant plants), biological control (predators, parasitoids, and pathogens), chemical control (synthetic or naturally derived), and human factors (restrictions on commodities, quarantines, etc.) (Kumar 2015; Vincent et al. 2003; Vincent et al. 2009). Traditional chemical control methodologies have often proven inadequate in preventing economic losses both before and after harvest. With the emergence of pesticide resistance to multiple active ingredients and formulations in a variety of systems, including pests in horticultural crops (Horowitz et al. 2020; Tabashnik et al. 2023) and stored product insects, both for contact insecticides (Daglish et al. 2013; Sehgal & Subramanyam 2014; Daglish & Nayak, 2018) and fumigants, especially phosphine (Agrafioti et al. 2019; Nayak et al. 2020; Machuca-Mesa et al. 2023), the significant impact of pests on global food security necessitates a continuous and dynamic innovation of pest management strategies. Ideally, integrated pest management (IPM) should rely on diverse tactics that focus on the utilization of -more than one active ingredient (or mode of action) to manage the pest below the economic injury level. However, in reality, the primary approaches for controlling the pest have focused predominantly on contact insecticides and fumigation (Metcalf 1989; Fields & White 2002; Kuhar & Kamminga 2017).

Increasing problems, such as resistance of various insects to major insecticides, environmental concerns, and strict regulations in the registration and use of various active ingredients (AIs), drastically limit the use of chemical control measures.

In response to these challenges, researchers have explored innovative strategies to sustain and diversify IPM programs, among which the deployment of long-lasting insecticide-incorporated netting (LLIN, hereafter) emerges as a promising intervention. LLIN is a fine mesh net which is made of polyethylene or polyester material, with the incorporation of different concentrations of various AIs, such as 0.4% deltamethrin (Vestergaard Inc., Switzerland) or 0.34% alpha-cypermethrin (BASF Corp., Germany) or other AIs, such as permethrin or bifenthrin. LLINs were originally designed for the control of disease vectors such as mosquitoes in tropical regions of the world (Alonso et al. 1991; Yang et al. 2018; Barker et al. 2023). Apart from controlling mosquitoes and the spread of malaria, LLINs have been used in other areas including public health pests to control insect-borne diseases (Bermejo & Veeken 1992; Hill et al. 2006). In the past few years, LLINs have been used in monitoring traps in fruit trees and evaluated for reducing nuisance insect issues associated with home invasion (Kuhar et al. 2017; Bergh & Quinn 2018). More recently, LLIN have been studied for stored product pest management (Morrison et al. 2018; Rumbos et al. 2018; Athanassiou et al. 2019; Wilkins et al. 2020; Gerken et al. 2021a; Gerken et al. 2021b; Scheff et al. 2021, Altunç et al., 2024).

In urban and tropical settings, LLINs are resistant to multiple washes and can have an effective lifespan of up to 5 years (Guillet et al. 2001). In postharvest systems, a lifespan of 1–2 years appears more realistic (Scheff et al. 2024). The reason for decreased efficacy after harvest compared to the tropics may be that in food facilities (e.g., mills, retail stores, and some warehouses), indoor temperatures are significantly elevated from ambient outside temperatures (Gerken and Campbell 2022). According to prior literature for tropical regions

like Ghana, mean temperatures ranged from 25–28°C depending on region and year (Srivastava et al. 2017), whereas mean temperatures inside grain silos from Kansas it ranged from 23–45°C (Ranabhat et al. 2024a; Toews et al. 2006). Thus, it appears that temperatures inside food facilities are elevated both when comparing to ambient outside conditions and when comparing to the tropics.

To the authors' best knowledge, the first implementation of LLIN in preharvest agriculture was carried out to protect cabbage in Africa in the early aughts (Martin et al. 2006). In that study, the authors found that the number of all lepidopteran larvae with deltamethrin-based LLIN and foliar insecticide sprays was significantly lower than the unprotected control, but only the treatments with LLIN provided 100% protection against aphids (Martin et al. 2006). Since that time, there has been an increasing number of publications on the preharvest use of LLIN to protect a variety of commodities, especially during the last decade.

It was not until seven years after the initial preharvest implementation of LLIN that Msango and Longwe (2013) adapted alpha-cypermethrin LLINs for the protection of stored commodities. The rationale behind the utilization of LLINs lie in addressing several critical aspects of stored product pest management simultaneously. Depending on its mesh size, LLIN can serve as a chemical barrier or offer a dual mode of action as a physical barrier as well as a mean to prevent pests from reaching stored foods. Msango and Longwe (2013) reported that the utilization of alpha-cypermethrin LLIN (Carifend[®], BASF, Germany) after phosphine fumigation of tobacco showed complete protection of the product from the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) infestation for more than three months of storage. This first look in a limited setting by one species showed promise after harvest for the use of LLIN in managing stored product insects as an additional IPM strategy.

More recently, there has been a renewed interest in LLINs for stored product protection as there have been mounting pressures on industry to diversify IPM programs. Building on Msango and Longwe (2013), Rumbos et al. (2018) investigated the knockdown and mortality effect of an alpha-cypermethrin coated LLIN on *L. serricorne* and the tobacco moth, *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae). Results demonstrated high immediate knockdown effect, which also led to high delayed mortality in both species (Rumbos et al. 2018). Research on the sublethal effects of LLIN showed that a large mesh ($2 \times 2 \text{ mm}^2$), deltamethrin-based LLIN acted like a chemical barrier, preventing dispersal of stored product insects while reducing progeny production in warehouses compared to controls without netting (Morrison et al. 2018; Wilkins et al. 2020; Wilkins et al. 2021). A LLIN has been found effective against both phosphine-susceptible and resistant colonies of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Ranabhat et al. 2022). Moreover, the dispersal ability of *T. castaneum* and *R. dominica* was drastically reduced even after one minute of exposure to deltamethrin-based LLIN (D-Terrence[®], Vestergaard Inc., Switzerland) (Morrison et al. 2018).

In addition to investigating the strong potential of LLINs for stored product pest management strategies, numerous studies have been conducted covering various aspects such as field evaluations (Msango & Longwe 2013; Athanassiou et al. 2019; Wilkins et al. 2021), sublethal effects (Wilkins et al. 2020; Domingue et al. 2021, 2022; Gerken et al. 2021a; Scheff et al. 2021; Ranabhat et al. 2022, 2023), different coating materials (Agrafioti et al. 2020), scenarios involving multiple exposures (Gerken et al. 2021b), coating the nets with different concentrations of insecticide (Athanassiou et al. 2023), residual efficacy (Agrafioti et al. 2021) and delayed effects (Agrafioti et al. 2023) on a wide range of insect species. All the

above studies illustrate a LLIN-based approach can be reliable in many pest control applications scenarios.

This review aims to provide a comprehensive analysis of existing literature on the utilization of LLINs in the management of pre- and postharvest pest insect species (excluding those in urban systems and vectors) (Figure 1.1, Figure 1.2). By synthesizing findings from a broad spectrum of studies, we aim to discern patterns relative to system and experimental design and develop a new synthesized understanding of the effectiveness of LLINs in agriculture. Moreover, our review intends to identify and address gaps in current knowledge. Through a meta-analytical approach, this review aims to distill key insights that can inform future research directions, and practical applications in the integrated management programs of pre- and postharvest insect pests.

Materials and Methods

We followed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines (Page et al. 2021) for reporting the meta-analysis, providing insights into the effectiveness of LLINs for a variety of agricultural pests, including those before and after harvest.

Data Collection and Literature Review

We used databases such as Web of Science, Scopus, and Google Scholar to find studies related to LLINs. First, we carried out a literature search on the topic of insecticide netting. We used various keyword combinations to identify a broad initial dataset, including various terms such as ‘netting’, ‘LLIN’ ‘insecticide netting’ in combination with either ‘insects’, ‘preharvest’, ‘postharvest’, and ‘agriculture’. Papers were individually checked to ensure they met criteria for inclusion in the meta-analysis, which included that 1) articles were published between 1990–2024, 2) the focus was on agricultural crops (not urban systems or vectored

diseases), 3) netting was not solely used for exclusion (e.g., in the absence of an insecticidal ingredient), and 4) included a proper negative or stakeholder standard control. At the end, this left us with a total of 44 peer-reviewed publications (23 postharvest + 22 preharvest studies, one common study for pre and postharvest insects) with 285 data points on the efficacy of LLINs in agriculture. However, an additional required criterion was that all studies needed to present means, standard errors, and samples sizes for both controls and LLIN treatment. After this additional criterion, there was a final total of 31 peer-reviewed publications (19 postharvest + 12 preharvest studies) (Figure 1.3). Additionally, potential publication bias was assessed by using funnel plots, and statistical tests were evaluated.

Data Analysis

Two primary tests are used. We followed the procedure for calculating metaanalysis statistics found in Luo et al. 2018. First, we calculated the standardized mean difference (MD) between the treatment and control in each study, and plotted with 95% confidence intervals, then inferred significant differences between factors that did not have overlapping intervals. We employed the *meta* package in R, with a call to the *metacont* function and all analysis was performed using R Software (R Core Team, 2024) with $\alpha = 0.05$. In addition, we assessed bias using the *metabias* function from the same package, which implements a test for funnel plot asymmetry based on rank correlation (Kendall's tau as described in Begg and Mazumdar, 1994), and also tests for differences in heterogeneity. Significance testing employed t- or z-scores.

Results

To date, all existing studies have focused mainly on exposure to LLINs through two main effects, namely direct lethality and/or sublethal effects. Sublethal effects include those on pest insect feeding behavior, movement, dispersal or progeny production after exposure to LLIN.

Here, we review the effects of LLINs in preharvest and postharvest setting for these two main effects.

Direct lethality of LLIN on insect pests

Small study bias was ruled out in the meta-analysis for direct mortality (τ bias = 145 ± 210 ; $z = 0.69$; $P = 0.49$; $n = 73$) (Figure 1.4 top panel). There were a total of 21 laboratory and 3 field studies in the postharvest environment, while there were 13 laboratory and 9 field studies in the pre-harvest environment. This included 8 postharvest studies and 7 preharvest studies assessing direct lethality (Figure 1.4, top panel, Table 1.1). The effect size on the right side from the vertical line represented positive lethal effects whereas on the left side represented negative lethal effects of LLIN on the species of specific studies. Importantly, with the exception of two studies, all the studies included found a uniformly and significantly higher direct mortality after exposure to LLIN compared to controls (Figure 1.4, top panel; $Q = 1.21 \times 10^5$, $df = 72$, $P < 0.0001$). The mean difference in effect size was similar for direct mortality caused by LLIN in preharvest (e.g., 30.6 ± 6.2 , mean $\pm$ CI) and postharvest (e.g., 33.0 ± 2.8) systems (Figure 1.5, top panel). The overlapping of the 95% confidence interval (CI) of the lethal effect size caused by LLIN in pre- and postharvest studies indicates that there was no significant difference in the mean effect size on the direct mortality between the settings. Exposure to LLIN was 8.6-fold more effective in causing direct mortality in the laboratory compared to field deployments but broad generalization here is limited because of low sample size for field studies (only 6 data points compared to 67 data points from the laboratory). Deltamethrin and alpha-cypermethrin-incorporated netting inflict similar direct mortality across taxa (Figure 1.7, top panel). Overall, LLIN exposure has shown direct efficacy against 19 out of 23 tested species above the controls included in treatments. A total of 13 of these species were from postharvest systems, while six were from preharvest systems (Figure 1.8, top panel).

Sublethal effects of LLIN on insect pests

A total of 17 studies (11 postharvest + 6 preharvest) were used for the quantitative analysis of the literature to determine the mean sublethal effect after exposure by LLIN (Figure 1.4, bottom panel, Table 1.2). Sublethal effects are defined as those on pest insect feeding behavior, movement, dispersal or progeny production after exposure to LLIN. We were able to rule out the effect of small study bias (τ bias = 0.54 ± 6.3 ; $t = 0.09$, $df = 63$; $P = 0.93$). For the sublethal effects, the effect size on the left side from the vertical line represented a greater effect size of LLIN because the behaviors of the exposed insects were decreasing compared to the control in their specific studies. Significant sublethal effects after exposure to LLIN were more prevalent in postharvest systems (8 out of 11 studies or in 72% of cases) than in preharvest systems (3 out of 6 studies or in 50% of cases; Figure 1.4, bottom panel). Furthermore, when we compared the overall sublethal effects of LLIN in preharvest and postharvest systems, we observed that LLIN resulted in sublethal effects that were 4.9-fold stronger in postharvest environments (Figure 1.5, bottom panel). LLIN exposure was 257-fold more effective in causing sublethal effects in the laboratory compared to field deployments but broad generalization here is limited because of low sample size for field studies ($n = 3$ in the field compared to $n = 48$ in the laboratory; Figure 1.6, bottom panel). Insecticide netting with deltamethrin incorporated caused sublethal effects that were 19-fold greater than netting with alpha-cypermethrin (Figure 1.7, bottom panel). LLIN induced sublethal effects in 10 out of 16 tested species. A total of 78% (7 out of 9) of the postharvest insects exhibited sublethal effects, while 57% (4 out of 7) of the preharvest insects showed sublethal effects.

Case studies for use of LLIN in preharvest systems

The efficacy of LLINs was investigated on a diverse range of species before harvest (Figure 1.2, top). Implementations can be successfully used in preharvest systems. For instance, Arthurs et al. (2018) reported that exposure to LLIN with 0.4 % deltamethrin (D-Terrence®)

had a significant effect on adults of the Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) reproduction on bean plants when compared with untreated (insecticide control) and no-net treatments. This study also reported that LLIN caused the mortality of almost all male and female adults of *F. occidentalis* at 2 h following a 15-min exposure ($97.9\text{--}100 \pm 0\text{--}1.3\%$). Marianelli et al. (2019) demonstrated that LLIN with deltamethrin (ZeroFly[®], Vestergaard, Switzerland) caused 100% mortality of Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) even after 5 s of exposure, while exposure to another LLIN with alpha-cypermethrin (Storanet[®], BASF, Germany) caused 89% mortality. The brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) was shown to be generally susceptible to LLIN after exposure, both to LLINs that were impregnated with deltamethrin (Kuhar et al. 2017; Krawczyk et al. 2019; Ibrahim et al. 2020) and with alpha-cypermethrin (Sabbatini Peverieri et al. 2018; Suckling et al. 2019).

In pre-harvest systems, there can be some difficulties in implementing LLIN. For example, the small mesh size may lead to undesired results as they can exclude not only the harmful insect pests but also the beneficial insects (Chouinard et al. 2017). Dáder et al. 2015 demonstrated that LLINs led to a notable decrease in the migration of the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and the cotton aphid, *Aphis gossypii* (Glover) (Hemiptera: Aphididae) without impacting the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidinae). However, field trials indicated that LLIN effectiveness diminished upon sun exposure, whereas the efficacy of both bifenthrin and deltamethrin was deemed insufficient in controlling populations of the silverleaf whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleurodidae) due to their small size (Dáder et al. 2015). Further, it can also affect the ventilation and air capacity of the enclosed area by reducing permeability which restricts the application of nets in plant production environments

such as greenhouses and other contexts where air exchange is critical (Bell & Baker 2000, Parra et al. 2004).

Case studies for use of LLIN in postharvest systems

Immediate efficacy of LLINs

Studies conducted on the effect of LLINs on stored product insect species have primarily focused on their lethal and sublethal impacts (Figure 1.2, bottom). One of the main goals of LLINs is to kill pests as soon as they come into contact with them. However, studies have shown that the effectiveness of LLINs in killing pests immediately (e.g., within 24 h of contact) varies depending on the insect species and how long they are exposed. For example, some insects such as *E. elutella* and *L. serricorne* exhibited no immediate mortality even after 24 h of exposure to LLIN with alpha-cypermethrin (Carifend®) (Rumbos et al. 2018). For the adults of *T. castaneum* and the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), at least 5 d of exposure to Carifend® were needed to cause a lethal effect (Paloukas et al. 2020). However, at the larval stage, *T. castaneum* was found to be less susceptible to Carifend®, with 37% mortality after 7 d of exposure (Galán et al. 2024). Conversely, adults of a wide range of stored product beetle species were highly susceptible, showing immediate mortality starting from just 1 d of exposure and reaching 100% with longer exposure to Carifend® (Paloukas et al. 2020).

Apart from killing insects, LLINs can also knock down insects shortly after they are exposed to toxic agent, which, apparently is a direct consequence of the exposure to pyrethroids. Initial studies showed that 24 h contact with Carifend® net caused 90% knock down in *L. serricorne* and *E. elutella* (Rumbos et al. 2018), around 80% in *P. truncatus* and *R. dominica*, but less than 9% in *S. zeamais* and the confused flour beetle *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) (Paloukas et al. 2020). Despite variations, the knockdown effect of LLINs offers a promising approach for pest control. The immediate

effect is indeed a crucial parameter as immobilized insects after exposure that remain on the treated substrate after immobilization eventually die (Athanassiou et al. 2019). Hence, in the case of Carifend[®], LLIN can be placed as vertical “curtains” that cover some layer on the ground, in order for knocked-down insects to remain in contact with the insecticide.

Along with the immediate knockdown and lethal effects, studies have explored the impact of LLINs on the behavioral characteristics of stored product pest insects, such as mobility and dispersal ability. Initial findings showed that exposure to LLINs can significantly reduce the distance moved by adults of *T. castaneum* and *R. dominica* (using D-Terrence[®] nets) by 2–4 fold immediately after up to 10 min of exposure (Morrison et al. 2018). This is particularly important, as immediate knockdown can prevent further dispersal of the affected insects within the facility. Immature insects also move less after exposure to LLINs, with movement decreases of 90% and 83% observed after 10 min of exposure in *T. castaneum* and the warehouse beetle, *Trogoderma variabile* Ballion (Coleoptera: Dermestidae), respectively (Wilkins et al. 2020).

Delayed efficacy of LLINs

Delayed efficacy of LLINs extend beyond immediate impacts, as the influence of insecticide AIs persists even after exposure. Lethal and sublethal effects continue to manifest over time, becoming more pronounced with increasing post-exposure time. For instance, despite the absence of immediate lethal impact initially, 24 h exposure to Carifend[®] caused 55% mortality in *L. serricorne*, and 100% mortality in *E. elutella* in the period of 7 d from the exposure (Rumbos et al. 2018). Similarly, delayed mortality in *P. truncatus* ranged between 95–100% depending on exposure time (2, 8, 24 h), with initial mortality rates as low as 10% (Agrafioti et al. 2023). Other pest species such as the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) and *R. dominica* also exhibited delayed mortality, reaching 72% and 98% after 7 d from exposure, respectively (Agrafioti et al. 2023). The deltamethrin-

based LLIN (D-Terrence[®]) produced a similar pattern of increasing mortality over time in the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *T. castaneum* and *R. dominica* (Scheff et al. 2021).

The immediate knockdown effect following LLIN contact can persist into the post-exposure period in three ways: first, the temporary maintenance of knockdown status, second, delayed mortality, and third, the potential recovery of initially knocked-down individuals within the post-exposure period. For example, exposure to D-Terrence[®] led to a transition from knockdown to mortality within the first 24 h for *T. castaneum* (Morrison et al. 2018). While knockdown status persisted for up to 7 d, some individuals recovered after 3 and 7 d. In contrast, *R. dominica* exhibited less recovery from knockdown during the post-exposure period (Morrison et al. 2018). A similar trend was observed in the case of Carifend[®] and *L. serricornis*, where the immediate knockdown rate decreased with increasing post-exposure duration (Rumbos et al. 2018). While the majority of knocked-down individuals succumbed to delayed mortality, few individuals recovered (Rumbos et al. 2018). However, the likelihood of delayed mortality in knocked-down individuals depended strongly on the duration of exposure, with less contact with the insecticide resulting in more recoveries during the post-exposure period (Rumbos et al. 2018; Altunç et al. 2024). The feeding ability and availability of food also influenced recovery over time (Scheff et al. 2021; Ming et al. 2024a).

In addition to delayed mortality, LLINs also affect the dispersal of exposed individuals during the post-exposure period. The mobility response of *T. variabile* larvae decreased further after 24 h from exposure, and movement speed and ability to reach new food resources for *T. variabile* and *T. castaneum* adults and larvae were negatively affected with prolonged period after contact with D-Terrence[®] (Wilkins et al. 2020). The distance moved and velocity level were also significantly decreased within the post-exposure duration of up to 7 d in *R. dominica* and *T. castaneum*, regardless of the phosphine-resistant status of the populations

tested (Ranabhat et al. 2022). This finding highlights the potential of LLINs as 'resistance-breaker' for phosphine specifically, the most common type of resistance (Afful et al. 2018) in stored product pest control.

The long in the long-lasting shield

Although LLINs are advertised to maintain their efficacy for 2-5 years, depending on the brand, there is a notable absence of studies that definitively prove this longevity concerning stored product insects. Recent data suggest that the longevity of deltamethrin-based LLIN on a south-facing wall is about a year to a year and a half (Scheff et al. 2024). The same deltamethrin netting -had no decrease in efficacy over a six-month deployment season even with food dust accumulation at a rice processing facility against *T. castaneum* and *R. dominica* (McKay et al. 2024). Finally, in a two-year field study in 110-metric tonne capacity grain bins, Ranabhat et al. (2024a) found that the use of alpha-cypermethrin netting was effective in reducing fumigation by 68-91%, while insect dispersal and progeny production were both reduced by many-fold compared to positive and negative controls. Research conducted in stored tobacco facilities demonstrated LLIN's ability to effectively control *L. serricornis* and *E. elutella* for over a year, without loss in efficacy through time (Athanassiou et al. 2019). Moreover, studies investigating the residual impact of Carifend® in laboratory settings have yielded encouraging results. Tests conducted over a period of 3 months on pests like *S. oryzae*, *T. confusum*, *O. surinamensis*, and *P. truncatus* revealed that the efficacy of Carifend® remained consistent throughout the duration of the study, mirroring its initial performance (Agrafioti et al. 2021). Similarly, D-Terrence® is able to maintain its effectiveness for over a year (Scheff et al. 2020). Thus, D-Terrence® and Carifend® have shown promising potential for long-term pest control. A field trial conducted by Dáder et al. (2015) indicated that the concentration of bifenthrin in net exposed in field conditions for 2 months during the autumn decreased from 3.8 to 3.1 g × kg⁻¹. This study mentioned that the

durability of insecticides incorporated seemed to be jeopardized after one month, indicating that nets may partially lose their effectiveness after sun exposure. Further, in the Mediterranean area because of the intense light and UV radiation, the bifenthrin content in net significantly decreased from $3.6 \text{ g} \times \text{kg}^{-1}$ at the beginning of the trial to 2.2 after one month of exposure, and further declined to 1.9 by the end of the three-month trial (Fernández et al. 2017). Consequently, as the bifenthrin content in the net decreased during the study, the population levels of both pests and natural enemies were notably increased (Fernández et al. 2017). However, the net longevity studies by Krawczyk et al. (2019) revealed high effectiveness of LLINs used for two consecutive seasons. The new nets and one-year-old D-Terrence[®] (used during the last season and stored for the winter in the cold storage) collected much higher numbers of *H. halys* adults and nymphs than the 1.2 m tall pyramid traps baited with the same number of lures (Krawczyk et al. 2019). Additionally, the net trap which was not stored during the winter, but was kept outside the whole year including summer and winter, captured a similar number of *H. halys* as pyramid traps. The higher captures of *H. halys* instars observed in the old net (2 year net) traps might be linked to elevated levels of aggregation pheromone residues. It is possible that some residues, still active, remained attached to the nets from exposure to pheromones during the previous season (Krawczyk et al. 2019). Further, deltamethrin-based LLIN exposed to outdoor conditions provided high mortality (>90%) of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) up to 12 weeks and the efficacy decreased from 14–16 weeks. Thus, the recommended replacement of the developed “attract and kill” devices consisting of LLIN, on citrus trees is every 12 weeks to control psyllid (Trujillo 2014). Regardless of the lack of comprehensive studies on the extended durability of LLINs, Carifend[®] and D-Terrence[®] have promising potential for long-lasting protection against pests. Despite the detrimental effect of certain abiotic factors in the residual efficacy of LLINs, their insecticidal effect can remain

for longer intervals than those of insecticides that are applied through regular spraying. Further research is needed in real field settings to confirm and expand upon these findings.

Standard materials and active ingredients in LLIN

The most widely used LLINs contain the AIs deltamethrin and alpha-cypermethrin (Table 1.4). A total of 84% (=37) of the studies used these two active ingredients, with alpha-cypermethrin comprising 43% (=16) of those, and deltamethrin comprising 57% (=21). The remaining nets used were experimental and consisted of propylene glycol, bifenthrin, SiO₂, and carrageen, but together comprised less than 16% of the studies. The vast majority of nets are sodium channel modulators. Mesh size for commercial nets varied from 12-100 holes/cm². One of the most widely used insecticide nettings, Carifend[®], was also the smallest mesh netting (e.g., 100 holes/cm²). The three main companies producing LLIN are Vestergaard, BASF, and Sumitomo Chemical, though there are at least four other companies also producing LLIN. In total, we found 17 products available in different parts of the world, but only 59% are used in agriculture, and of those only 41% are or have had an EPA label in the US. A total of 41% are made of polyester, while 59% are made of polyethylene, and these are the only two materials.

Exploring Alternative Coating Materials

The journey towards enhancing LLINs with alternative active ingredients for the management of stored product pests began with an innovative approach involving SiO₂ nanoparticles (Agrafioti et al. 2020). The effectiveness of silica-coated nets was evaluated with different doses targeting *S. oryzae* and *T. confusum*. The results showed the potential of silica-coated nets in controlling *S. oryzae* adults, achieving a 100% mortality rate within 7 days, even after just 15 minutes of exposure. However, in that study, the authors indicated that the same exposure had little effect in the case of *T. confusum* larvae (Agrafioti et al. 2020). This study shed light on the promising role of silica-coated nets in stored product pest management

strategies, signaling the quest for alternative materials for LLINs. Another investigation aimed to combine botanical insecticides with nets for the management of stored product pests. Incorporating nets with thyme essential oil-based nano-emulsions proved to be effective in causing high mortality rates in *T. castaneum*, despite the unsuccessful repellent activity of the LLIN (Mangang et al. 2022).

In yet another study, researchers delved into enhancing the potency of LLINs by improved concentrations of alpha-cypermethrin, across a range of doses from 10 to 325 mg $\times$ m⁻² targeting stored tobacco pests (Athanassiou et al. 2023). While the commercial alpha-cypermethrin-based LLIN Carifend® contains 163.2 mg $\times$ m⁻² of active ingredient, the findings illustrated that even a concentration as low as 10 mg $\times$ m⁻² proved highly effective against both *L. serricorne* and *E. elutella*, with efficacy being proportionally increased with the increase of concentrations (Athanassiou et al. 2023). These studies highlight ongoing efforts to find better coating materials for insect nets, offering potential improvements for managing stored product pests.

Discussion

The use of LLINs in managing pests before and after harvest in agriculture has emerged as a promising strategy for mitigating the significant economic losses caused by insect infestations. However, while our work shows that LLINs can be effective in both preharvest and postharvest systems, our meta-analysis shows that LLINs are especially effective in durable stored products. Conventional chemical control methods have often proven insufficient due to the emergence of pesticide resistance among many agroecosystems. In this context, the efficacy and sustainability of LLINs, which can be layered on top of other tactics in a holistic IPM program and act in the background, offers a compelling solution to reduce pre- and postharvest losses while enhancing global food security. LLINs are by-and-large chemical barriers, and depending on mesh size can also be physical barriers as well. They

generally apply the active ingredient at much higher concentrations than residual contact insecticides and may thus garner certain advantages over other delivery methods for insecticides. For example, one incontestable advantage of LLINs is their targeted application over the rather broadscale application of insecticides, aerosols, or fumigants. In addition, it is possible to eliminate contact in the postharvest application of LLINs with the commodity, and they generally have an increased efficacy against a wide range of insect species at the postharvest stages of durable agricultural commodities at least in some cases (Agrafioti et al. 2021; Wilkins et al. 2021).

Research into the effectiveness of LLINs in agriculture has expanded over the years (Figure 1.1), encompassing various aspects of LLIN dynamics and application scenarios. From the early investigations conducted by Martin et al. (2006) and Msango and Longwe (2013) to recent advancements, the field has matured, with evolving methodologies and insights. Recent empirically-informed ecological modeling has found that LLIN reduces fecundity and survivorship of *T. castaneum* at food sufficiently that populations end up extirpating in 15 generations when no immigration is present (Ranabhat et al. 2024b). This synthesis and temporal analysis underscores the growing recognition of LLINs as an effective strategy for mitigating pre- and postharvest losses caused by insect infestations. Early investigations have focused on immediate lethal effects, demonstrating the potential of LLINs to kill pests upon first contact. However, recent advancements have revealed sublethal and delayed effects, elucidating the persistent effect of insecticidal ingredients following exposure. While immediate effects are crucial for initial efficacy assessments, understanding delayed effects is equally essential for evaluating long-term pest control strategies. These delayed and sublethal effects may be even more important than immediate ones in the case of LLINs, as the majority of these nets are based on pyrethroids, that cause immediate knockdown, that can either lead to mortality or recovery (Agrafioti et al. 2023). This is why

the placement of these nets should be in such a way to maximize the insect contact with the treated substrate, even after the initial knockdown (Rumbos et al. 2018; Athanassiou et al. 2019). In this context, we consider that post-exposure interval and immobilization are equally important with the initial contact with the insecticide.

The increasing resistance of stored product pests, particularly to insecticides like phosphine, underscores the potential importance of LLINs as an alternative IPM tool. It may be possible to leverage the efficacy of LLIN against these resistant pests by comparing results against both resistant and susceptible populations. A notable study by Ranabhat et al. (2022) found that the deltamethrin-based LLIN D-Terrence[®] resulted in similar lethal and sublethal outcomes, such as distance moved and velocity, in both phosphine-resistant and susceptible populations of *R. dominica* and *T. castaneum*. This finding can be considered further as the potential utilization of LLINs as 'resistance-breaker' for the resistance to the most commonly used fumigant phosphine (Afful et al. 2018) in stored product pest control. The potential of the utilization of LLINs as phosphine resistance breakers requires additional investigation. For instance, Athanassiou et al. (2019) tested Carifend[®] on tobacco that had been previously fumigated with phosphine, under the “start clean- stay clean” approach. Further in the preharvest system as well, the use of LLINs could be a novel approach to control the insect pests that developed resistance to most of the major categories of insecticides and/or fumigants (except pyrethroids), as well as cross-resistance to many insecticide classes. However, there were very few studies for assessing the efficacy of LLINs against resistant insect species. For instance, Gökçe et al. (2018) conducted contact toxicity bioassays to test the efficacy of four different types of ZeroFly[®] (deltamethrin-incorporated) net against the susceptible and insecticide-cocktail resistant third-instar Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), and reported that all treated LLIN, except JLCB061009 (ZeroFly[®] Vestergaard trial batch, 12 × 10 woven

polyethylene monofilament) caused >90% mortality within 24 h of exposure for both susceptible and resistant *L. decemlineata*. More studies should be assessed to evaluate the possible role of LLIN in resistance management in preharvest systems, as it may compliment ongoing efforts to combat resistance in preharvest systems.

The most commonly assessed AIs in LLINs have been alpha-cypermethrin and deltamethrin due to limitations in the availability of commercial options on the international market. Only a handful of studies employed other AIs, including the one conducted by Agrafioti et al. (2020), which employed silica-coated nets, with good results. Other studies have employed permethrin, bifenthin, and propylene glycol. At present, one of the possible risks in the consistent utilization of LLINs before or after harvest in pest management programs is the potential development of pyrethroid resistance. Taking an innovative approach, Ming et al. (2024b) found adding the synergist piperonyl butoxide could increase the efficacy of Carifend® against *R. dominica* by six-fold. Other novel approaches in urban entomology, such as employing LLINs containing dual AIs with different modes of action and targets in the insect (Barker et al. 2023), show promise and can be adapted for use in preharvest and postharvest production systems.

Currently, the insecticide AIs incorporated into LLINs are mainly pyrethroids with knockdown activity against insects. As new LLINs are deployed before and after harvest in pest management strategies, it is possible that other insecticide groups can be potentially utilized. However, other insecticides may lack the immediate knockdown effect associated with neurotoxic pyrethroids. This knockdown effect has yet been crucial, as immobilized individuals within the first few minutes of contact can remain on the substrate, extending their exposure duration to the nets until eventual death (Wilkins et al. 2021; Gerken et al. 2021a). Since other neurotoxic insecticides lack this knockdown effect, research focusing on reducing the killing time of these insecticides to be incorporated into LLINs will be essential for

informing their deployment in agriculture. Additionally, the simultaneous use of other insecticides and/or insecticide synergists (e.g., piperonyl butoxide) along with pyrethroids may provide the inferences necessary for an increase in efficacy, even after the removal of the exposed insects from the treated substrate. Silica-based compounds can effectively serve towards that direction (Agrafioti et al. 2020).

Considering that LLINs may require extended exposure times to achieve optimal mortality rates, integrating them into traps could present an effective attract-and-kill strategy (Krawczyk et al. 2019; Suckling et al. 2019; Gerken et al. 2021b; Wilkins et al. 2021). These traps in pre- or postharvest environments are equipped with LLINs and combined with pheromonal or kairomonal stimuli to attract insects and facilitate their elimination, offering a targeted and efficient method for managing pest populations (Morrison et al. 2021) while addressing the need for prolonged exposure associated with LLINs. Initial findings indicate that combining LLINs with interception traps is highly effective in eliminating *T. castaneum*, *R. dominica* and *T. variable* (Wilkins et al. 2021). Furthermore, traps equipped with D-Terrence[®] have no adverse effect on pest attraction while effectively inhibiting the progeny production of captured insects. Research findings also demonstrate the enhanced impact of LLINs when combined with attractant lures, supporting the promising approach of attract-and-kill (Kuhar et al. 2017; Krawczyk et al. 2019; Suckling et al. 2019; Wilkins et al. 2021). Overall, by leveraging natural attractants in combination with LLINs, attract-and-kill strategies could offer potential synergistic effects in controlling insect populations. Further research exploring the feasibility and efficacy of integrated attract-and-kill approaches can unlock new techniques for sustainable pest management.

Different life stages of insects may display varying susceptibilities to LLINs, which can influence the overall efficacy of these interventions. Furthermore, the developmental stage of certain insect species can affect their behavior and interactions with treated

substrates, impacting factors such as feeding habits, movement patterns, and susceptibility to insecticidal effects. For instance, exposure to the ZeroFly[®] for 10 s resulted in >90% mortality of *H. halys* nymphs and just >40% mortality of *H. halys* adults. Exposure to the net for 10 min resulted in 100% of nymphal and 84% of adult mortalities in *H. halys*. The immediate mortality of *T. castaneum* adults after 7 days of exposure to Carifend[®] is approximately 16% (Paloukas et al. 2020), whereas the same duration results in a 37% mortality in the larval stage (Galán et al. 2024). However, these findings may vary depending on species and life stage characteristics. Larval stages of species like *T. variable* and *T. inclusum* may have limited contact with LLINs due to their dense hairy bodies (Ranabhat et al. 2023). Similarly, the mobility and dispersal ability of *T. castaneum* and *T. variable* larvae are less affected than adults after exposure to D-Terrence[®] (Wilkins et al. 2020). However, there is still inadequate information about the lethal and sublethal effects of LLINs on different life stages of stored product pests. Investigating the potential impact of LLINs on reproductive capacity and population dynamics at different life stages can provide valuable insights into the long-term efficacy and sustainability of LLIN-based interventions. Apparently, adults and larvae can be prioritized in future research, as these are the mobile life stages of holometabolous insects, and hence, these are the life stages that can be in contact with LLINs. Indirect delayed effects on certain life table characteristics, e.g. adult fertility after exposure, pupal formation and adult emergence from previously exposed larvae, can be also included in future research.

The utilization of LLINs shows promise for agriculture in multiple systems, yet their adoption remains limited, and they are not yet considered a standard tool in IPM strategies. Several factors may contribute to this limited adoption and highlight the need for additional research to address existing challenges and uncertainties (Table 1.3). One significant barrier to the widespread adoption of LLINs may be the lack of comprehensive field studies

reflecting real-world application scenarios (Athanasios et al. 2019). While laboratory experiments provide valuable insights into LLINs dynamics and effectiveness, field trials are essential for assessing practical feasibility and scalability, elucidating the influence of environmental factors. Field studies were notably the least represented among our dataset, but the few field studies there are suggests great promise for LLINs. For example, in bulk storage, Ranabhat et al. (2024a) found using alpha-cypermethrin netting could reduce pest dispersal and progeny production by multiple fold, while fumigations could be reduced by 68–91% in 110 metric tonne grain bins. Additionally, the effects of LLINs on natural enemies that exist on stored commodities, either naturally or through biological control programs, remains largely unknown. In preharvest settings, some studies reported the use of exclusion netting without any insecticide incorporation in field conditions (Tasin et al. 2008; Sauphanor et al. 2012; Chouinard et al. 2023; Leach et al. 2023). However, there are very few field studies of netting with insecticide in real-world, commercial settings in preharvest agroecosystems. One of the field trials revealed that LLINs led to a significant decrease in the migration of the *M. persicae* and *A. gossypii* without impacting the parasitoid *A. colemani* which showed the promising use of LLIN in the real setting (Dáder et al. 2015). However, another study showed that bifenthrin incorporated LLIN was deleterious to adults of both natural enemies the predatory mite *Amblyseius swirskii* and the parasitoid *Eretmocerus mundus* (Hymenoptera: Aphelinidae) after 72 h exposure to LLIN in lab (Fernández et al. 2017). Further, this study also reported that both of these natural enemies were neither attracted nor repelled by LLIN and in the semi-field trial, no negative effects were observed on the beneficial capacity of *E. Mundus* and in field trial, LLIN deployment was compatible with *A. swirskii* while decreasing pest densities. However, more field trials are required in different agricultural settings to determine the effect of LLIN on various natural enemies. Dáder et al. (2015) demonstrated that sun exposure diminished the effectiveness of LLIN. Furthermore, due to the size of the

holes in the LLIN, the potency of both bifenthrin and deltamethrin was deemed insufficient in controlling small body size *B. tabaci* populations ((Dáder et al. 2015). It is crucial to consider the mesh size of LLINs, which can affect the behavioral dynamics of the species involved. Compact mesh dimensions may yield unintended outcomes by potentially excluding not only harmful insect pests but also beneficial ones (Chouinard et al. 2023). Furthermore, developing clear guidelines for deploying, maintaining, and monitoring LLINs is crucial for ensuring consistent and effective pest control in agriculture supply chain. Comprehensive research that also includes a grower or stakeholder standard protocol is needed to evaluate the efficacy of LLINs across a wide range of pest species, systems, and environmental conditions to identify optimal deployment strategies and maximize their impact on pest populations.

Critical research gap: field studies

The initial exploration of LLINs in stored product protection took shape through a field trial conducted in a tobacco facility with the aim of curbing *L. serricorne* infestation (Msango & Longwe 2013). This trial revealed promising results, indicating that the combination of phosphine fumigation with Carifend® nets offered superior protection against *L. serricorne* infestation, thereby mitigating re-infestation risks post-phosphine treatment. These findings attracted the interest among researchers regarding the potential of LLINs in stored product pest management systems.

Ranabhat et al. (2024a) conducted a two-year field trial in grain bins showing that alpha-cypermethrin LLIN can be leveraged in bulk storage to protect wheat and reduce fumigations by 68–91%. In addition, damage in silos was reduced by 50–99% compared to controls, while progeny production was reduced by 89–99% compared to insecticide-free netting and no-netting controls (Ranabhat et al. 2024a). In addition, both deltamethrin- and alpha-cypermethrin LLIN were used successfully over 6 months by McKay et al. (2024) to induce mortality of *T. castaneum* and *R. dominica* at a commercial rice processing facility.

Athanassiou et al. (2019) emphasized on the efficacy of Carifend[®] as an alternative to traditional insecticidal treatments (such as phosphine fumigation) in managing pests in stored tobacco, in a series of field trials. Through semi-field and field experiments, Carifend[®] demonstrated its effectiveness in preventing infestations by *L. serricorne* and *E. elutella* for over 6 months, providing valuable protection to tobacco boxes (Athanassiou et al. 2019). Another significant study delved into the implementation of D-Terrence[®] nets in trapping systems for stored product management is that of Wilkins et al. (2021). In that study, the authors reported that LLINs significantly reduced insect infestations in warehouses and interception traps positioned around facility perimeters, highlighting their potential as a barrier against stored product insects. Furthermore, D-Terrence[®] proved adept at blocking insects from reaching commodities, achieving remarkable reductions in infestation rates of up to 93% and progeny production reductions of up to 100%. Nevertheless, up to date, no more field studies have been carried out; however, the existing findings underscore the robust potential of LLINs in stored product pest management.

An important consideration in assessing the efficacy of LLINs is the potential repellent effect, particularly in pyrethroid-based LLIN. Such side effects of pyrethroids in repelling insects are also recognized in combination with nets used against disease vector mosquitoes (Kawada et al. 2014). The repellent effect of pyrethroid-incorporated nets has been consistently demonstrated, even on populations of mosquitoes that are resistant to these chemicals (Bowman et al. 2018). However, the pattern is quite different on stored product insects. For example, repellent effects on stored product pests have been investigated by Scheff et al. (2021), who found no evidence of long-distance or contact repellency or attraction by D-Terrence[®] on *T. castaneum*, *R. dominica*, and *S. oryzae*. Beyond this, research has found when stored product insects are given the choice between LLIN and untreated netting in a Petri dish, they spend an equal time on both sides (Scheff et al. 2021). Finally,

almost 10 species of stored product insects have been assessed for repellency to deltamethrin netting, and none was found for any of them. However, this has been a research gap in preharvest systems, and follow-up work should evaluate potential repellency by pests in multiple agroecosystems. The efficacy of LLINs could be compromised if insects are repelled from direct contact with treated surfaces, particularly in scenarios where alternative pathways remain accessible. LLIN exhibiting repellency to preharvest pests may form the basis for the development of behavioral resistance, and the exploitation of alternative pathways to enter fields and orchards to infest the commodity.

Another research gap alluded to above is the lack of studies investigating response of pyrethroid-resistant insects to deltamethrin or alpha-cypermethrin. These tests have neither been done in a preharvest nor a postharvest setting. However, understanding the response of insects from both systems would be beneficial in determining an adequate resistance management plan for growers and food facility managers.

Conclusions

In summary, addressing existing knowledge gaps and challenges is essential for LLIN's broader adoption and integration into IPM strategies. Continued research efforts focused on field-level understanding of LLIN efficacy, standardization, resistance management, and environmental impact assessment are necessary to unlock the full potential of LLINs and promote their use as a standard tool in stored product pest management programs. However, challenges such as limited field studies, uncertainties about their impact on natural enemies, and the absence of clear deployment guidelines hinder their widespread adoption. Addressing these challenges through comprehensive research is essential for maximizing the effectiveness and sustainability of LLIN-based pest management strategies. Ultimately, continued efforts to optimize the deployment of LLINs hold promise for enhancing pest control practices and

reducing postharvest losses in stored product environments, in conjunction with other reduced-risk control methods.

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Figures

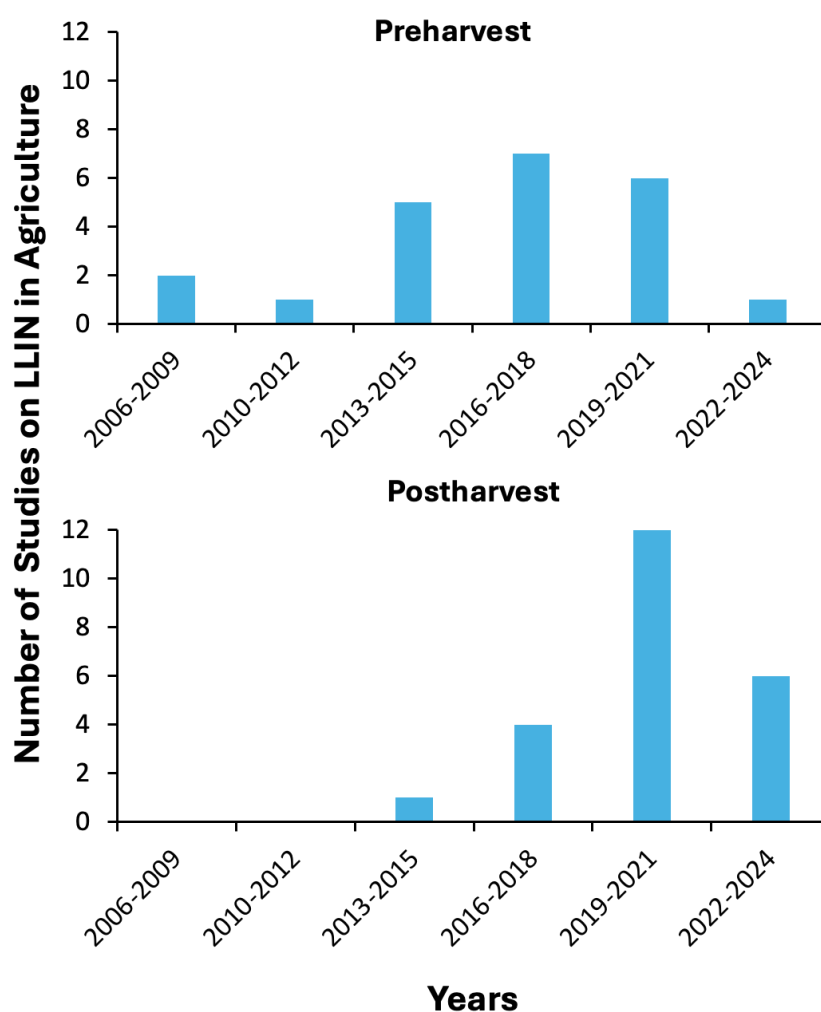


Figure 1.1 Increasing publication trend of peer-reviewed manuscripts investigating the efficacy of LLINs in agriculture

Increasing publication trend in the number of peer-reviewed manuscripts over the years investigating the efficacy of LLINs on pre- and post-harvest pest insects in agriculture.

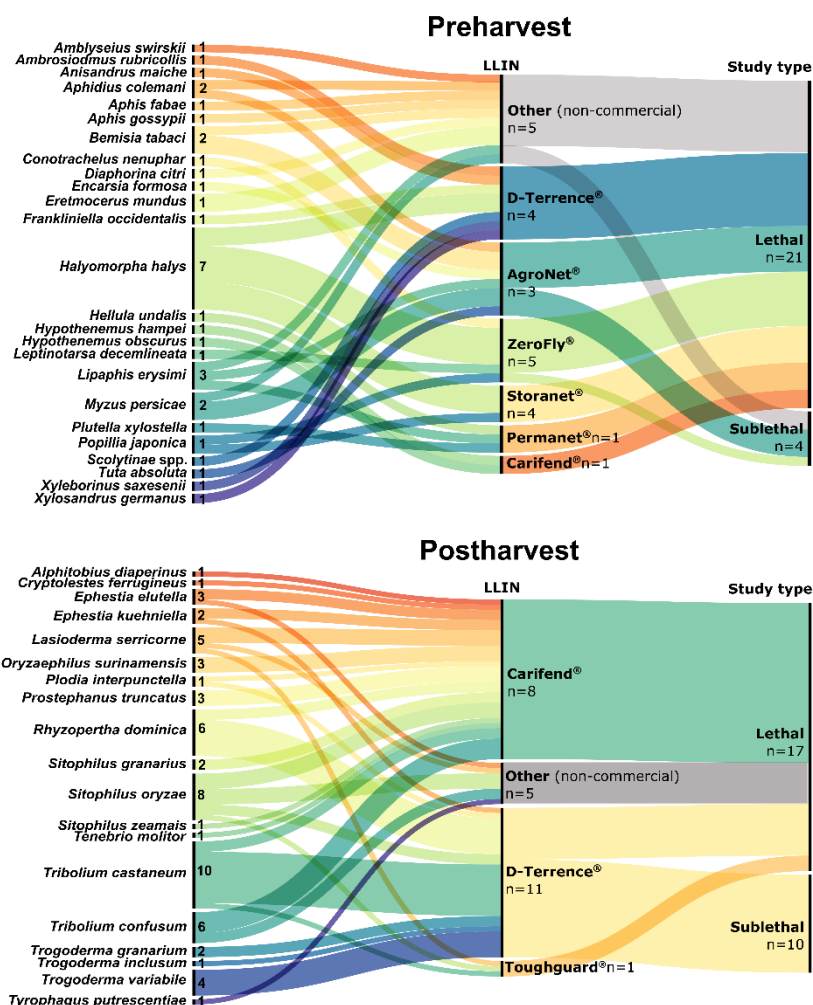


Figure 1.2 Distribution of investigated preharvest and postharvest species, utilized LLINs, and type of studies

The distribution of investigated preharvest (top) and postharvest species (bottom), utilized insecticide-incorporated nets (LLIN), and types of studies. "n" indicates the number of studies.

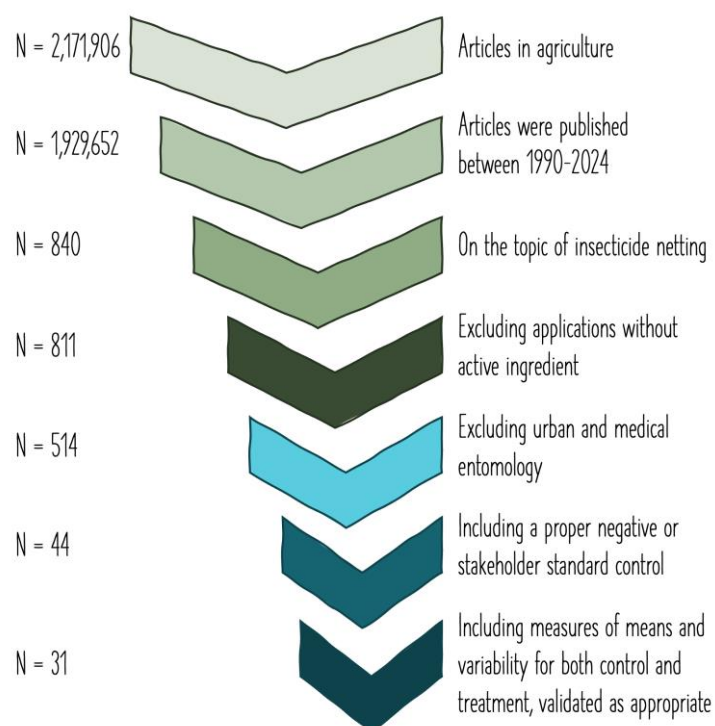


Figure 1.3 Criteria used for inclusion of studies and for the metaanalysis on the effects of LLIN on agricultural insects

The criteria used for inclusion of studies and winnowing of the pool of data for the metaanalysis on the effects of LLIN exposure on agricultural insects.

Metaanalysis Studies on LLIN in Agriculture

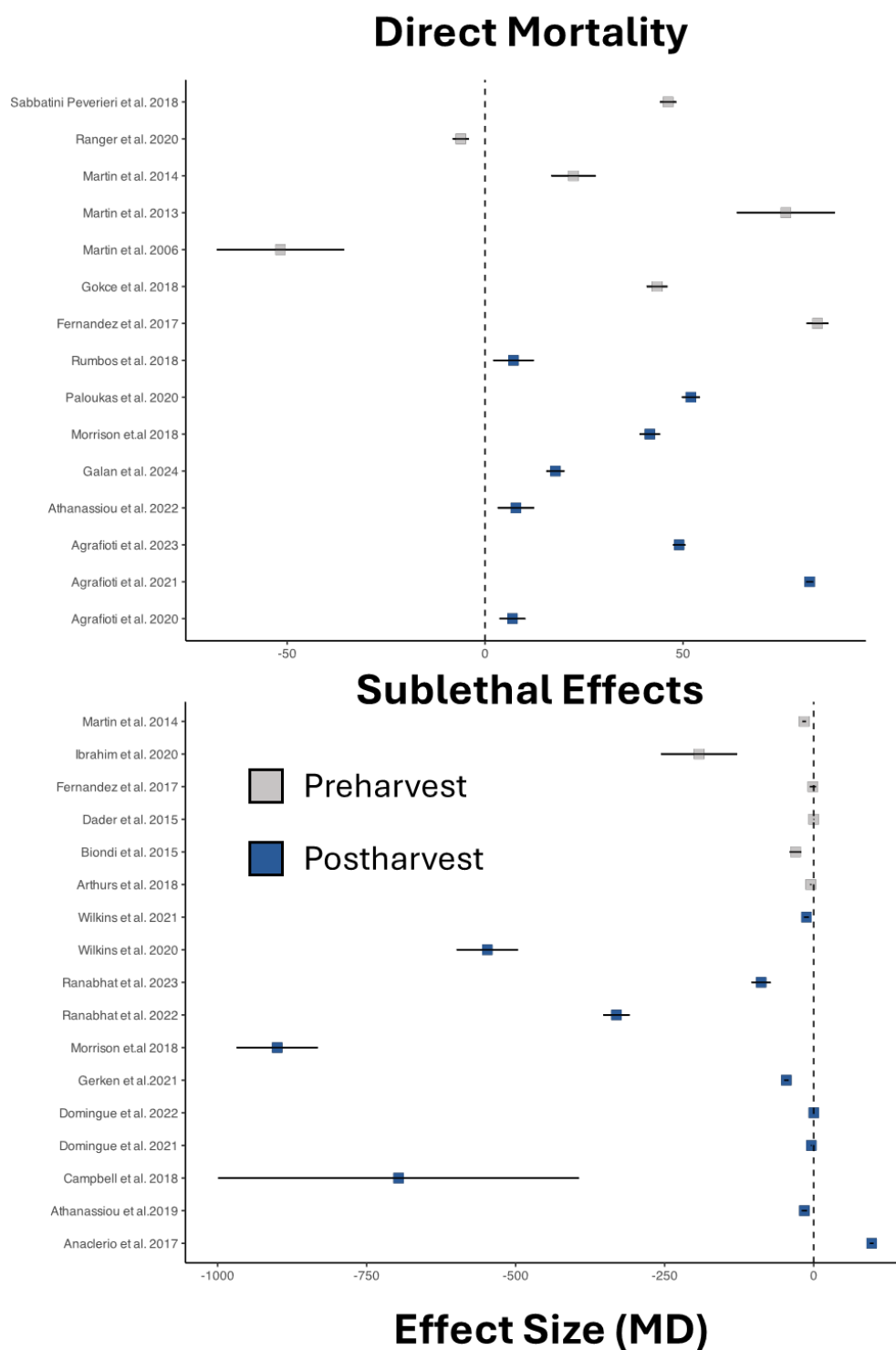


Figure 1.4 Effect size of exposure to LLIN on direct mortality and sublethal effects in preharvest and postharvest agricultural systems

Effect size (standardized mean difference) of exposure to long-lasting insecticide-incorporated netting (LLIN) on direct mortality (top panel) and sublethal effects in preharvest (grey) and postharvest (blue) agricultural systems among multiple studies. Bars represent $\pm$ 95% confidence intervals, and bars not overlapping with a mean effect of zero (dotted line) show a significant effect (Z-score, $\alpha = 0.05$).

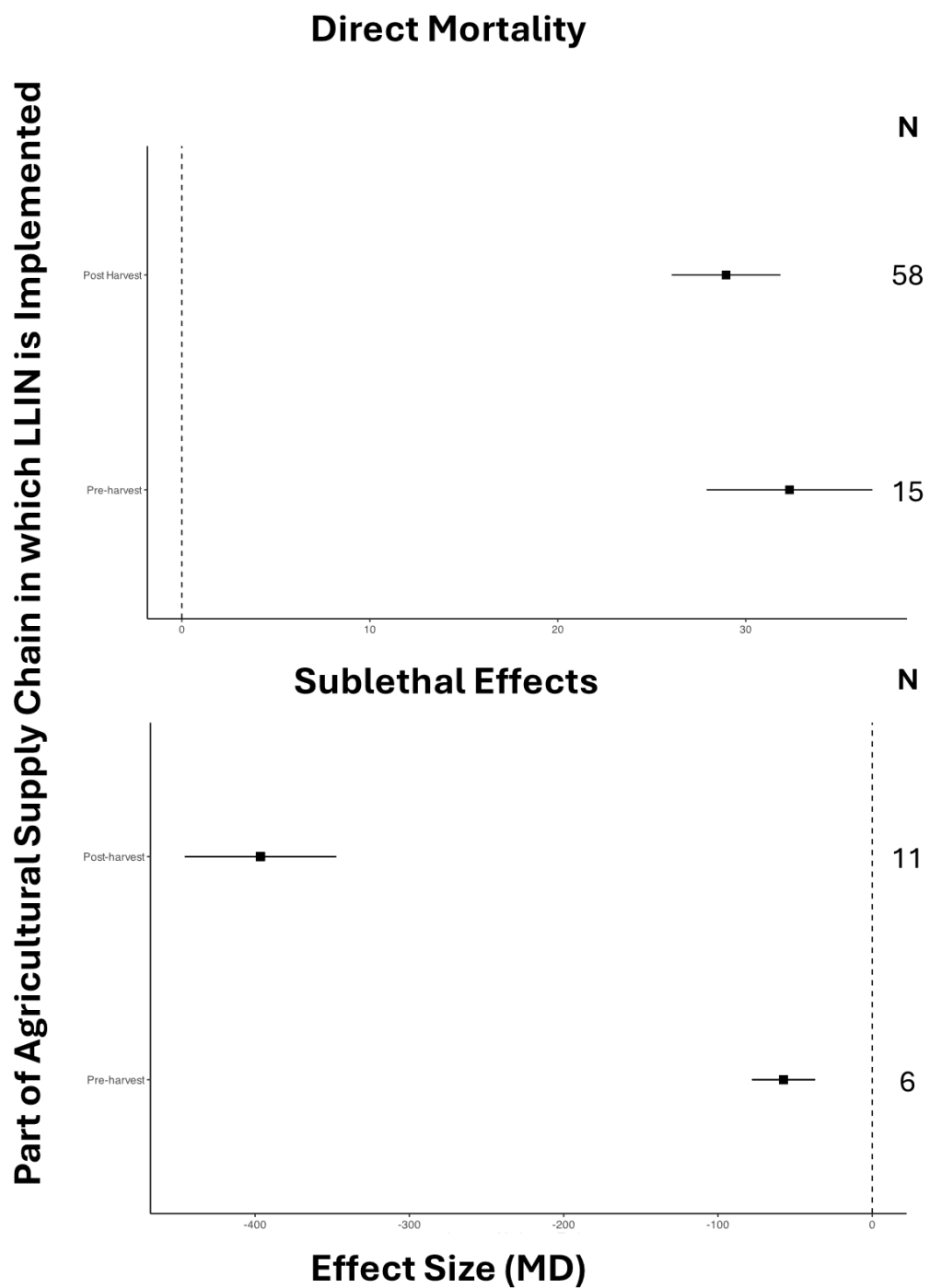


Figure 1.5 Overall effect size of exposure to LLIN in preharvest and postharvest agricultural systems

Overall effect size (standardized mean difference) of exposure to long-lasting insecticide-incorporated netting (LLIN) in preharvest and postharvest agricultural systems. Direct mortality is shown on top, and sublethal effects on bottom. Bars represent $\pm$ 95% confidence intervals, and bars not overlapping with a mean effect of zero (dotted line) show a significant effect.

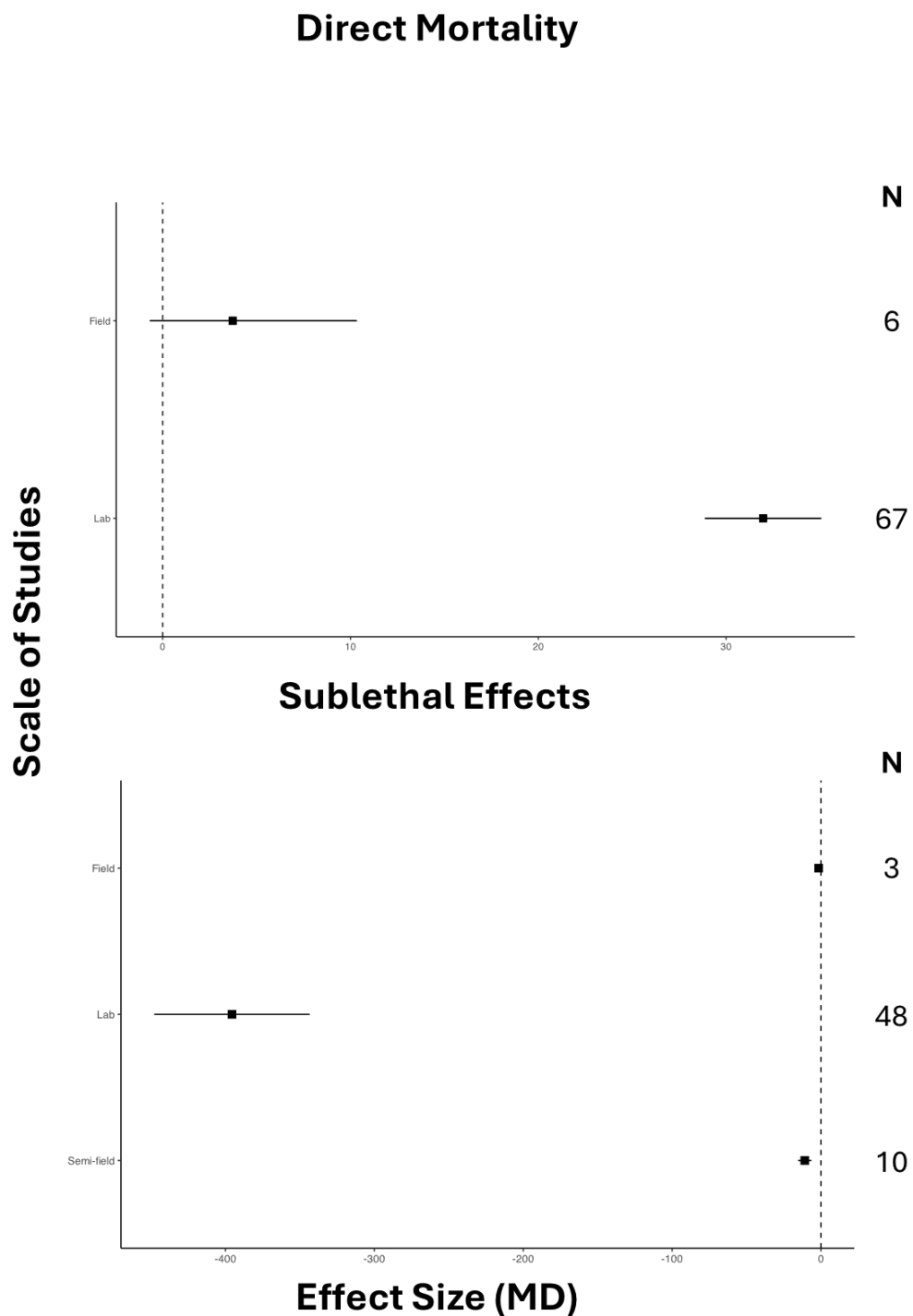


Figure 1.6 Overall effect size of exposure to LLIN at different scales, including, laboratory, semi-field, and field on direct mortality and sublethal effects

Overall effect size (standardized mean difference) of exposure to long-lasting insecticide-incorporated netting (LLIN) at different scales, including, laboratory, semi-field, and field on direct mortality (top panel) and sublethal effects (bottom panel). Bars represent $\pm$ 95% confidence intervals, and bars not overlapping with a mean effect of zero (dotted line) show a significant effect.

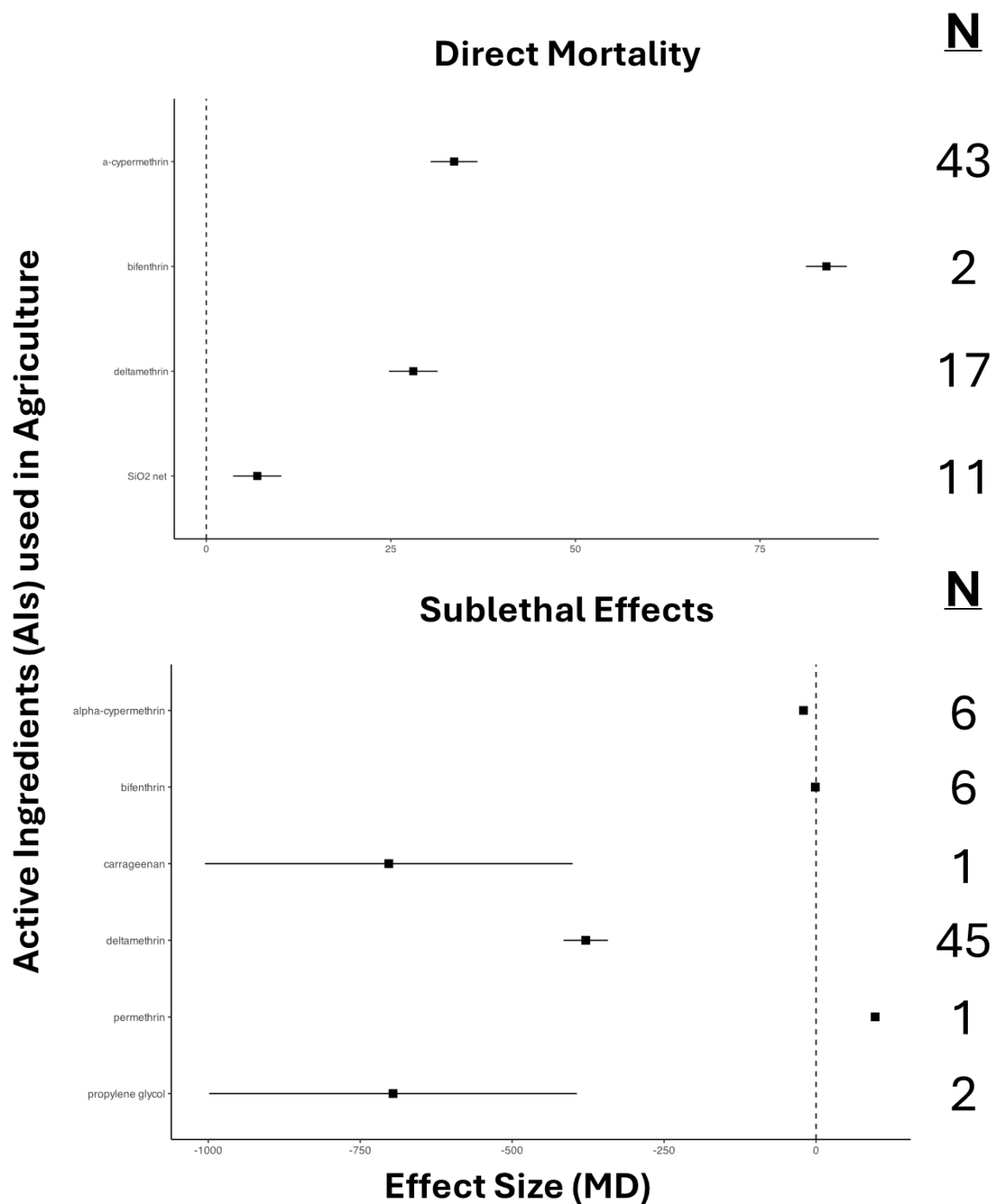


Figure 1.7 Overall effect size of exposure to different active ingredients in LLIN on direct mortality and sublethal effects

Overall effect size (standardized mean difference) of exposure to different active ingredients in long-lasting insecticide-incorporated netting (LLIN) on direct mortality (top panel) and sublethal effects (bottom panel). Bars represent $\pm$ 95% confidence intervals, and bars not overlapping with a mean effect of zero (dotted line) show a significant effect.

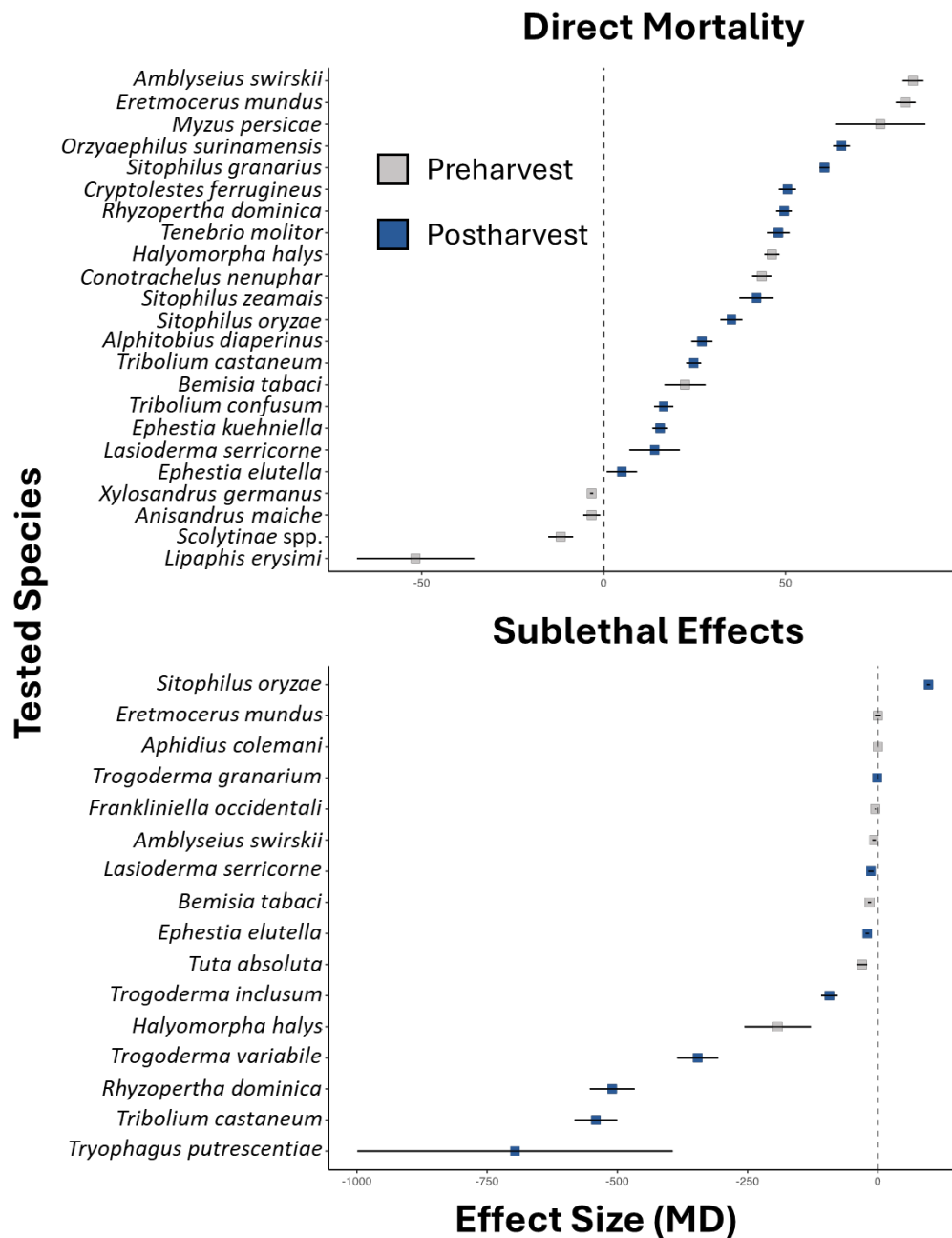


Figure 1.8 Overall effect size of exposure to LLIN on different species related with preharvest and postharvest via direct mortality and sublethal effects
 Overall effect size (standardized mean difference) of exposure to long-lasting insecticide-incorporated netting (LLIN) on different species related with preharvest (grey) and postharvest (blue) via direct mortality (top panel) and sublethal effects (bottom panel). Bars represent $\pm$ 95% confidence intervals, and bars not overlapping with a mean effect of zero (dotted line) show a significant effect.

Table 1.1 Mean difference and 95% CIs for mortality after exposure to long-lasting insecticide-incorporated netting (LLIN)

Studies	Mean Difference	95% (CIs)		Z	P value
		Lower	Upper		
Agrafioti et al. 2020	6.9	3.6	10	2.6	0.071
Agrafioti et al. 2021	82	81	83	170	< 0.001
Agrafioti et al. 2021	49	47	51	74	< 0.001
Athanassiou et al. 2022	7.8	3.2	12	3.4	0.210
Fernandez et al. 2017	84	81	87	60	< 0.001
Galán et al. 2024	18	15	20	12	0.140
Gökçe et al. 2018	43	41	46	39	< 0.001
Martin et al. 2006	-52	-68	-36	-6.3	< 0.001
Martin et al. 2013	76	64	88	12	< 0.001
Martin et al. 2014	22	17	28	8.0	< 0.001
Morrison et al. 2018	42	39	44	33	< 0.001
Paloukas et al. 2020	52	50	54	48	0.010
Ranger et al. 2020	-6.1	-8.2	-4.1	-8.2	0.002
Rumbos et al. 2018	7.2	2.0	12	1.6	0.150
Sabbatini Peverieri et al. 2018	46	44	48	45	< 0.001

Table 1.2 Mean difference and 95% CIs for sublethal effects after exposure to long-lasting insecticide-incorporated netting (LLIN)

Studies	Mean Difference	95% (CIs)		Z	P value
		Lower	Upper		
Anaclerio et al. 2018	97	94	100	60	< 0.001
Arthurs et al. 2018	-4.9	-5.9	-3.9	-9.6	< 0.001
Athanassiou et al. 2018	-16	-20	-11	-7.4	< 0.001
Biondi et al. 2015	-30	-40	-21	-4.3	0.280
Campbell et al. 2018	-700	-1,000	-390	-4.5	< 0.001
Dáder et al. 2015	0.070	-0.63	0.77	0.20	0.850
Domingue et al. 2021	-4.0	-4.7	-3.3	-11	< 0.001
Domingue et al. 2022	0.13	-0.037	0.30	1.7	0.180
Fernandez et al. 2017	-1.4	-6.6	3.9	-1.0	0.290
Gerken et al. 2021	-46	-50	-42	-16	0.120
Ibrahim et al. 2020	-190	-260	-130	-14	0.190
Martin et al. 2014	-16	-19	-13	-9.9	< 0.001
Morrison et al. 2018	-900	-970	-830	-27	< 0.001
Ranabhat et al. 2022	-330	-350	-310	-32	< 0.001
Ranabhat et al. 2023	-88	-100	-72	-11	< 0.001
Wilkins et al. 2020	-550	-600	-500	-21	< 0.001
Wilkins et al. 2021	-12	-16	-7.9	-6.0	< 0.001

Table 1.3 Research gaps on long-lasting insecticide-incorporated netting (LLIN) and future directions, using the current meta-analysis as the basis

Rank	Importance	Gap	Description
1		Guidelines for deployment	For each system and species, need a comprehensive set of deployment guidelines, length of time, and optimal locations to facilitate stakeholder confidence
2		Field – scale research	Need field – scale and/or commercial implementation of LLIN showing success relative to a grower or stakeholder standard (at least positive control or negative control)
3		Role in resistance management	Require testing on whether LLIN can break key resistance in some systems, and/or on whether it won't worsen the development of existing resistance problems, especially for preharvest systems
4		Focus on improving attributes of LLIN	Ideal if novel active ingredients were options in commercial LLIN in agriculture and/or if action of existing netting could be improved with a synergist
5		Assessing repellency of LLIN	Especially in preharvest agroecosystems, assessing the potential repellency of LLIN to target insects and life stages

Table 1.4 Summary of information about commercially available (globally) long-lasting insecticide-incorporated netting (LLIN)

Net Product	Net Manufacturer ^a	Materials	Mesh size	Concentrations of AIs	IRAC#	Mode of Action	EPA Label in USA	Used in Agriculture	No. published studies in Ag from review
D-Terrence®	Vestergaard Inc.	Polyethylene	32 holes/cm ²	0.4% (w/w) deltamethrin	3A	Sodium channel modulator	Yes	Yes	12
Carifend®	BASF	Polyester	100 holes/cm ²	0.34% (w/w) alpha-cypermethrin	3A	Sodium channel modulator	Yes	Yes	8
Zerofly®	Vestergaard Inc.	Polyethylene	32–33 holes per cm ²	3.85–4 mg AI/g deltamethrin	3A	Sodium channel modulator	Yes	Yes	4
Storanet®	BASF	Polyester	24 holes/cm ²	1.57mg AI/g alphacypermethrin	3A	Sodium channel modulator	No	Yes	2
Toughguard®	Sumitomo Chemical	Polyethylene	1 × 1 mm ²	2% permethrin	3A	Sodium channel modulator	No	Yes	1
AgroNet	A to Z Textile Mills Ltd.	Polyethylene	0.9 mm pore diameter	1–2% alpha-cypermethrin	3A	Sodium channel modulator	No	Yes	3
PermaNet®	Vestergaard Inc.	Polyester	40 holes/cm ²	55mg a.i./m ² deltamethrin	3A	Sodium channel modulator	Yes	Yes	1
PermaNet® 2.0	Vestergaard Inc.	Polyester	12–24 holes/cm ²	1.4–1.8 g/kg deltamethrin	3A	Sodium channel modulator	Yes	Yes	–
PermaNet® 3.0	Vestergaard Inc.	Polyester	61–79 holes/cm ²	4g/kg (400 mg/m ²) deltamethrin + 25g/kg (2500 mg/m ²) PBO ^b	3A	Sodium channel modulator + Metabolic enzyme inhibitor	No	Yes	–
PermaNet® Dual	Vestergaard Inc.	Polyester	24 holes/cm ²	84 mg/m ² or 2.1 g/kg deltamethrin + 200 mg/m ² or 5 g/kg chlorfenapyr	3A + 13	Sodium channel modulators + Uncouplers of oxidative phosphorylation via disruption of proton gradient	No	No	–
Olyset®	Sumitomo Chemical	Polyethylene	61 holes/cm ²	2% permethrin	3A	Sodium channel modulator	Yes	No	–

Olyset® Pro	Sumitomo Chemical	Polyethylene	61 holes/cm ²	2% permethrin	3A	Sodium channel modulator	Yes	Yes ^c	–
Olyset™ Plus	Sumitomo Chemical	Polyethylene	61 holes/cm ²	2% permethrin + 1% PBO	3A	Sodium channel modulator + Metabolic enzyme inhibitor	No	No	–
Interceptor® G2	BASF	Polyester	61 holes/cm ²	100 mg/m ² alpha-cypermethrin + 200 mg/m ² chlorfenapyr	3A + 13	Sodium channel modulators + Uncouplers of oxidative phosphorylation via disruption of proton gradient	No	No	–
DawaPlus® 2.0	Tana Netting	Polyethylene	61 holes/cm ²	0.05% deltamethrin	3A	Sodium channel modulator	No	No	–
DuraNet©	Shobikaa Impex Pvt. Ltd.	Polyethylene	~61 holes/cm ²	0.55% (w/w) alpha-cypermethrin	3A	Sodium channel modulator	No	No	–
Royal Sentry®	Disease Control Technologies, LLC	polyethylene	61–79 holes/in ²	5.8 g/kg or 261 mg/m ² alpha-cypermethrin	3A	Sodium channel modulator	No	No	–

^a Location of manufacturers: Vestergaard Inc., Lausanne, Switzerland; BASF AG, Ludwigshafen, Germany; Sumitomo Chemical, Tokyo, Japan; A to Z Textile Mills Ltd., Arusha, Tanzania; Tana Netting, United Arab Emirates; Shobikaa Impex Pvt. Ltd., Tamil Nadu, India; Disease Control Technologies, LLC, Greenville, SC, USA

^b PBO - piperonyl buoxide, a synergist

^c Used in agriculture in Japan

**Chapter 2 - Mobility of phosphine-susceptible and -resistant
Rhyzopertha dominica (F.)(Coleoptera: Bostrichidae) and *Tribolium
castaneum* (Herbst) (Coleoptera: Tenebrionidae) after exposure to
controlled release materials with existing and novel active
ingredients**

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Abstract

There is interest in developing controlled release materials (CRMs) with novel modes of action to improve resistance management. Long-lasting insecticide-incorporated netting (LLIN) with deltamethrin has been effectively used against stored-product pests. Here, we evaluated the efficacy of different CRMs (LLIN or packaging) with each of four active ingredients (AI) (deltamethrin, permethrin, indoxacarb, and dinotefuran) and compared them to control CRMs in reducing movement and increasing mortality of phosphine-susceptible and -resistant *Rhyzopertha dominica* and *Tribolium castaneum*. Adults were exposed for 0.5, 2, or 60 min, and their movement was assessed immediately or after 24, or 168 h using video-tracking and Ethovision software. We recorded total distance and velocity traveled by adults. Finally, we tested higher rates of each AI on surrogate netting material (e.g., standardized-sized cheesecloth) and varied exposure time to obtain median lethal time (LT₅₀) for each compound and susceptibility. Exposure to LLIN with deltamethrin significantly reduced the movement of both species compared to the other CRMs regardless of their susceptibility to phosphine. Deltamethrin was the most effective AI for both species, while dinotefuran and indoxacarb were the least effective for *R. dominica* and *T. castaneum* adults, respectively. Most AIs resulted in appreciable and approximately equivalent mortality at higher concentrations among phosphine-susceptible and resistant strains. Our results demonstrate that CRMs can be an additional approach to combat phosphine-resistant populations of stored product insects around food facilities. Other compounds such as permethrin, dinotefuran, and indoxacarb are also effective against phosphine-resistant populations of these key stored product insects except indoxacarb for *T. castaneum*.

Keywords: insecticide netting, stored product insects, phosphine resistance, integrated pest management, lesser grain borer, red flour beetle

Introduction

Stored products include cereals grains, legumes, and other durable or processed commodities. Together, these products significantly contribute a high value to the economy as well as to global food security. In the US, the production for rice, wheat, and corn alone represents a value of \$62 billion USD (USDA-NASS 2019). As commodities move along the supply chain from farms to end consumers, insects may readily attack at each link. Insect damage causes economic losses by reducing the quality and quantity of food for human consumption. Annually, 2–50% of commodities are lost after harvest from insect infestation (Davis 1991), translating to over \$100 billion in losses of food products globally (Wacker 2018). As a result of insect contamination, stored products may act as allergens or health hazards if not managed appropriately after harvest (Hubert et al. 2018). Therefore, it is critical to develop effective pest management strategies to mitigate losses.

The most common pest management tactic after harvest is fumigation in bulk storage of commodities. Historically, methyl bromide and phosphine were the most common fumigants used, but the former was phased out of usage in most applications due to its deleterious role in depleting the ozone layer in the atmosphere (Fields and White 2002). Phosphine is still widely used as the main fumigant after harvest but has experienced increasing problems with its use. For example, there has been a dramatic rise in phosphine-resistant populations of at least eight stored product taxa around the world (Nayak et al. 2020). Furthermore, there has been a growing demand for low or no insecticide residues by consumers even after harvest (Batte et al. 2007). As a result, there has been an increasing push to diversify integrated pest management (IPM) programs for stored products.

Stored product insects regularly are moved around food facilities through two primary methods. First, stored product insects may be moved among facilities or different parts within a facility through human-mediated movement. This may periodically happen when receiving new commodities at a facility, when moving commodities to different parts of a facility internally, or when insects move independently in a facility after storage of new commodities (Campbell et al. 2002). Secondly, stored product insects may immigrate into food facilities from the surrounding landscape. It is well-known that stored product insects have wild refugia and hosts in the landscape, even far away from food facilities such as in prairie ecosystems (Jia et al. 2008). Additionally, in wooded and open sites, stored product insects such as the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), may move 261–375 m in a short period (Mahroof et al. 2010) and the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), is known for switching between wooded habitats and grain stores (Quellhorst et al. 2021). Indeed, others have called for insect movement to be explicitly considered in IPM programs (Jian 2019).

One way to prevent insect movement both by insects within a food facility and those immigrating from the landscape may be to use controlled release materials (CRMs). CRMs may be defined as formulated polymer matrices containing active ingredients (such as insecticides), designed to be effective for specific periods of time under specified conditions, affecting insect pests in one or all of the following ways; reduced fecundity, knock down, and mortality (Calvert and Billingham 1979, Limm and Hollifield 1996, Focke and Van Parea 2011, Gesta et al. 2015). A subset of CRMs are known as long-lasting insecticide-incorporated netting (LLIN), which have historically been used as bed nets to prevent the spread of arthropod-borne disease in tropical regions (Alonso et al. 1991, Barlow et al. 2001), but more recently are being assessed in

pre-harvest (Kuhar et al. 2017, Bergh and Quinn 2018) and post-harvest agriculture (Morrison et al. 2018, Rumbos et al. 2018). LLINs have the added benefit of allowing airflow because of their larger mesh size, enabling them to be used on vents and in other areas where food dust accumulation may otherwise be a problem. Another subset of CRMs are insecticide-incorporated packaging, which act as a barrier to insect immigration on a smaller scale (Paudyal et al. 2017, Scheff et al. 2021). The expanded use of CRMs may provide an additional tactic to diversify postharvest IPM programs.

Over the last four years, the efficacy of a deltamethrin-incorporated LLIN has been intensively investigated for both sublethal and lethal effects. Prior work has found that exposure to LLIN by multiple stored product insects resulted in movement that is reduced by 50–75%, while dispersal was reduced by 95–100% compared to controls (Morrison et al. 2018, Wilkins et al. 2020). Direct lethality for five out of eight stored product species ranged from 75–93% after a 5-minute exposure (Scheff et al. 2022), while multiple exposures to netting resulted in the same lethality as a single longer exposure (Gerken et al. 2020). LLIN has been shown to be effective for over a year (Scheff et al. 2022), while their deployment in pilot-scale warehouses, regardless of specific method, resulted in 93% fewer insects dispersing to a commodity as well as 99% fewer progeny produced after 6 weeks compared to a control (Wilkins et al. 2021). Thus, LLIN has exhibited great promise for diversifying IPM programs for stored products.

However, despite the positive results, one concern about using LLIN is its role in the resistance management of stored product insects at food facilities, including whether its use may promote resistance by stored product insects to its primary active ingredient, deltamethrin, and whether LLIN could be effective at controlling phosphine-resistant insects. There has been a dearth of studies testing CRMs with different AIs containing other modes of action. In Greece,

there have been tests using Carifend®, an LLIN that uses α -cypermethrin and which has shown good efficacy in the laboratory and field (Rumbos et al. 2018, Athanassiou et al. 2019, Paloukas et al. 2020). A recent study tested silica-coated nets for efficacy against adults of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and larvae of the confused flour beetle, *Tribolium confusum* du Val (Coleoptera: Tenebrionidae), and found that while mortality reached 100% for weevils, it never rose above 34% for the larvae (Agrafioti et al. 2020). Anaclerio et al. (2018), on the other hand, tested a permethrin-incorporated netting for control of *S. oryzae* and found it induced 98% mortality. However, most of these studies have assessed compounds with similar modes of action, and no study has assessed whether LLINs are effective at preventing movement by or inducing mortality of phosphine-resistant populations of stored product insects. Consequently, there is both a critical need to assess CRMs with new AIs, as well as assess how existing and new AIs may be effective against phosphine-resistant stored product insects. In particular, the incorporation of CRMs with novel modes of action might improve resistance management and significantly reduce the risk of phosphine-resistant populations at food facilities by providing multiple barriers to infestations.

Potential alternative AIs for consideration in CRMs include permethrin, dinotefuran, and indoxacarb. Permethrin is a type 1 pyrethroid, that has been shown to be less irritant than the currently used type 2 pyrethroid deltamethrin for operators when yarns are produced, and also when handled by rice mill warehouse workers in tropical climates such as Thailand (Bingham G.V. unpublished data), but may still provide the desired quick knockdown. Dinotefuran is an agonist against nicotinic acetylcholine receptors, and has been found to be highly effective against six species of stored product insects (Arthur and Fontenot 2013). Indoxacarb is a reduced-risk oxadiazine that has also been shown to have some efficacy against stored product

insects (Khan 2020). Each of these may be good candidates for inclusion in CRMs to diversify the options of AIs available to stakeholders at food facilities.

Two critical and cosmopolitan stored product pest species for assessing LLIN-based management strategies are the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and *R. dominica*, because phosphine-resistant populations for both are widespread in the US and elsewhere (Afful et al. 2018, Nayak et al. 2020). These two species represent highly mobile species capable of dispersing far (Campbell et al. 2002, Mahroof et al. 2010), as well as two extremes in other life history parameters among stored product insects (Hagstrum and Subramanyam 2006). *Rhyzopertha dominica* is a pest that feeds and develops on kernels internally as larvae (Hagstrum and Subramanyam 2006, Morrison et al. 2020); it is also a strong flier (Edde and Phillips 2006) and long-distance disperser (Mahroof et al. 2010). By contrast, *T. castaneum* is an important pest of processing facilities, which feeds externally on damaged or broken grain (Hagstrum and Subramanyam 2006), is a strong walker (Morrison et al. 2019), but a comparatively weak flier that is largely confined to food facilities and areas around where grain is handled (Drury et al. 2009, Ridley et al. 2011). Thus, our aims in the current study were to: 1) evaluate the efficacy of different controlled release materials (CRMs) with alternative AIs, including a high concentration of deltamethrin, indoxacarb, permethrin, or dinotefuran mimicking that found in commercially available CRMs compared to untreated control CRMs using movement of *R. dominica* and *T. castaneum* as a surrogate measure of efficacy, and 2) determine the efficacy of the CRMs against phosphine-susceptible and resistant strains of *R. dominica* and *T. castaneum*.

Materials and Methods

Experimental insects

Phosphine-susceptible and -resistant strains of *T. castaneum* and *R. dominica* were used in this study. For both, four to eight-week-old adults were used. Cultures of susceptible strains of *T. castaneum* and *R. dominica* have been maintained in the laboratory since 1958 and 1972, respectively, at the USDA Center for Grain Animal Health Research in Manhattan, KS. Resistant strains of both species were derived from field populations collected in Enid, Garfield County, Oklahoma, USA in 2009. The resistance ratios for *R. dominica* and *T. castaneum* were 1519 and 119 based on LC₉₉ (Opit et al. 2012). *Tribolium castaneum* was reared on a mixture of 95% unbleached, organic flour and 5% brewer's yeast, while *R. dominica* was reared on organic whole wheat. All the strains were maintained at 25–27.5°C, 65% RH, and 14:10 or 16:8 (L:D) h photoperiod.

Treatments

Two different controlled release materials (CRMs) were used, including insecticide netting and insecticide-incorporated packaging. In total, there were six treatments, including packaging (Vestergaard SA., Lausanne, Switzerland) with either 0.1% (w/w AI) indoxacarb, 0.1% permethrin, or 0.2% dinotefuran, or no AI but identical physical properties, and long-lasting insecticide-incorporated polyethylene netting (2 × 2 mm mesh, Vestergaard SA., Lausanne, Switzerland) with 0.4% deltamethrin, or control netting without insecticide but otherwise identical in physical properties. These were used with the movement assay.

In order to evaluate whether the CRMs were more effective at higher concentrations of each AI, we used cheesecloth (100 % cotton, Loins Services, Inc. Charlotte, NC) as a common CRM surrogate material sprayed with technical grade AIs dissolved with acetone. We prepared

solutions (containing 1% of each AI above) with each technical grade insecticide in acetone (Table 1) and sprayed 2 ml of each insecticide solution uniformly on glass petri dishes (5 cm diameter) containing a 4.8 cm diameter piece cheesecloth by using an artist's airbrush sprayer (Badger 100 series, Badger Corporation, Franklin Park, IL, US) which is commonly used in stored product insect research (e.g. Arthur and Morrison 2020; Morrison et al. 2021). A 1% concentration of AI was used to approximate the far higher concentration of AI in commercially available incorporated long-lasting materials compared to the concentration typically used in direct spray applications. The insecticide-treated cheesecloth was allowed to dry at room temperature overnight (~18 h) inside a fume hood to avoid affecting the behavior of insects.

Sublethal movement assay for CRMs

Mixed-sex adult beetles were exposed to the CRMs mentioned above. Cohorts of 5–20 adults were exposed for 0.5, 2, or 60-min interval on CRMs affixed to a $24 \times 24 \text{ cm}^2$ petri dish in the laboratory. After exposure, effects of the insecticides on adults were assessed either immediately or after held for 24 or 168 h in petri dishes under the same environmental chamber conditions as the colonies but without supplemental food, and then assayed using the video-tracking system described below. The movement of adults was tracked in six individual petri dishes ($100 \times 15 \text{ mm D: H}$) with a piece of filter paper (85 mm D, Grade 1, GE Healthcare, Buckinghamshire, United Kingdom) lining the bottom for 1 h using a network camera (GigE, Basler AG, Ahrenburg, Germany) affixed 80 cm above the dishes. The petri dishes were backlit using a LED light box ($42 \times 30 \text{ cm W:L}$, LPB3, Litup, Shenzhen, China) to increase contrast and affixed in place with white foam board.

Video was streamed to a computer and processed in Ethovision (v.14.0, Noldus Inc., Leesburg, VA). The program automatically calculated the total distance moved (cm) and the

mean instantaneous velocity (cm/s) over the 1-h period for each adult. An input filter was created that specific distance was only accumulated if it was less than the length of two beetles (~8 mm) per 0.03 s to avoid cursor bounce. Each adult was considered a replicate and was never used more than once. Only adults classified as alive or affected (as defined in Morrison et al. 2018) were used in the assay. Briefly, alive adults were defined as moving with normal speed and activity and able to right themselves if flipped, while affected adults exhibited sluggish or drunken movements, could not right themselves if flipped, and some or all of their limbs exhibited twitching. Dead adults were completely immobile. In total, 15 replicates were performed per treatment combination, translating to 97,200 min of video for a total of 1,620 adults tested for each species.

Time to Lethal Exposure Assay with Higher Concentrations on Cheesecloth

Cohorts of 20 mixed-sex (~1:1 M: F sex ratio) adult beetles were exposed continuously up to 96 h or 1 week in the laboratory on cheesecloth (e.g., as an absorptive CRM surrogate material) at constant conditions ($27.5^{\circ} \pm 0.1$ C, 65% RH, 14:10 L:D) in an environmental chamber. Exposure times were added iteratively at the same 1% concentration to yield a sufficient number of points to calculate median lethal time (LT_{50}) up to 1 week. At each time point, the condition of insects was checked, and rated as alive, affected, or dead according to the definitions in Morrison et al. (2018) for each of the different treatments. Dead insects were completely immobile, even after prodding, and were removed from the tested arenas, but retained in analyses. There were 4 replicate cohorts for each treatment combination of insecticide, exposure, phosphine susceptibility, and species.

Statistical analysis

First, the total distance moved, and instantaneous velocity were analyzed as separate response variables with an overall linear mixed model, using run date as a random variable. Exposure time (30 s, 2, or 60 min), controlled release materials (netting or packaging), AIs (control netting, 0.4% deltamethrin netting, control packaging, 0.1% indoxacarb packaging, 0.1% permethrin packaging, or 0.2% dinotefuran packaging), phosphine susceptibility of insects (susceptible or resistant), and post-exposure holding duration (0, 24, or 168 h) were fixed explanatory variables. Separate models were run for each species. Each model had the same form as above.

Assumptions of normality and homogeneity in variances were checked using residuals and histograms, and to correct issues, log- transformation was used. Multiple comparisons were not performed with the overall models, because the goal of the overall model was to determine where significant interactions may be occurring and to parse appropriately. Movement analyses were run using R software (R Core Team 2020) and tests were considered significant at $\alpha = 0.05$.

Based on significant interactions with exposure time in the overall models for distance moved and instantaneous velocity by each strain and species, we separately ran simplified models for each exposure time (30 s, 2, or 60 min). Each model used AIs (control netting, 0.4% deltamethrin netting, control packaging, 0.1% indoxacarb packaging, 0.1% permethrin packaging, or 0.2% dinotefuran packaging), phosphine susceptibility of insects (susceptible or resistant), and post-exposure holding duration (0, 24, or 168 h) as fixed, explanatory variables. As a result, Tables with these summaries of these model results lack entries for any terms with exposure time (e.g., Tables 2, 4, 6, 8). Assumptions of normality and homoscedastic variances were checked using residuals and histograms, and log- transformation were used to correct issues

where appropriate. Tukey HSD test was used for multiple comparisons upon a significant result from the model through the *HSD.test* function in the *agricolae* package (de Mendiburu and Yaseen 2020).

Based on the initial results of the overall model with all the CRMs showing the greatest effect with the deltamethrin-LLIN, a follow-up analysis was performed that focused solely on this CRM and its interaction with phosphine-susceptible or -resistant strains of each species. Again, the total distance moved, and instantaneous velocity were analyzed as separate response variables within a linear mixed model framework, using run date as a random variable. The following were included as fixed, explanatory variables: exposure time (30 s, 2, or 60 min), treatment (control netting, or 0.4% deltamethrin netting), and post-exposure holding duration (0, 24, or 168 h). Separate models were run for each strain (e.g., phosphine-susceptible or -resistant) and species. Assumptions of normality and homogeneity in variances were checked using residuals and histograms, and were corrected with log-transformation where deviations were detected in assumptions. Upon a significant result from the model, Tukey HSD test was used for multiple comparisons and was implemented with the function *HSD.test* from the *agricolae* package (de Mendiburu and Yaseen 2020).

To determine the LT_{50} , the total insects, and the number of dead insects were broken down for each strain, species, and AI, then analyzed by probit analysis using the procedure PROC PROBIT from SAS software (SAS Institute Inc 2018) with $\alpha = 0.05$.

Results

Movement assay: *R. dominica*

Overall Distance Moved Model

Overall, neither the phosphine susceptibility nor the CRMs significantly affected the distance moved by *R. dominica* (Table 2.2, Figure 2.1). Further, exposure time also did not significantly affect the distance moved by adults. However, post-exposure holding duration significantly affected the distance moved by *R. dominica*, with adults moving only 30 and 5% of the distance after 24 and 168 h, respectively, compared to the adults moving immediately after exposure (0 h). None of the two-way interactions between the susceptibility and the other factors were significant (Table 2.2). However, the interaction between treatment and post-exposure holding duration significantly affected the distance moved. Adults exposed to deltamethrin LLIN moved 36, 6, and 7% of the distance moved by those exposed to other CRMs with post-exposure holding durations of 0 h, 24 h, and 168 h, respectively. Similarly, exposure time by post-exposure holding duration also significantly affected the distance moved by *R. dominica*. The 3-way interaction among susceptibility, treatment, and exposure time as well as among susceptibility, exposure time, and post-exposure holding duration were not significant. By contrast, the 3-way interactions among susceptibility, treatment, and post-exposure holding duration as well as treatment, exposure time, and post-exposure holding duration significantly affected distance moved by *R. dominica*. Finally, the 4-way interaction between all the factors also significantly affected the distance moved by *R. dominica* (Table 2.2).

LLIN-Specific Distance Moved Model

As the deltamethrin-incorporated insecticide netting was more effective than other CRMs, we focused our results more on this particular CRM. Overall, neither treatment nor post-exposure holding duration and exposure time significantly affected the distance moved by *R. dominica* (Table 2.3; Figure 2.2). However, the phosphine susceptibility did significantly affect the distance moved by *R. dominica*, but in most pairwise comparisons of resistant to susceptible

strain, there was no significant difference in distance moved by adults (Figure 2.2). The two-way interaction between the susceptibility and treatment was not significant. Similarly, the interaction between treatment and exposure time was also not significant. However, all the other two-way interactions between post-exposure holding duration and the other factors significantly affected the distance moved by *R. dominica*. Similarly, the interaction between the susceptibility and exposure time was significant. Importantly, the 3-way interactions between all factors and the 4-way interaction between all the factors significantly affected the distance moved by *R. dominica* (Table 2.3; Figure 2.2). These were mostly quantitative interactions. For example, the distance moved by *R. dominica* immediately after exposure was 3–5-fold less than compared to adults exposed to control netting, but this was to 16–30-fold less 24 h after exposure but equilibrated to no effect by 168 h later because of the general lack of movement, even in the controls (Figure 2.2). Adult *R. dominica* exposed for 30 s moved 6-fold less compared to control netting exposed individuals, while those exposed for 2 min and 60 min moved 4- and 35-fold less, respectively.

Overall Velocity Model

The velocity of *R. dominica* movement was neither affected by the susceptibility nor the CRMs (Table 2.4, Figure 2.3). Additionally, the exposure time also did not significantly affect the velocity by adults. However, post-exposure holding duration significantly affected the velocity of *R. dominica* movement, with adults moving only 31 and 5% the distance after 24 and 168 h compared with adults moving immediately after exposure. The two-way interaction between the susceptibility and the other factors were not significant. However, the interaction between the treatment and post-exposure holding duration significantly affected the velocity. Adults exposed to deltamethrin LLIN moved with velocities that were only 36, 6, and 7% of the velocity moved by the adults exposed to other CRMs at 0, 24, and 168 h, respectively. Similarly, exposure time

by post-exposure holding duration also significantly affected the velocity of *R. dominica* movement. The 3-way interaction among the susceptibility, treatment, and the exposure time was not significant. However, the 3-way interactions among the susceptibility, exposure time, and post-exposure holding duration and susceptibility, treatment, and post-exposure holding duration as well as treatment, exposure time, and post-exposure holding duration significantly affected velocity of *R. dominica* movement. Lastly, the 4-way interaction between all the factors also significantly affected the velocity of *R. dominica* movement (Table 2.4).

LLIN-Specific Velocity Model

The velocity of *R. dominica* movement was neither affected by post-exposure holding duration nor exposure time (Table 2.5, Figure 2.2). Further, the treatment also did not affect the velocity. However, the susceptibility did significantly affect the velocity of *R. dominica*. The two-way interaction between the susceptibility and treatment on the velocity of adults was not significant nor was the interaction between the treatment and exposure time. However, all the other two-way interactions between post-exposure holding duration and the other factors significantly affected the velocity of *R. dominica*. Likewise, the interaction between the susceptibility and exposure time was significant. Importantly, all the 3-way interactions and the 4-way interactions between all the factors significantly affected the velocity of *R. dominica* (Table 2.5, Figure 2.2). In particular, LLIN-exposed adults moved 3–5-fold to 143–177-fold slower than the control netting-exposed individuals immediately after 0–24 h, but there was no effect by 168 h later because movement was almost nonexistent (Figure 2.2). Exposing *R. dominica* to LLIN for 30 s to 2 min resulted in adults moving 2–6-fold slower than the control netting exposed individuals, while those exposed for 60 min moved 57-fold less.

Movement assay: *T. castaneum*

Overall Distance Moved Model

The distance moved by *T. castaneum* was neither affected by the phosphine susceptibility nor by the exposure time (Table 2.6, Figure 2.4). However, post-exposure holding duration significantly affected the distance moved by the adults, with them moving 94% of the distance after 24 h compared to the distance adults moved initially and 12% farther after 168 h compared to initially. Similarly, the CRMs also significantly affected the distance moved by *T. castaneum*. Adults exposed to deltamethrin-LLIN moved 2.8-, 2.7-, and 2.8- fold less than the adults exposed to packaging materials that were incorporated with dinotefuran, indoxacarb, and permethrin respectively. None of the two-way interactions between susceptibility and the other factors were significant. Further, exposure time by post-exposure holding duration also did not significantly affect the distance moved by *T. castaneum*. However, the treatment and post-exposure holding duration significantly affected the distance moved. Adults exposed to deltamethrin LLIN moved 32, 25, and 49% of the distance moved by the adults exposed to other AIs at 0, 24, and 168 h, respectively. Similarly, the treatment and exposure time did significantly affect the distance moved. Adults exposed to deltamethrin LLIN moved 63, 47, and 2% of the distance moved by adults exposed to other AIs at 0.5, 2, and 60 min, respectively (Figure 2.4). The 3-way interactions among the susceptibility, treatment, and exposure time as well as among the susceptibility, exposure time, and post-exposure holding duration were not significant, nor was the 3-way interactions among the treatment, post-exposure holding, and exposure time. By contrast, the interactions between susceptibility, treatment, and post-exposure holding duration significantly affected the distance moved by *T. castaneum*. Finally, the 4-way interactions

between all the factors did not significantly affect the distance moved by *T. castaneum* (Table 2.5).

LLIN-Specific Distance Moved Model

Similar to the results for *R. dominica*, the deltamethrin incorporated insecticide netting was also more effective in reducing *T. castaneum* movement compared to other CRMs. Neither the susceptibility nor the exposure time significantly affected the distance moved by *T. castaneum* (Table 2.7, Figure 2.5). However, the treatment affected the distance moved by adult *T. castaneum*. LLIN-exposed *T. castaneum* moved 35% of the distance moved by the adults exposed to the control netting. Further, the post-exposure holding duration also significantly affected the distance moved by *T. castaneum*. At 24 h, adults exposed to deltamethrin LLIN moved 75% of the distance moved by the adults exposed at day of treatment and at 168 h post-exposure holding duration moved 69% farther. The two-way interactions between the susceptibility and the other factors were not significant. Further, the interactions between the exposure time by post-exposure holding duration also did not significantly affect the distance moved by *T. castaneum*. However, the interactions between the treatment and post-exposure holding duration as well as the interactions between the treatment and exposure time did significantly affect the distance moved by *T. castaneum*. All the three-way interactions among the susceptibility and all the other factors did not significantly affect the distance moved by *T. castaneum*. By contrast, the interactions between the treatment, exposure time, and post-exposure holding duration significantly affected the distance moved by *T. castaneum*. Finally, the 4-way interactions among all the factors did not significantly affect the distance moved by *T. castaneum*.

Overall Velocity Model

Overall, the main effects of the phosphine susceptibility, post-exposure holding duration, and exposure time did not significantly affect the velocity of *T. castaneum* movement (Table 2.8, Figure 2.6). However, the type of CRM significantly affected the velocity moved. Adults exposed to deltamethrin LLIN, for example, moved with velocity that was 2.8-, 2.7-, and 2.8-fold less than the adults exposed to packaging materials that incorporated dinotefuran, indoxacarb, and permethrin, respectively. None of the two-way interactions between the susceptibility and the other factors were significant. Further, the exposure time by post-exposure holding duration also did not significantly affect the velocity of *T. castaneum* movement. Adults exposed to deltamethrin LLIN moved with a velocity 32, 25, and 50% of the velocity moved by the adults exposed to other CRMs at immediately after exposure, 24, and 168 h, respectively. Similarly, the treatment and exposure time significantly affected the velocity moved. Adults exposed to deltamethrin LLIN moved with a velocity of 63, 47, and 2% of the velocity moved by adults exposed to other treatments at 0.5, 2, and 60 min, respectively (Figure 2.6). The only significant 3-way interactions were among the susceptibility, treatment, and post-exposure holding duration, which significantly affected the velocity of *T. castaneum* movement. Finally, the 4-way interactions between all the factors did not significantly affect the distance moved by *T. castaneum* (Table 2.8).

LLIN-Specific Velocity Model

Overall, neither the susceptibility nor the exposure time significantly affected the velocity of *T. castaneum* movement (Table 2.9), but the treatment did. Adults exposed to deltamethrin-LLIN moved 35% slower than the adults exposed to the control netting. Further, post-exposure holding duration significantly affected the velocity of *T. castaneum* movement. At 24 h, adults exposed to deltamethrin LLIN moved 75% of the distance moved by the adults exposed at day of

treatment and at 168 h post-exposure holding duration moved 69% farther. The two-way interactions between the susceptibility and the other factors were not significant. Further, the two-way interactions between the exposure time by post-exposure holding duration and the interactions between the exposure time and the treatment did not significantly affect the velocity of *T. castaneum* movement. However, the interactions between the treatment and the post-exposure holding duration significantly affected the velocity of *T. castaneum* movement. None of the three-way interactions between the susceptibility and the other factors significantly affected the velocity of *T. castaneum* movement. By contrast, the interactions between the treatment, exposure time, and post-exposure holding duration significantly affected the velocity of *T. castaneum* movement. Finally, the 4-way interactions between all the factors significantly affected the velocity of *T. castaneum* movement.

Lethality Assay on Cheesecloth: *R. dominica*

The results of LT₅₀ analysis indicated that the deltamethrin killed both phosphine-susceptible and resistant *R. dominica* relatively quickly and equally (Table 2.10, Figure 2.7). The fastest acting AI was deltamethrin with an LT₅₀ value that was 9.8–17.2-fold less than that of dinotefuran. In each case, the LT₅₀ value was separated by a negligible amount for the phosphine-susceptible and -resistant strains of *R. dominica*. Based on the LT₅₀ values of the four AIs against *R. dominica*, deltamethrin showed the highest efficacy followed by permethrin, indoxacarb, and dinotefuran. The control mortality was 7.5 % at 96 h for both the phosphine-susceptible and –resistant strains of *R. dominica* (Figure 2.7).

Lethality Assay on Cheesecloth: *T. castaneum*

Similar to the results for *R. dominica*, LT₅₀ analysis indicated that the deltamethrin killed both the phosphine-susceptible and resistant *T. castaneum* relatively quickly and equally (Table 2.11,

Figure 2.8). The fastest acting AI was deltamethrin with an LT_{50} that was 16.1–23.3-fold less than dinotefuran. Based on the LT_{50} values of the four AIs against *T. castaneum*, deltamethrin showed the highest efficacy followed by permethrin, dinotefuran, and indoxacarb. *T. castaneum* was not susceptible to indoxacarb, because the mortality caused by the indoxacarb at 168 h for both phosphine-susceptible and resistant strains of *T. castaneum* was only 7.5%. Thus, we didn't calculate an LT_{50} for indoxacarb. The control mortality was 1.25% for phosphine-susceptible and 0% for resistant strain of *T. castaneum* at 168 h (Figure 2.8).

Discussion

This is the first study to assess the mobility of phosphine-susceptible and -resistant strains of *R. dominica* and *T. castaneum* and after exposure to LLIN and other CRMs with different AIs. Overall, we demonstrated that deltamethrin LLIN is the most effective CRM among those tested in this study at reducing the movement of *R. dominica* and *T. castaneum*, but other CRMs were also effective at inducing mortality at higher concentrations. Further, we found deltamethrin and other CRMs at higher concentrations successfully and equally affected phosphine-susceptible and resistant strains for both species. Additionally, we found that deltamethrin caused mortality faster than permethrin, dinotefuran, and indoxacarb regardless of phosphine susceptibility.

We found that the distance moved and velocity was reduced for both phosphine-susceptible and resistant *R. dominica* and *T. castaneum* after exposure to the AIs tested in the study. These are the crucial movement variables and they mediate immigration into food facilities, foraging, mating, egg-laying and dispersal (Jian 2019). Indirect toxicity was observed through reduced movement, especially when exposed to deltamethrin LLIN. Further, the sublethal effects from LLIN exposure for both species were present immediately after exposure and lasted up to 168 h. This indicates that exposure to deltamethrin LLIN has both immediate

and lasting effects on the movement of both species regardless of their susceptibility to phosphine. Previous work has reported that deltamethrin-incorporated LLIN was successful at reducing the movement of *R. dominica* and *T. castaneum* (Morrison et al. 2018) and other life stages in the laboratory and field (Wilkins et al. 2020, 2021), but had not determined how it may change with phosphine susceptibility. We found that deltamethrin LLIN was effective at reducing movement in highly phosphine-resistant strains of *R. dominica* and *T. castaneum* from Enid, Oklahoma (Opit et al. 2012). Thus, along with other work demonstrating the utility of pyrethroid-incorporated netting to manage stored product insects (Morrison et al. 2018, Rumbos et al. 2018, Paloukas et al. 2020), our data suggests LLIN is a valuable additional tool to manage phosphine-resistant populations of stored product insects.

At low concentrations (e.g., 0.1–0.2%), the alternative CRMs did not significantly affect the movement or mortality of either *R. dominica* or *T. castaneum*, but the movement and mortality of both species were dramatically affected at higher concentrations (e.g., 0.4–1%) when tested on a surrogate CRM. In this study, two different types of CRMs were used: netting and packaging materials. AIs included deltamethrin, permethrin, indoxacarb, and dinotefuran. We observed that deltamethrin-incorporated LLIN was the most effective, followed by permethrin for both species and they were equally effective for both phosphine-susceptible and resistant strains. While this was followed in order by indoxacarb and dinotefuran in effectiveness for *R. dominica*, the order was reversed for *T. castaneum*. Insecticide efficacies may depend on various factors like environmental, biological, and physical factors including specific insecticide and formulation, modes of action, actual application rate (Arthur et al. 2020), the time interval insects are exposed to the pesticide (Morrison et al. 2021), target insect species (Arthur and Morrison 2020), and surface substrate (Zettler and Arthur 2000). Deltamethrin-incorporated

LLIN, the most effective CRM, used a label rate of 0.4%, which was twice as high as the other CRMs with other AIs. In order to produce a fair comparison and assess a higher concentration, the same surrogate substrate (cheesecloth) and the same concentration (1%) was used. When this was performed, we found that continuous exposure to many of the alternative AIs were suitable alternatives to deltamethrin at inducing mortality in *R. dominica* and *T. castaneum*, resulting in equally low LT₅₀ in both phosphine-susceptible and resistant insects. It is interesting to note that in commercially labeled LLIN, the deltamethrin concentration is 4000 ppm, which is a far higher rate than what would typically be used in a residual or grain protectant spray with the same compound (Arthur and Morrison et al. 2020); this was the partial justification for using a higher rate of AIs in this study. We found that *T. castaneum* was not susceptible to indoxacarb, causing only 7.5% mortality by 168 h. Our results were similar to Daglish and Nayak (2011), which showed indoxacarb was effective as a residual treatment against *R. dominica* but not against *T. castaneum* even at a high dose. Overall, permethrin, dinotefuran, and to a lesser extent, indoxacarb, appear suitable for inclusion in CRMs targeting stored product insects.

Rotation of insecticide mode of action is required for the successful stewardship of insecticidal products, and this is required to limit the development of insecticide-resistance at food facilities. Relying on a single active ingredient or mode of action will increase the probability of developing resistant populations over time. In this study, deltamethrin and permethrin are pyrethroids that act on the insect's central nervous system by altering the gating kinetics of voltage-gated sodium channels (Soderlund 2010). When insects are exposed to these two compounds, it typically causes tremors, involuntary extremity movements, and reduced coordination, which results in insect mortality (Narahashi 1971). Dinotefuran is a neonicotinoid that acts agonistically on the nicotinic acetylcholine receptor, disrupting synapses in the central

nervous system (Tomizawa and Yamamoto, 1993), while indoxacarb is an oxadiazine that blocks neuronal sodium channels in insects (Lapied et al. 2001). All the compounds tested in this study resulted in significant numbers of affected and dead insects, except for indoxacarb in the case of *T. castaneum*. It has been reported that indoxacarb bioactivates very slowly for some insects, which may cause the slow onset of symptoms (Wing et al. 2000), making it less useful for inclusion in CRMs.

The present study has provided information about important alternative compounds with a different mode of action for potential inclusion in CRMs to manage stored product insects. The inclusion of new AIs in CRMs will support appropriate resistance management programs at food facilities and their inclusion in comprehensive IPM program to prevent future resistance issues. With increasing phosphine resistance among stored product insects around the world (Zettler et al. 1989, Pimentel et al. 2009, Opit et al. 2012, Nguyen et al. 2016, Cato et al. 2017), tools that are effective against both phosphine-susceptible and -resistant populations are necessary. Our study demonstrated that the use of deltamethrin-incorporated netting reduces the mobility of both *R. dominica* and *T. castaneum* adults regardless of their phosphine susceptibility, while deltamethrin and other AIs are effective against phosphine-susceptible and resistant populations of both species. A future active ingredient worth testing extensively is alpha-cypermethrin against a variety of stored product insects, as it has demonstrated promise in prior work (Rumbos et al. 2018, Athanassiou et al. 2019, Paloukas et al. 2020, Agraftoti et al. 2021). Overall, our study suggests a variety of new AIs are effective against phosphine-resistant populations of key stored product insects, and LLIN may be used as an additional tool to combat phosphine resistance.

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Figures:

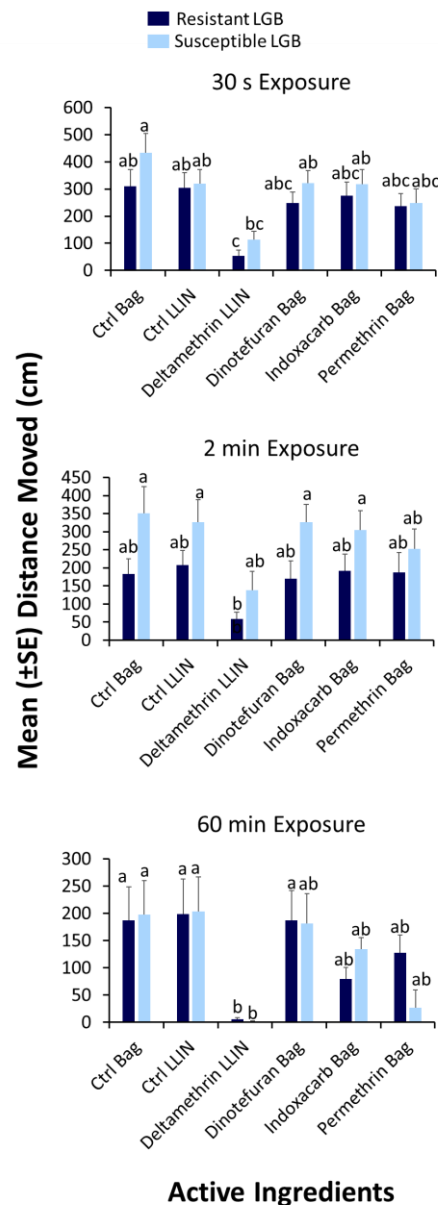


Figure 2.1 Distance moved ($\pm$ SE) by susceptible and resistant *R. dominica* after varying exposure time to different controlled release materials

Distance moved ($\pm$ SE, cm) by susceptible (light blue) and resistant (dark blue bars) *R. dominica* (LGB) after varying exposure for 30 s (top panel), 2 min (middle panel), or 60 min (bottom panel) to different controlled release materials in the sublethal movement assay during tracking with a video camera coupled with Ethovision for 60-min periods in the laboratory. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Letters illustrate multiple comparisons for the interaction among exposure time, susceptibility, and controlled release materials. Abbreviations: Ctrl – control (no insecticide), and LLIN – long-lasting insecticide-netting.

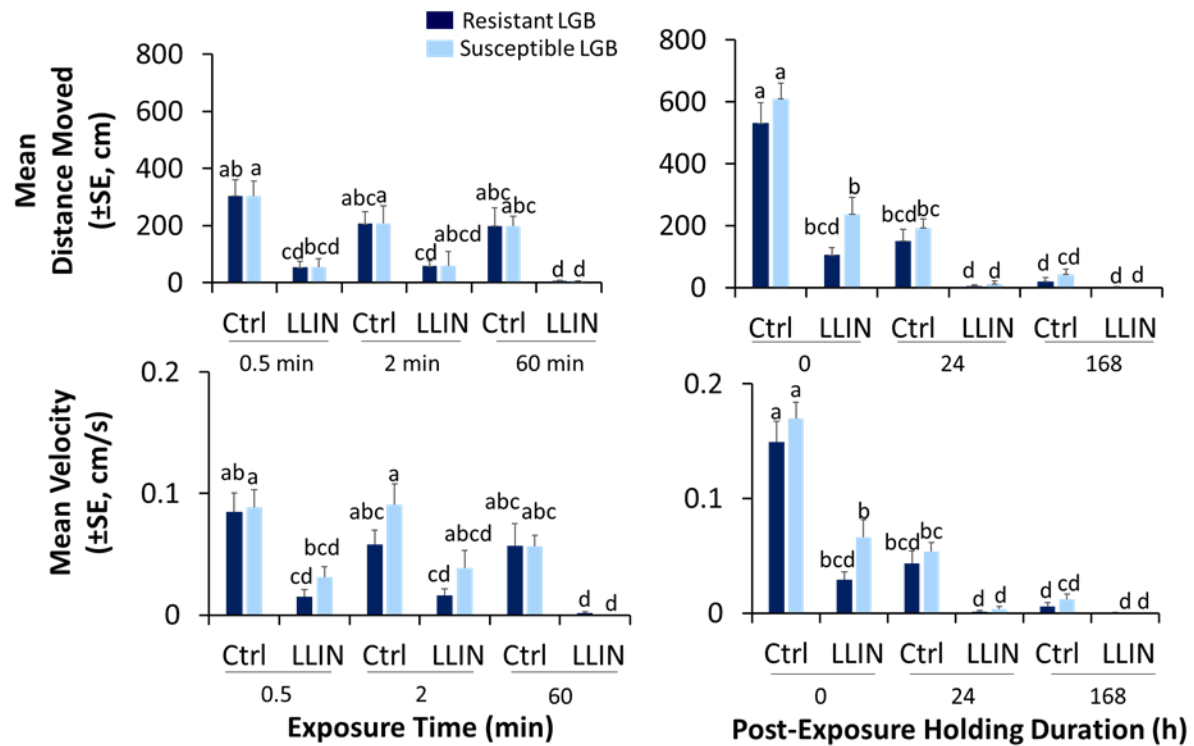


Figure 2.2 Distance moved ($\pm$ SE) or velocity ($\pm$ SE) by susceptible and resistant *R. dominica* after exposure to LLIN or control netting after various exposure times and varying post-exposure holding durations

Distance moved ($\pm$ SE) (top panels) or velocity ($\pm$ SE) (bottom panels) by susceptible (light blue) and resistant (dark blue bars) *R. dominica* (LGB) after exposure to long-lasting 0.4% deltamethrin-incorporated netting (LLIN) or control netting (Ctrl) in the sublethal movement assay during tracking for 60-min periods on Ethovision after various exposure times (left) and varying post-exposure holding durations (right). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Letters represent multiple comparisons between the interaction among exposure time, susceptibility, and LLIN (left column) or among post-exposure holding duration, susceptibility, and LLIN (right column).

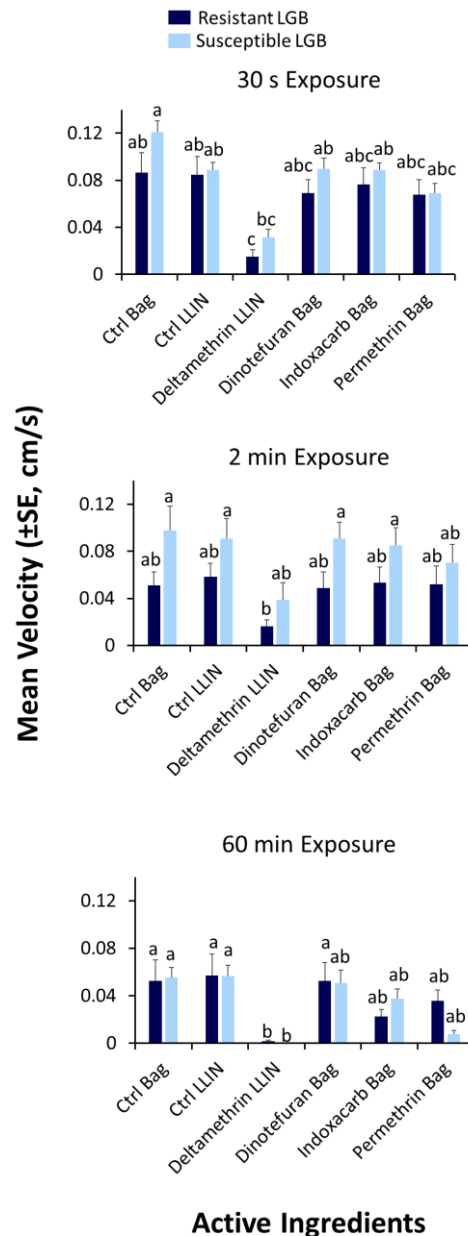


Figure 2.3 Velocity ($\pm$ SE) by susceptible and resistant *R. dominica* after varying exposure times to different controlled release materials

Velocity ($\pm$ SE, cm/s) by susceptible (light blue) and resistant (dark blue bars) *R. dominica* (LGB) after varying exposure for 30 s (top panel), 2 min (middle panel), or 60 min (bottom panel) to different controlled release materials in the sublethal movement assay during tracking with a video camera coupled with Ethovision for 60-min periods in the laboratory. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Letters illustrate multiple comparisons for the interaction among exposure time, susceptibility, and controlled release materials. Abbreviations: Ctrl – control (no insecticide), and LLIN – long-lasting insecticide-netting.

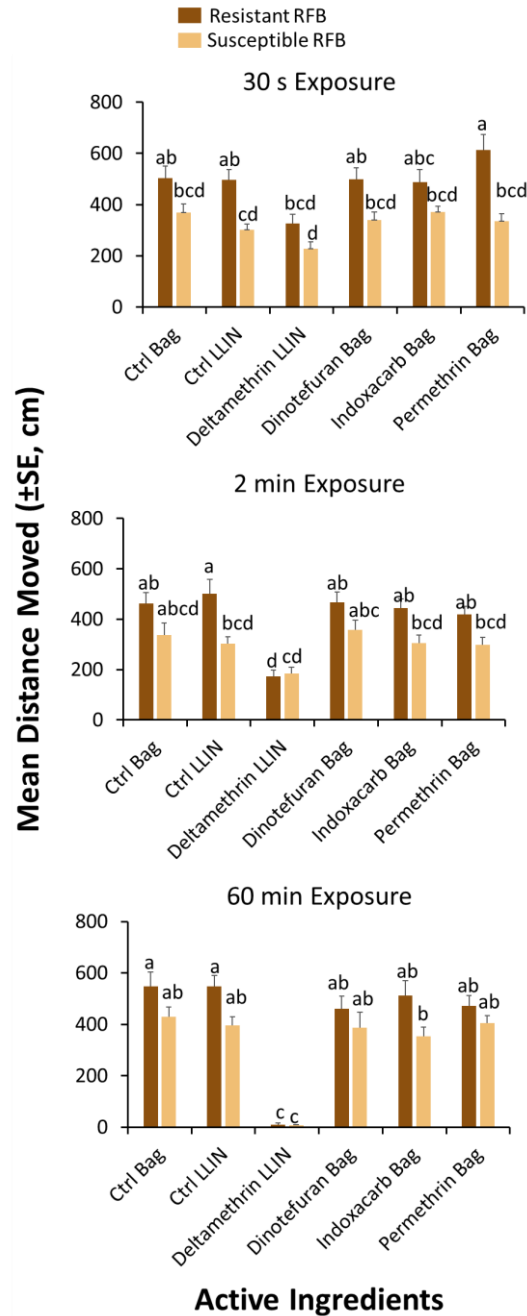


Figure 2.4 Distance moved ($\pm$ SE) by susceptible and resistant *T. castaneum* after varying exposure times to different controlled release materials

Distance moved ($\pm$ SE, cm) by susceptible (light brown) and resistant (dark brown bars) *T. castaneum* (RFB) after varying exposure for 30 s (top panel), 2 min (middle panel), or 60 min (bottom panel) to different controlled release materials in the sublethal movement assay during tracking with a video camera coupled with Ethovision for 60-min periods in the laboratory. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Letters illustrate multiple comparisons for the interaction among exposure time, susceptibility, and controlled release materials. Abbreviations: Ctrl – control (no insecticide), and LLIN – long-lasting insecticide-netting.

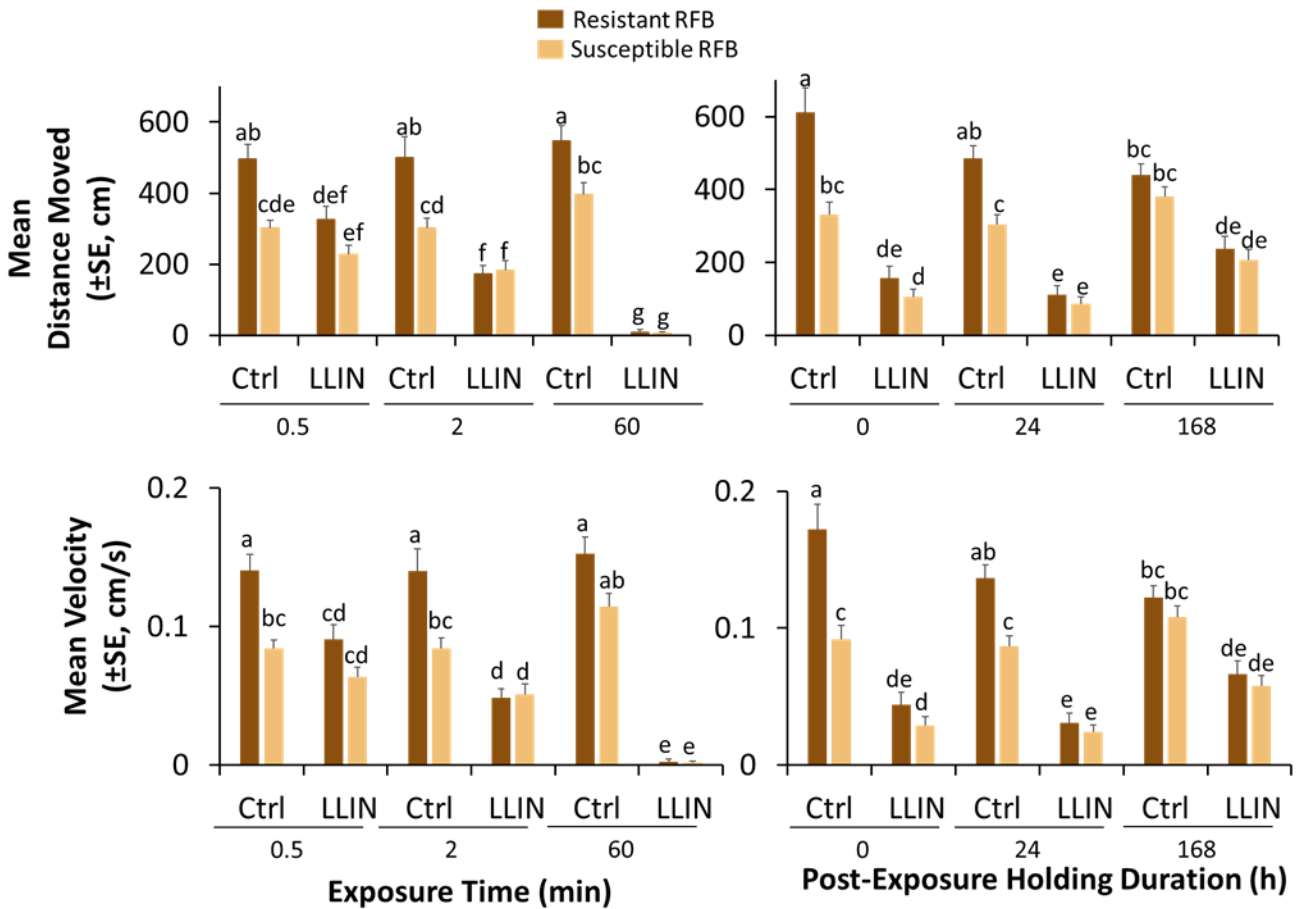


Figure 2.5 Distance moved ($\pm$ SE) or velocity ($\pm$ SE) by susceptible and resistant *T. castaneum* after exposure to LLIN or control netting various exposure times and varying post-exposure holding durations

Distance moved ($\pm$ SE) (top panels) or velocity ($\pm$ SE) (bottom panels) by susceptible (light brown) and resistant (dark brown bars) *T. castaneum* (RFB) after exposure to long-lasting 0.4% deltamethrin-incorporated netting (LLIN) or control netting (Ctrl) in the sublethal movement assay during tracking for 60-min periods on Ethovision after various exposure times (left) and varying post-exposure holding durations (right). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Letters represent multiple comparisons between the interaction among exposure time, susceptibility, and LLIN (left column) or among post-exposure holding duration, susceptibility, and LLIN (right column).

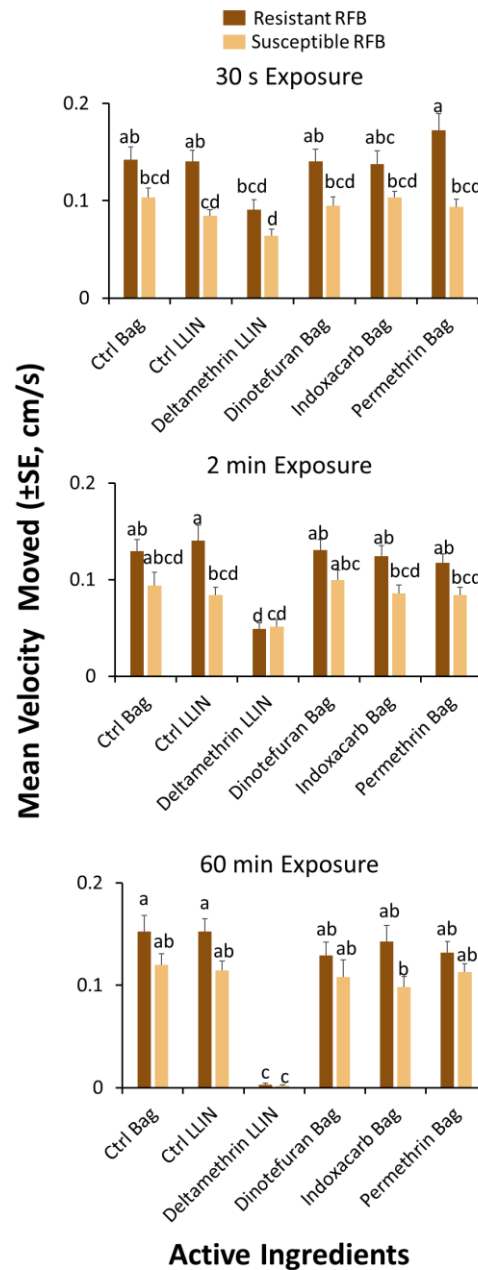


Figure 2.6 Velocity ($\pm$ SE) by susceptible and resistant *T. castaneum* after varying exposure times to different controlled release materials

Velocity ($\pm$ SE, cm/s) by susceptible (light brown) and resistant (dark brown bars) *T. castaneum* (RFB) after varying exposure for 30 s (top panel), 2 min (middle panel), or 60 min (bottom panel) to different controlled release materials in the sublethal movement assay during tracking with a video camera coupled with Ethovision for 60-min periods in the laboratory. Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Letters illustrate multiple comparisons for the interaction among exposure time, susceptibility, and controlled release materials. Abbreviations: Ctrl – control (no insecticide), and LLIN – long-lasting insecticide-netting.

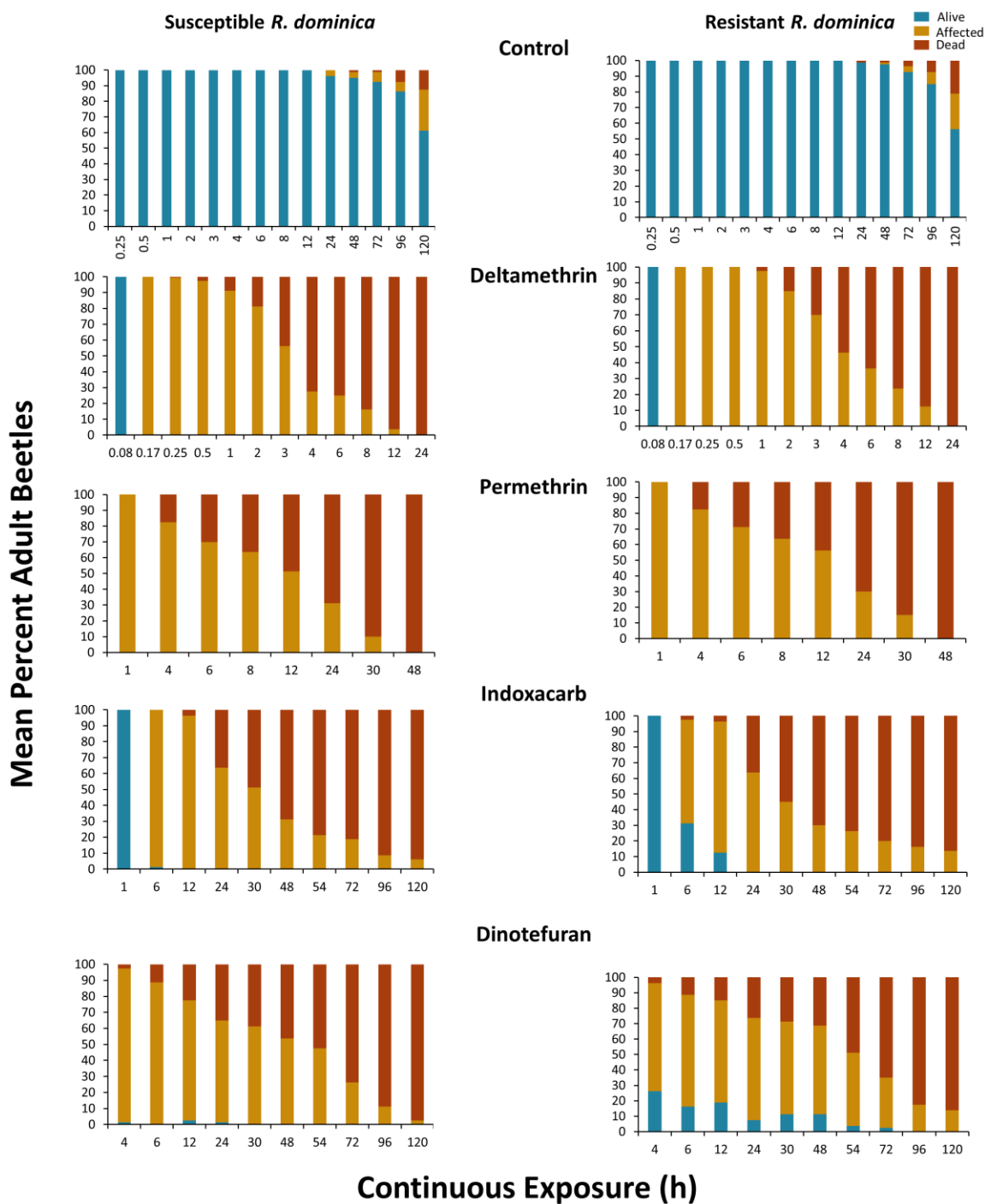


Figure 2.7 Percentage mortality of susceptible and resistant *R. dominica* after exposure to treated cheesecloths with 1% concentration of AIs

The percentage of susceptible (left) and resistant (right) *R. dominica* adults that were alive (turquoise), affected (yellow), or dead (red) after exposure to treated cheesecloths with 1% concentration of AI for deltamethrin, permethrin, dinotefuran, indoxacarb, or solvent only control (e.g., acetone) after various exposure times.

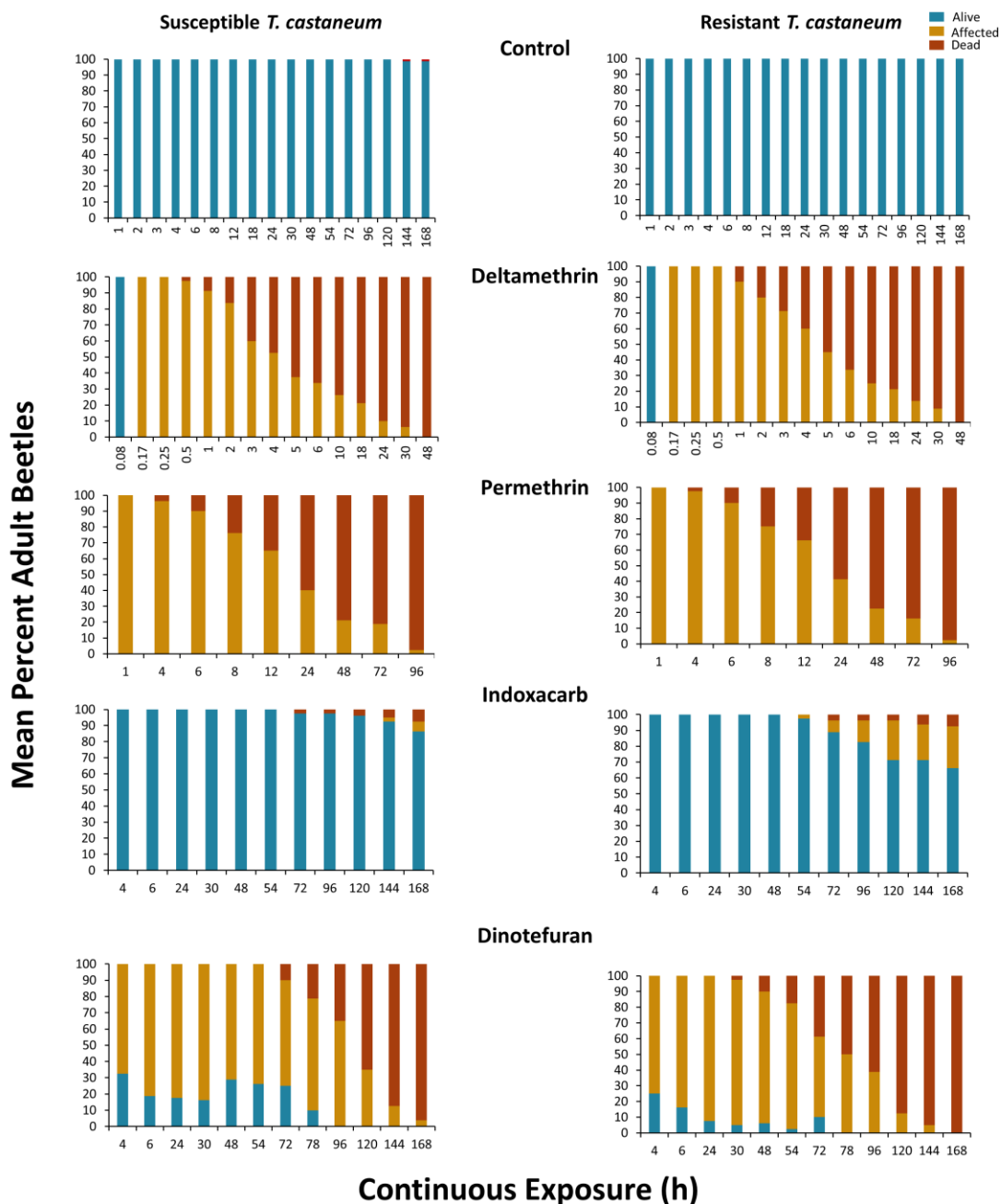


Figure 2.8 Percentage of susceptible and resistant *T. castaneum* after exposure to treated cheesecloth with various 1% concentration of AIs

The percentage of susceptible (left) and resistant (right) *T. castaneum* adults that were alive (turquoise), affected (yellow), or dead (red) after exposure to treated cheesecloth with various 1% concentration of AI for deltamethrin, permethrin, dinotefuran, indoxacarb, or solvent only control (e.g., acetone) after various exposure times.

Tables:

Table 2.1 Summary of AIs used in the time to lethal exposure assay to higher concentrations on cheesecloth.

	Purity (%)	Concentration (mg/ml)	Manufacturer
Deltamethrin	98.9	0.64	Chem Service Inc, West Chester, PA, USA
Indoxacarb	99.5	0.63	Chem Service Inc, West Chester, PA, USA
Permethrin	91.8	0.69	MilliporeSigma Supelco, Milwaukee, WI, USA
Dinotefuran	98	0.65	TCI America, Portland, OR, USA

Table 2.2 Summary of statistical model results for the log of the distance moved by *R. dominica* after exposure to controlled release materials in the sublethal movement assay over a 60-min period.

Variable	Exposure Time-Specific Models ¹								
	Overall model			60 min ²		2 min ³		30 s ⁴	
	Df	F	P	F	P	F	P	F	P
susceptibility	1	0.71	0.40	7.05	0.01	0.85	0.36	0.13	0.71
treatment	5	0.61	0.69	1.54	0.18	1.19	0.31	1.69	0.14
post-exposure	2	4.79	0.01	7.07	0.00	16.54	0.00	1.75	0.18
exposuretime	2	0.14	0.87	-	-	-	-	-	-
susceptibility:treatment	5	1.17	0.32	2.14	0.06	0.78	0.56	0.56	0.73
susceptibility:post-exposure	2	1.44	0.24	0.95	0.39	5.42	0.00	0.66	0.52
treatment:post-exposure	10	9.84	0.00	20.66	0.00	12.58	0.00	20.15	0.00
exposuretime:post-exposure	4	3.69	0.01	-	-	-	-	-	-
susceptibility:exposuretime	2	0.51	0.60	-	-	-	-	-	-
treatment:exposuretime	10	0.64	0.78	-	-	-	-	-	-
susceptibility:treatment:post-exposure	10	3.54	0.00	1.89	0.04	8.62	0.00	6.18	0.00
susceptibility:treatment:exposuretime	10	0.97	0.47	-	-	-	-	-	-
susceptibility:exposuretime:post-exposure	4	0.26	0.91	-	-	-	-	-	-
treatment:exposuretime:post-exposure	20	5.40	0.00	-	-	-	-	-	-
susceptibility:treatment:exposuretime:post-exposure	20	4.65	0.00	-	-	-	-	-	-
Residuals	1523								

¹Models specific to each exposure time do not include exposure time as a fixed, explanatory variable in the model structure, and thus entries containing exposure time have been left blank.

² Residual df is 518

³ Residual df is 498

⁴ Residual df is 507

Table 2.3 Summary of the specific statistical model results for the log distance moved by *R. dominica* after exposure to 0.4% long-lasting deltamethrin-incorporated netting in the sublethal movement assay over a 60-min period.

Variable	Overall model		
	Df	F	P
susceptibility	1	5.51	0.02
treatment	1	0.59	0.44
post-exposure	2	0.80	0.45
exposuretime	2	1.82	0.16
susceptibility:treatment	1	1.36	0.24
susceptibility:post-exposure	2	7.22	0.00
treatment:post-exposure	2	29.70	0.00
exposuretime:post-exposure	4	9.82	0.00
susceptibility:exposuretime	2	4.75	0.01
treatment:exposuretime	2	0.22	0.81
susceptibility:treatment:post-exposure	2	18.85	0.00
susceptibility:treatment:exposuretime	2	3.76	0.02
susceptibility:exposuretime:post-exposure	4	7.10	0.00
treatment:exposuretime:post-exposure	4	14.77	0.00
susceptibility:treatment:exposuretime:post-exposure	4	11.40	0.00
Residuals	506		

Table 2.4 Summary of statistical model results for the log of the velocity moved by *R. dominica* after exposure to controlled release materials in the sublethal movement assay over a 60-min period.

Variable	Exposure Time-Specific Models ¹								
	Overall model			60 min ²		2 min ³		30 s ⁴	
	Df	F	P	F	P	F	P	F	P
susceptibility	1	1.06	0.30	6.78	0.01	0.98	0.32	0.07	0.79
treatment	5	1.73	0.12	2.38	0.04	1.10	0.36	6.71	0.00
post-exposure	2	13.75	0.00	12.28	0.00	11.35	0.00	1.49	0.19
exposuretime	2	0.58	0.56	-	-	-	-	-	-
susceptibility:treatment	5	1.12	0.35	1.15	0.33	1.80	0.11	0.42	0.83
susceptibility:post-exposure	2	0.60	0.55	0.82	0.44	2.92	0.06	1.28	0.28
treatment:post-exposure	10	9.13	0.00	4.23	0.00	3.28	0.00	27.29	0.00
exposuretime:post-exposure	4	3.01	0.02	-	-	-	-	-	-
susceptibility:exposuretime	2	1.85	0.16	-	-	-	-	-	-
treatment:exposuretime	10	0.52	0.87	-	-	-	-	-	-
susceptibility:treatment:post-exposure	10	5.42	0.00	1.80	0.06	9.91	0.00	12.50	0.00
susceptibility:treatment:exposuretime	10	1.47	0.14	-	-	-	-	-	-
susceptibility:exposuretime:post-exposure	4	2.51	0.04	-	-	-	-	-	-
treatment:exposuretime:post-exposure	20	6.85	0.00	-	-	-	-	-	-
susceptibility:treatment:exposuretime:post-exposure	20	6.25	0.00	-	-	-	-	-	-
Residuals	1523								

¹Models specific to each exposure time do not include exposure time as a fixed, explanatory variable in the model structure, and thus entries containing exposure time have been left blank.

² Residual df is 518

³ Residual df is 498

⁴ Residual df is 507

Table 2.5 Summary of the specific statistical model results for the log velocity moved by *R. dominica* after exposure to 0.4% long lasting deltamethrin-incorporated netting in the sublethal movement assay over a 60-min period.

Variable	Overall model		
	Df	F	P
susceptibility	1	5.51	0.02
treatment	1	0.00	0.96
post-exposure	2	2.05	0.13
exposuretime	2	1.07	0.34
susceptibility:treatment	1	3.35	0.07
susceptibility:post-exposure	2	4.14	0.02
treatment:post-exposure	2	6.25	0.00
exposuretime:post-exposure	4	19.04	0.00
susceptibility:exposuretime	2	3.53	0.03
treatment:exposuretime	2	1.08	0.34
susceptibility:treatment:post-exposure	2	14.87	0.00
susceptibility:treatment:exposuretime	2	3.22	0.04
susceptibility:exposuretime:post-exposure	4	10.08	0.00
treatment:exposuretime:post-exposure	4	11.98	0.00
susceptibility:treatment:exposuretime:post-exposure	4	9.48	0.00
Residuals	506		

Table 2.6 Summary of statistical model results for the log of the distance moved by *T. castaneum* after exposure to controlled release materials in the sublethal movement assay over a 60-min period.

Variable	Exposure Time-Specific Models ¹								
	Overall model			60 min ²		2 min ³		30 s ⁴	
	Df	F	P	F	P	F	P	F	P
susceptibility	1	3.50	0.06	0.37	0.54	1.43	0.23	2.24	0.13
treatment	5	4.71	0.00	6.72	0.00	1.54	0.18	2.19	0.05
post-exposure	2	4.47	0.01	0.67	0.51	2.83	0.06	1.32	0.27
exposuretime	2	2.21	0.11	-	-	-	-	-	-
susceptibility:treatment	5	1.82	0.11	2.52	0.03	0.15	0.98	0.88	0.49
susceptibility:post-exposure	2	2.27	0.10	0.10	0.91	1.50	0.22	0.80	0.45
treatment:post-exposure	10	2.58	0.00	1.09	0.36	1.42	0.17	1.26	0.25
exposuretime:post-exposure	4	1.21	0.31	-	-	-	-	-	-
susceptibility:exposuretime	2	0.24	0.78	-	-	-	-	-	-
treatment:exposuretime	10	2.90	0.00	-	-	-	-	-	-
susceptibility:treatment:post-exposure	10	2.64	0.00	4.24	0.00	1.35	0.20	1.70	0.08
susceptibility:treatment:exposuretime	10	0.94	0.49	-	-	-	-	-	-
susceptibility:exposuretime:post-exposure	4	0.81	0.52	-	-	-	-	-	-
treatment:exposuretime:post-exposure	20	1.09	0.35	-	-	-	-	-	-
susceptibility:treatment:exposuretime:post-exposure	20	0.90	0.58	-	-	-	-	-	-
Residuals	1454								

¹Models specific to each exposure time do not include exposure time as a fixed, explanatory variable in the model structure, and thus entries containing exposure time have been left blank.

² Residual df is 491

³ Residual df is 492

⁴ Residual df is 471

Table 2.7 Summary of the specific statistical model results for the log distance moved by *T. castaneum* after exposure to 0.4% long lasting deltamethrin-incorporated netting in the sublethal movement assay over a 60-min period.

Variable	Overall model		
	Df	F	P
susceptibility	1	0.17	0.68
treatment	1	10.13	0.00
post-exposure	2	5.43	0.00
exposuretime	2	2.70	0.07
susceptibility:treatment	1	0.37	0.55
susceptibility:post-exposure	2	1.01	0.36
treatment:post-exposure	2	8.35	0.00
exposuretime:post-exposure	4	1.26	0.28
susceptibility:exposuretime	2	0.42	0.65
treatment:exposuretime	2	3.22	0.04
susceptibility:treatment:post-exposure	2	0.85	0.43
susceptibility:treatment:exposuretime	2	1.13	0.32
susceptibility:exposuretime:post-exposure	4	0.12	0.97
treatment:exposuretime:post-exposure	4	3.67	0.01
susceptibility:treatment:exposuretime:post-exposure	4	0.49	0.74
Residuals	750		

Table 2.8 Summary of overall statistical model results for the log of the velocity moved by *T. castaneum* after exposure to various controlled release materials with AI concentrations 0.1–0.4% in the sublethal movement assay over a 60-min period.

Variable	Exposure Time-Specific Models ¹								
	Overall model			60 min ²		2 min ³		30 s ⁴	
	Df	F	P	F	P	F	P	F	P
susceptibility	1	1.63	0.20	0.99	0.32	0.83	0.36	3.77	0.05
treatment	5	5.41	0.00	5.89	0.00	2.62	0.02	2.42	0.03
post-exposure	2	2.69	0.07	0.50	0.61	1.59	0.20	2.30	0.10
exposuretime	2	2.71	0.07	-	-	-	-	-	-
susceptibility:treatment	5	0.82	0.54	3.96	0.00	0.22	0.96	0.84	0.53
susceptibility:post-exposure	2	0.99	0.37	0.38	0.69	0.80	0.45	1.57	0.21
treatment:post-exposure	10	2.48	0.01	2.39	0.01	1.37	0.19	1.44	0.16
exposuretime:post-exposure	4	1.98	0.10	-	-	-	-	-	-
susceptibility:exposuretime	2	0.86	0.42	-	-	-	-	-	-
treatment:exposuretime	10	2.96	0.00	-	-	-	-	-	-
susceptibility:treatment:post-exposure	10	1.32	0.21	2.73	0.00	0.96	0.47	1.88	0.05
susceptibility:treatment:exposuretime	10	1.53	0.12	-	-	-	-	-	-
susceptibility:exposuretime:post-exposure	4	1.64	0.16	-	-	-	-	-	-
treatment:exposuretime:post-exposure	20	1.15	0.29	-	-	-	-	-	-
susceptibility:treatment:exposuretime:post-exposure	20	0.98	0.48	-	-	-	-	-	-
Residuals	1454								

¹Models specific to each exposure time do not include exposure time as a fixed, explanatory variable in the model structure, and thus entries containing exposure time have been left blank.

² Residual df is 491

³ Residual df is 492

⁴ Residual df is 471

Table 2.9 Summary of the specific statistical model results for the log velocity moved by *T. castaneum* after exposure to 0.4% long lasting deltamethrin-incorporated netting in the sublethal movement assay over a 60-min period.

Variable	Overall model		
	Df	F	P
susceptibility	1	0.02	0.89
treatment	1	5.87	0.02
post-exposure	2	5.68	0.00
exposuretime	2	2.73	0.07
susceptibility:treatment	1	0.08	0.78
susceptibility:post-exposure	2	1.74	0.18
treatment:post-exposure	2	13.11	0.00
exposuretime:post-exposure	4	2.39	0.05
susceptibility:exposuretime	2	0.56	0.57
treatment:exposuretime	2	2.73	0.07
susceptibility:treatment:post-exposure	2	1.19	0.30
susceptibility:treatment:exposuretime	2	2.84	0.06
susceptibility:exposuretime:post-exposure	4	0.70	0.59
treatment:exposuretime:post-exposure	4	7.85	0.00
susceptibility:treatment:exposuretime:post-exposure	4	7.03	0.00
Residuals	750		

Table 2.10 Comparison of model results for time when 50% of *R. dominica* adults have been killed when continuously exposed to cheesecloth treated with 1% concentration of each AI.

Treatment	Susceptibility	LT₅₀ (h)	95% CI			Slope	±	SE	R²	χ²	df	P
Deltamethrin	Susceptible	3.37	3.00	-	3.73	1.07	±	0.10	0.86	13.92	18	0.73
	Resistant	4.47	4.03	-	4.99	1.05	±	0.06	0.95	5.62	18	0.99
Permethrin	Susceptible	11.29	9.91	-	12.90	0.77	±	0.05	0.92	14.63	22	0.88
	Resistant	11.98	0.46	-	13.81	0.74	±	0.04	0.94	9.42	22	0.99
Dinotefuran	Susceptible	43.98	38.56	-	49.97	0.88	±	0.05	0.86	15.92	22	0.94
	Resistant	58.01	52.11	-	65.27	0.92	±	0.09	0.82	15.49	22	0.84
Indoxacarb	Susceptible	31.67	27.02	-	35.57	0.99	±	0.08	0.91	5.76	18	1.00
	Resistant	30.44	24.97	-	34.80	0.88	±	0.12	0.76	13.22	18	0.78

Table 2.11 Comparison of model results for time when 50% of *T. castaneum* adults have been killed when continuously exposed to cheesecloth treated with 1% concentration of each AI

Treatment	Susceptibility	LT ₅₀ (h)	95% CI			Slope	±	SE	R ²	χ ²	df	P
Deltamethrin	Susceptible	4.46	3.81	-	5.13	0.64	±	0.08	0.72	34.28	26	0.13
	Resistant	4.98	4.34	-	5.7	0.68	±	0.06	0.82	22.26	26	0.67
Permethrin	Susceptible	19.18	15.88	-	22.78	0.64	±	0.03	0.95	4.32	18	0.9996
	Resistant	19.19	15.96	-	22.73	0.65	±	0.04	0.94	6.57	18	0.99
Dinotefuran	Susceptible	104.02	99.49	-	108.98	2.49	±	0.30	0.79	21.83	18	0.24
	Resistant	80.00	75.69	-	84.48	1.98	±	0.16	0.89	15.86	18	0.60

Chapter 3 - Disruption of semiochemical-mediated movement by the immature *Trogoderma variable* Baillon and *Trogoderma inclusum* Le Conte (Coleoptera: Dermestidae) after exposure to long-lasting insecticide netting

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<https://doi.org/10.1002/ps.7903>

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 post-harvest supply chain. A 0.4% deltamethrin-incorporated long-lasting insecticide 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 larger cabinet beetle, *T. inclusum* LeConte (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 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% immediately after 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.

Keywords: Dermestids, movement, behavior, olfaction, integrated pest management

Introduction

The agricultural sector makes a significant contribution to the economy, and the post-harvest 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 2–50% of their harvested crops due to stored product insect infestations, which leads to an economic loss of over \$100 billion USD globally.³ Therefore, it is 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 an insects within a system, employing insecticides as a final resort.⁴ Movement of insects has always been an ongoing challenge to successful integrated pest management programs (IPM). 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 integrated pest management 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,¹⁵ awnings on grain silos,¹⁶ and pallets in warehouses.¹⁷

LLIN incorporated with deltamethrin or alpha-cypermethrin has been highly effective against numerous important stored product insects.^{12–14,18–21} 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*,²² and khapra beetle *T. granarium*.²³ 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),¹⁹ and larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae).²⁰ 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).²⁰ Ranabhat *et al.*²⁴ 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.²⁴

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. variable* 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. variable* 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 tunnel²³ and found interacting effects related to thigmotaxis and anemotaxis. In addition, Morrison *et al.*²⁵ 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. variable* 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. variable* 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.²⁶ Both species have short lifespans as an adult,²⁷ thus the larval stage comprises the majority of the life cycle, causing most of the damage.²⁸ 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 *et al.*²⁹ demonstrated how exposure to LLIN affected the movement and olfaction in the presence of important semiochemicals to different sexes of adult *T. variable* and *T. inclusum*. Our aim was to evaluate whether immature dermestids, including larval *T. variable* 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.

Materials and methods

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% RH, and 14:10 (L:D) h 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.

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, multi-species lure (PTL lure, IL-108-10, Batch#1288200321, Insects Limited, Westfield, IN, USA), including *Trogoderma* spp pheromone, (Z)-14-methyl-8-hexadecenal,²⁹ which is female-produced and cross-attractive to both species.³⁰ 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.

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 D: H) with a piece of filter paper (85 mm D, 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 W: 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. variable* or *T. inclusum* were exposed to the 0.4% deltamethrin LLIN or a control netting for 1 min in a 21 × 21 cm square Petri dish, then their movement was tracked individually after a post-exposure holding duration of 1 min or 24 h to assess immediate vs. 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.

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% deltamethrin LLIN and control netting for 1 min. After exposure, treated insects were released at one corner of the sanded plastic bin ($60 \times 41.6 \times 16.5$ cm L:W:H). 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 ($4.8 \times 2.1 \times 6$ m, L:W:H) walk-in environmental chamber (Percival Instruments, Dallas County, IA, USA) set at constant conditions (27.5°C, 60% RH, and 14:10 L:D). 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 non-stimulus half of the container were recorded. A total of $n = 12$ replicates were performed per treatment combination for the larvae of each species.

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 post-exposure 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 upon 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 overdispersion was a problem), with the same model structure as above. All analyses were run using R software,³¹ with $\alpha=0.05$, and Tukey HSD for multiple comparisons. Finally, we have used Student's t test for select post-hoc comparisons between treatment and control zones in the movement assay, denoted in figures with asterisks.

Results

***Trogoderma variable* movement**

None of the main effects (e.g., exposure to LLIN, semiochemical presence, or postexposure holding duration) significantly affected the distance moved or velocity of larval *T. variable* (Table 3.1, Table 3.2). The 2-way interactions of netting with the treatment or post-exposure holding duration and 3-way interaction significantly affected the distance and velocity moved by immature *T. variable* (Table 3.1, Table 3.2). 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 control netting (Figure 3.1, Tukey HSD). Similarly, the velocity was reduced by 62% in the presence of food 24 h after exposure to LLN compared to control netting (Figure 3.1).

Behavior of immatures *T. variable*

Frequency of entering each zone

Semiochemical treatment, exposure to netting, and post-exposure 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 3.3). 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 3.3, Figure 3.2). Further, post-exposure 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 3.3). 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 (Figure 3.2). The two-way interaction between semiochemical treatment and post-exposure 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 (Figure 3.3).

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 post-exposure holding duration (Table 3.4). The cumulative duration spent in each zone was also significantly impacted by each two-way interaction among those variables (Table 3.4). 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 3.4). 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 (Figure 3.2). 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 post-exposure 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. Further, 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 (Figure 3.2). Further, the cumulative duration spent by immature *T. variable* 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 (Figure 3.3). Additionally, the cumulative duration spent in the treatment half zone baited with pheromone was increased by 1.4 -fold 24 h after exposure to LLIN compared to the control netting (Figure 3.3).

Latency to each zone

For immature *T. variable*, the latency to finding each zone was significantly affected by the semiochemical treatment, exposure to netting, and post-exposure holding duration (Table 3.5). There was a 1.5-fold increase and an 18% reduction in the time it took *T. variable* larvae to find the treatment and control stimulus zones, respectively, at 24 h compared to 1 min (Figure 3.3). The latency to first entry of the control side of the arena was 9-fold greater in the presence of food by *T. variable* larvae after exposure to control netting compared to the LLIN (Figure 3.3; t-test: $t = 4.85$, $df = 26$, $P < 0.0001$). 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 (Figure 3.3). Additionally, 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 (Figure 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 3.6; Figure 3.4).

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 (Figure S3-1). 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 (Figure 3.4). 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 were 6-7-fold more larvae underneath in the control and pheromone treatment, respectively, compared to the traps with food after exposure to LLIN (Figure 3.4).

***Trogoderma inclusum* movement**

The main effects of semiochemical treatment, exposure to netting, and post-exposure holding duration significantly affected the distance moved by immature *T. inclusum* (Table 3.1). 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 immediately after exposure to LLIN compared to the control netting (Figure 3.5). However,

the velocity moved by the *T. inclusum* larvae was not significantly affected by any of the main effects nor any of the interactions among the variables (Table 3.2; Figure 3.5).

Behavior of *T. inclusum*

Frequency of entering each zone

For *T. inclusum* larvae, the frequency of entering each zone was significantly affected by the semiochemical present and post-exposure holding duration (Table 3.3). In addition, the 3-way interaction among all the main factors including semiochemical treatment, exposure to netting, and post-exposure holding duration, significantly affected the frequency of entering each zone (Table 3.3). 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 post-exposure 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 (Figure 3.6 and Figure 3.7). 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 (Figure 3.6). 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 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 (Figure 3.7).

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 3.4). However, none of the interactions except treatment by netting significantly affected the cumulative duration spent in each zone (Table 3.4). 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 (Figure 3.6). Further, 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 a 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 (Figure 3.6). After 24 h, there was a 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 (Figure 3.6). Further, 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 (Figure 3.6). 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 (Figure 3.7).

Latency treatment, control stimulus and half zone

All the main effects, including semiochemical treatment, exposure to netting, and post-exposure exposure holding duration, significantly affected the latency to finding each zone by larval populations of *T. inclusum* (Table 3.5). Further, the two-way interaction between semiochemical treatment and post-exposure 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 (Figure 3.7), but this was no longer statistically significant after exposure to LLIN. Further, 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 (Figure 3.7).

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 3.6; Figure 3.4). There was a 96% decrease in the capture of *T. inclusum* larvae in traps after exposure to LLIN compared to control netting (Figure 3.4). Exposure to LLIN led to decreased movement and significantly reduced capture of larval *T. inclusum* by all traps compared to control netting (Figure S3-1). There were 2.9-fold more *T. inclusum* larvae on the non-stimulus 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 (t 3.4). There were 7.2-fold more larvae underneath the trap after exposure to control netting compared to after exposure to LLIN.

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 (Figure 3.8). In particular, here 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* larvae, while the overall movement of *T. inclusum* larvae was much 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 m² when provisioned with food were found to be more effective at inducing mortality against adults of *T. variabile* and *T. inclusum* than larvae.²⁶ 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 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.³² 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.²⁵ 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.*²⁹ found that the olfaction of adult *T. inclusum* was more affected than *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.

Additionally, 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.²³

In addition, exposure to cuticular extracts from conspecifics resulted in aversion by *T. inclusum*, likely due to the presence of oleic acid.³³ 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 9:00-19:00 under constant conditions ($27.5 \pm 0.03^{\circ}\text{C}$, $65 \pm 3\%$ 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 hours, 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.³⁴ 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.³⁵ In that study, they found between 0–40% 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.³⁴ Prior work has

found that *Trogoderma* can be the predominate species in a food facilities, 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 two years, a prior study captured 4418 *T. inclusum*, including a few larvae in traps.³⁵ In the US, out of seven dried distillers grains (DDGS) facilities, heavy *T. variabile* infestations were found at all of them and heavy infestations of *T. inclusum* were found at five facilities.³⁶ 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.³⁷ 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.³⁸ As most chemosensation happens through nerve-mediated signals from the antennae in insects, and most larval dermestidae, including *Trogoderma* spp. have antennae and sensoria,³⁹ 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^{7,17}. 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, but it is possible the inclusion of other types of cues could have resulted in similar or different outcomes in the 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. variable* at high concentrations, but it was attractive at low concentrations to *T. variable*.³³ While necromones did not reduce attraction to traps with conspecifics, they did not hinder attraction and may enhance it in some cases by *T. variable* and *T. inclusum*.⁴⁰ In other situations, necromones may have little effect on the stability of the volatile emissions from traps.⁴¹ 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.*)⁴², to which stored product insects may have a conserved response,⁴³ and interspecific insect cues (e.g., Athanassiou *et al.*⁴⁴ Quellhorst *et al.*⁴⁵). 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.⁴⁶ Indeed, Quellhorst *et al.*⁴⁵ 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 includes 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 for 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.

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Tables:

Table 3.1 Summary of statistical model results for the distance moved by larval dermestids after exposure to 0.4% deltamethrin incorporated LLIN 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.

Variable	ANOVA		
	df	F	P
<i>Larval T. variabile</i>			
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		
<i>Larval T. inclusum</i>			
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 3.2 Summary of statistical model results for the instantaneous velocity moved by larval dermestids after exposure to 0.4% deltamethrin incorporated LLIN 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.

Variable	ANOVA		
	df	F	P
Larval <i>T. variabile</i>			
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 <i>T. inclusum</i>			
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		

Table 3.3 Summary of statistical model results for the frequency of entering each zone by larval dermestids after exposure to 0.4% deltamethrin incorporated LLIN 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.

Variable	Sequential ANOVAs										
	MANOVA			Freq. of Stimulus Trt Zone		Freq. of Stimulus Ctrl Zone		Freq. of Half Trt Zone		Freq. of Half Ctrl Zone	
	df	F	P	F	P	F	P	F	P	F	P
Immature <i>T. variabile</i>											
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 <i>T. inclusum</i>											
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										

¹Abbreviations: Freq. – Frequency, Trt – Treatment, Ctrl – Control

Figure S3-1

Table 3.4 Summary of statistical model results for the cumulative duration spent in each zone by larval dermestids after exposure to 0.4% deltamethrin incorporated LLIN 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.

Variable	MANOVA			Sequential ANOVAs							
	df	F	P	Cum. Dur. Stimulus Trt Zone		Cum. Dur. Stimulus Ctrl Zone		Cum. Dur. in Half Trt Zone		Cum. Dur. in Half Ctrl Zone	
				F	P	F	P	F	P	F	P
Larval <i>T. variabile</i>											
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 <i>T. inclusum</i>											
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										

¹Abbreviations: Cum. – Cumulative, Dur. – Duration, Trt – Treatment, Ctrl – Control

Table 3.5 Summary of statistical model results for the latency to finding each zone by larval dermestids after exposure to 0.4% deltamethrin incorporated LLIN 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.

in *Trachinotus*, Rev.

				Sequential ANOVAs							
				Lat. to Stimulus Trt Zone		Lat. to Half Trt Zone		Lat. to Stimulus Ctrl Zone		Lat. to Half Ctrl Zone	
MANOVA											
Variable	df	F	P	F	P	F	P	F	P	F	P
Larval <i>T.variabile</i>											
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 <i>T.inclusum</i>											
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										

¹Abbreviations: Lat. – Latency, Trt – Treatment, Ctrl – Control

Table 3.6 Summary of statistical model results for the release-recapture assay using larval dermestids after exposure to 0.4% deltamethrin incorporated LLIN 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.

					Sequential ANOVAs							
					Stimulus Half		Underneath Trap		Captured in Trap		Non-Stimulus Half	
MANOVA												
Variable	df	den df	Approx. F	P	F	P	F	P	F	P	F	P
<i>T. variabile</i>												
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											
<i>T. inclusum</i>												
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											

Figures:

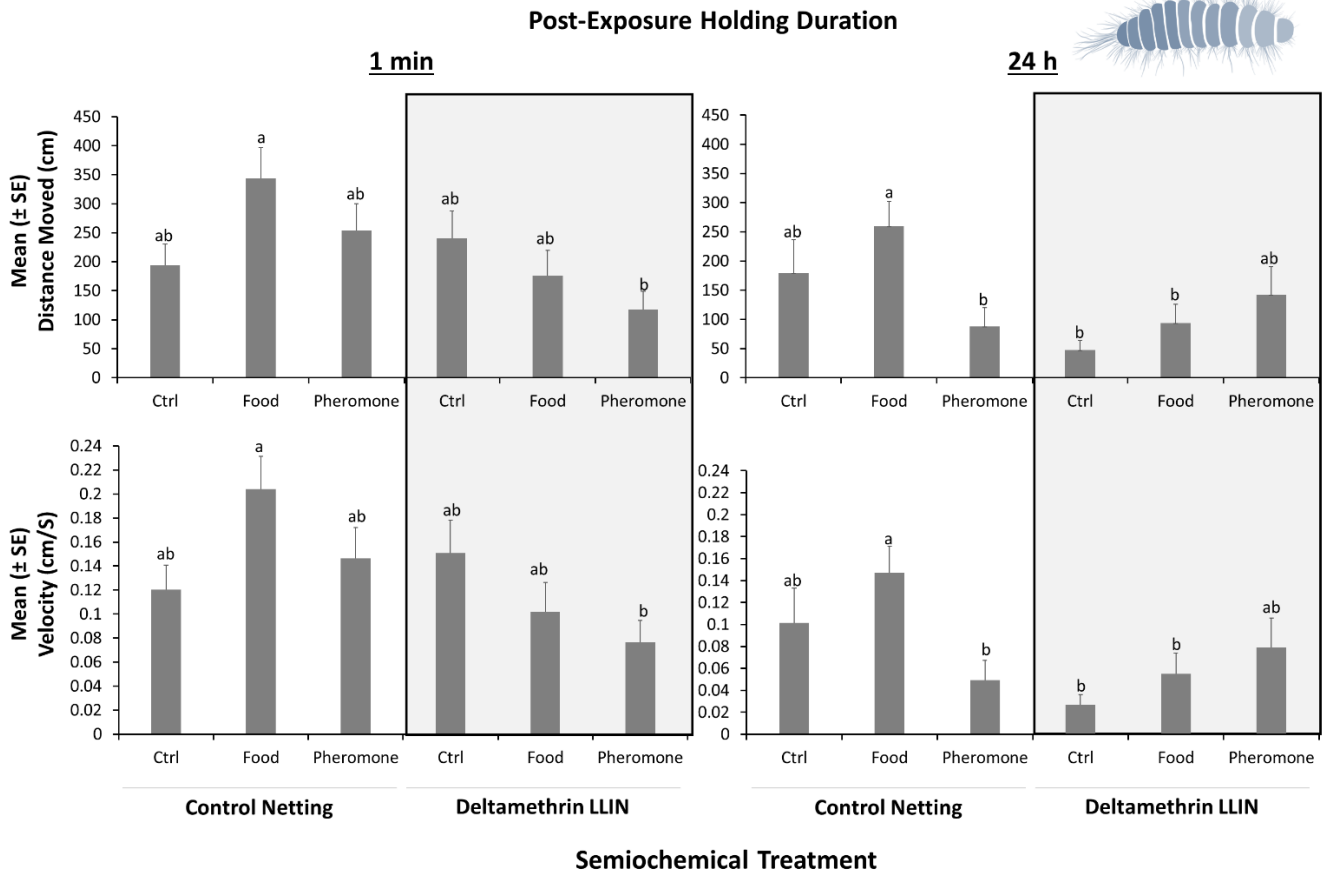


Figure 3.1 Distance moved ($\pm$ SE) or instantaneous velocity ($\pm$ SE) by the larval *T. variable* after exposure to control netting or to LLIN in the presence of semiochemicals

Distance moved ($\pm$ SE, cm) (top panels) or instantaneous velocity ($\pm$ SE, cm/s) (bottom panels) by larval *T. variable* after 1 min of exposure to control netting without insecticide or to 0.4% deltamethrin-incorporated long-lasting 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, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments.

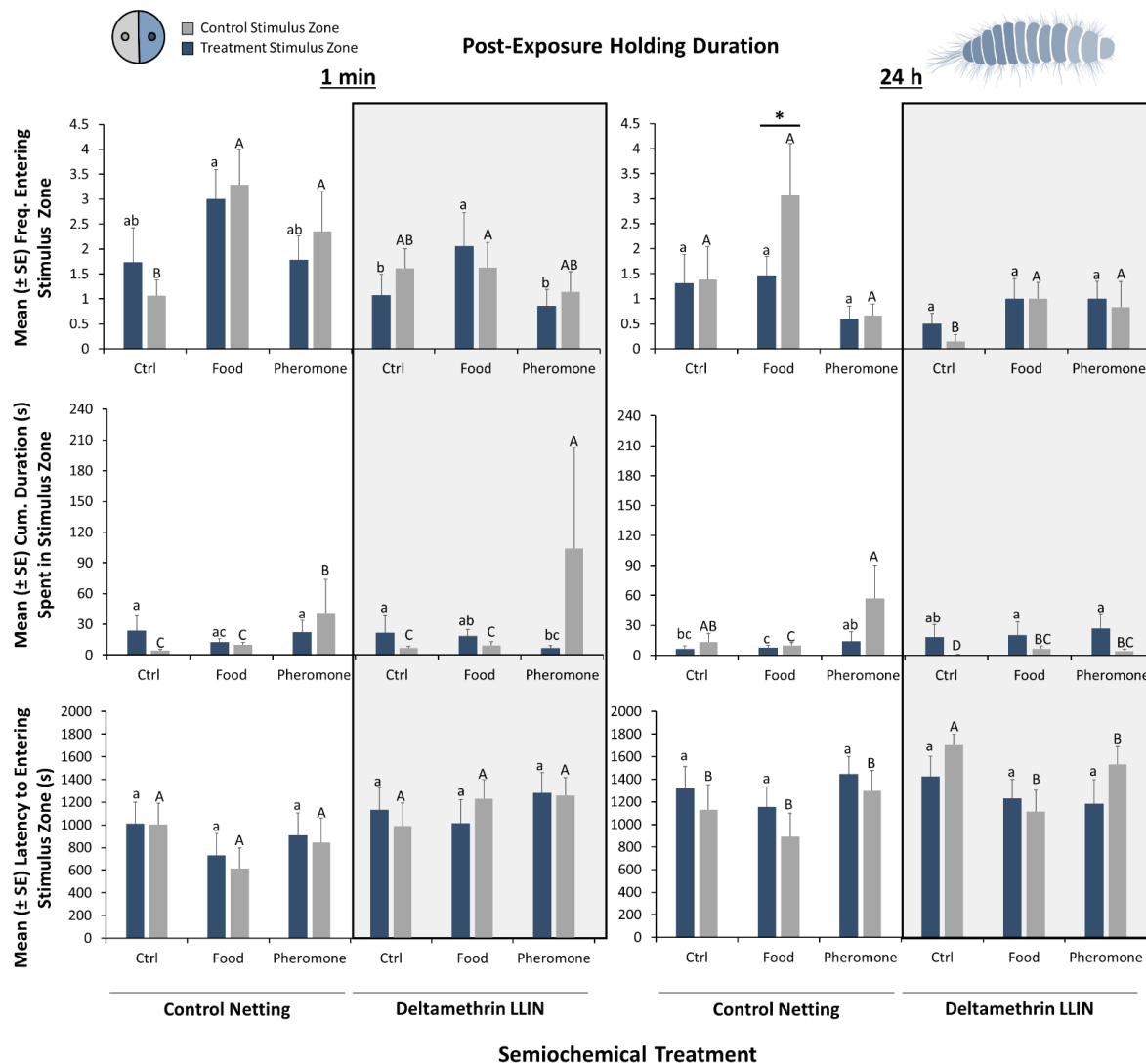


Figure 3.2 Frequency of entering ($\pm$ SE), cumulative duration spent in ($\pm$ SE) or latency to finding the treatment stimulus zone or control stimulus zone by larval *T. variabile* after exposure to the control netting and LLIN in the presence of important semiochemicals. Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels) or latency to finding (bottom panels) the treatment stimulus zone (blue bars) or control stimulus zone (gray bars) by larval *T. variabile* after 1-min exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting 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). Shaded grey area represents LLIN-exposed treatments.

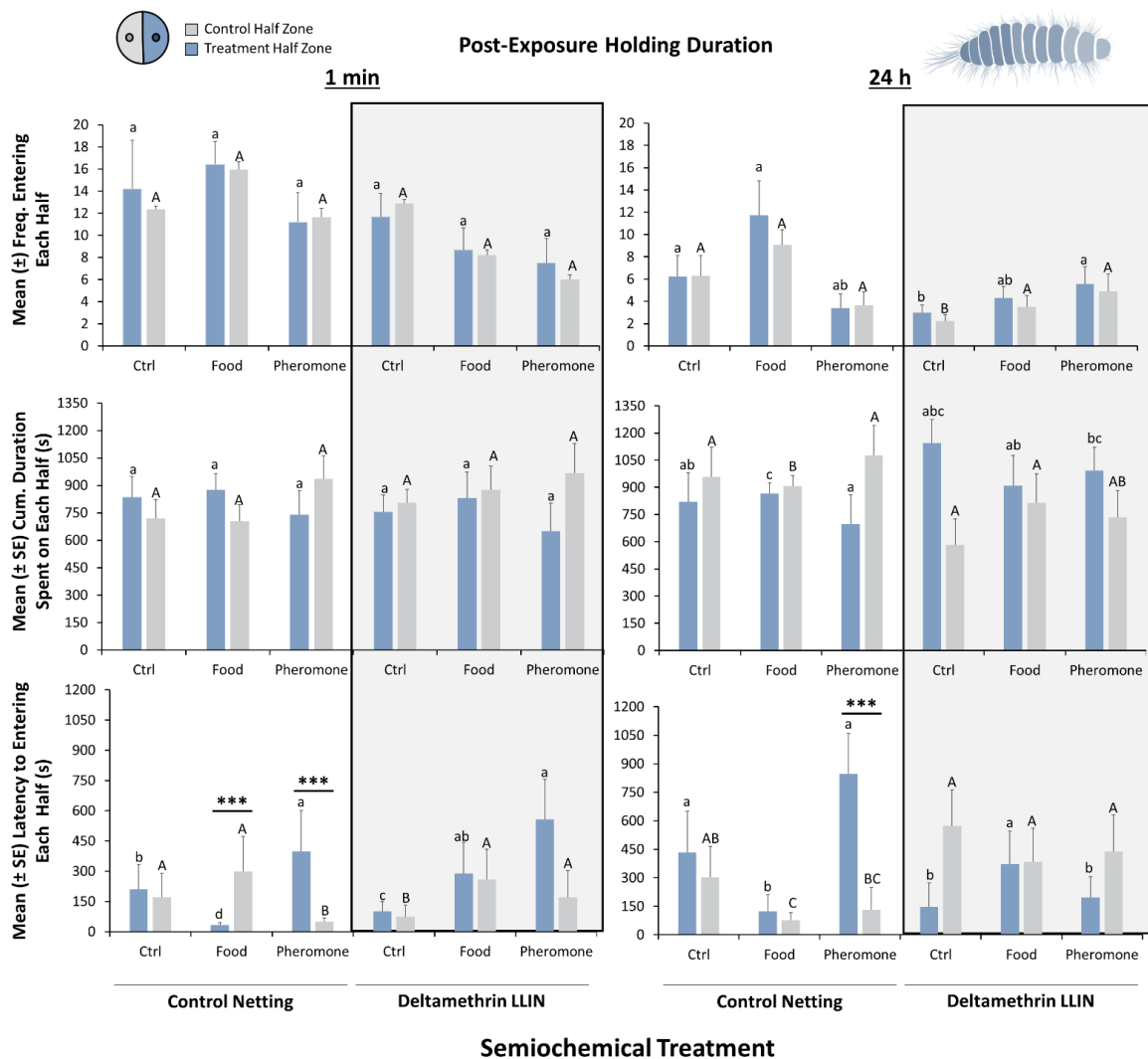


Figure 3.3 Frequency of entering ($\pm$ SE), cumulative duration spent in ($\pm$ SE) or latency to finding the treatment half or control half of the Petri dish by larval *T. variabile* after exposure to control netting or LLIN in the presence of important semiochemicals

Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ 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 *T. variabile* after 1-min exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting 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). Shaded grey area represents LLIN-exposed treatments.

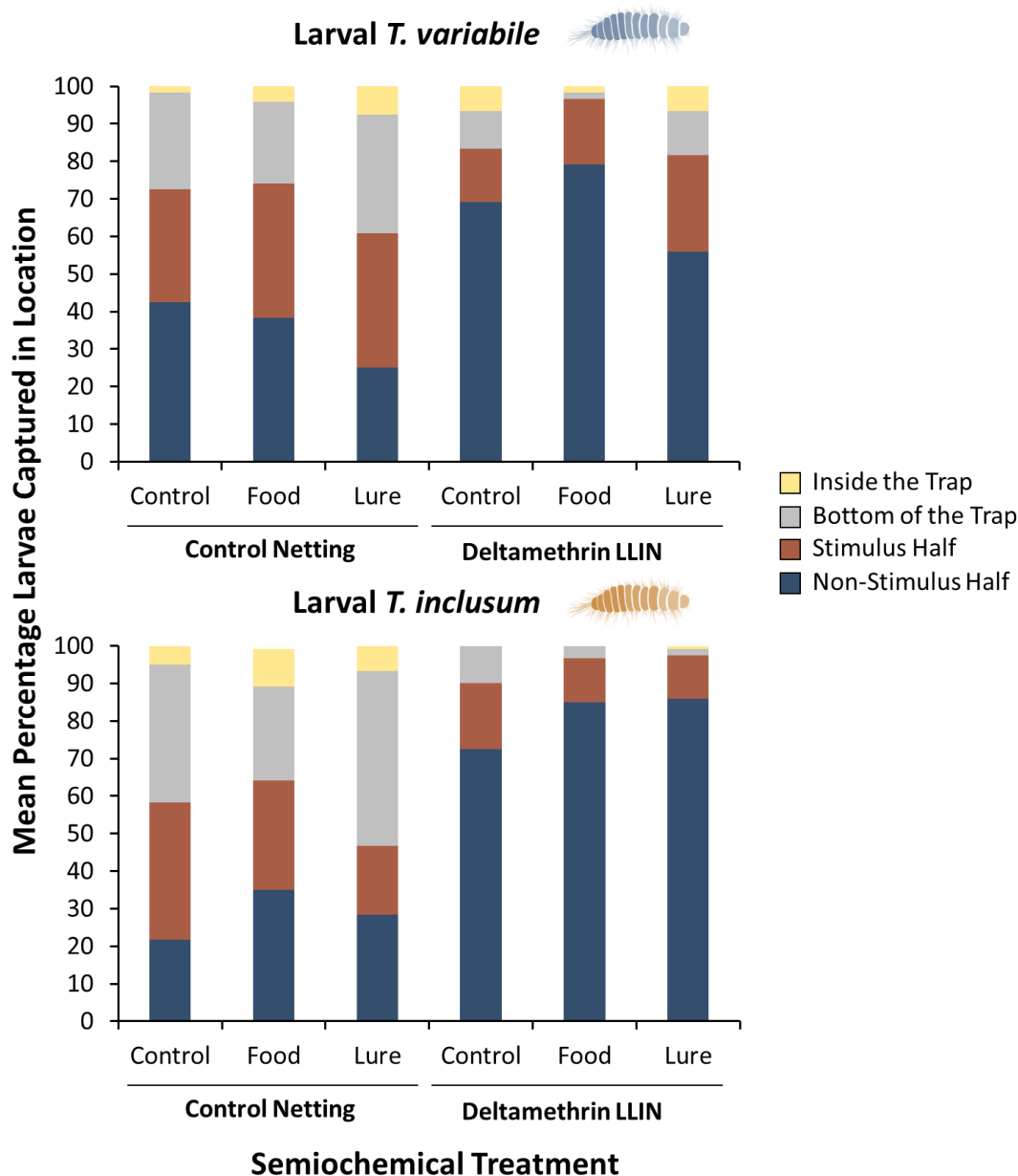


Figure 3.4 Mean percentage of recaptured larval *T. variabile* and larval *T. inclusum* after 1-min exposure to control netting or LLIN in a release-recapture assay

Mean percentage of larval *T. variabile* (top panel) and larval *T. inclusum* (bottom panel) recaptured inside the trap (yellow), underneath the trap (gray), on the stimulus half of the container (red), or the non-stimulus half of the container (blue) after 1-min exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting 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.

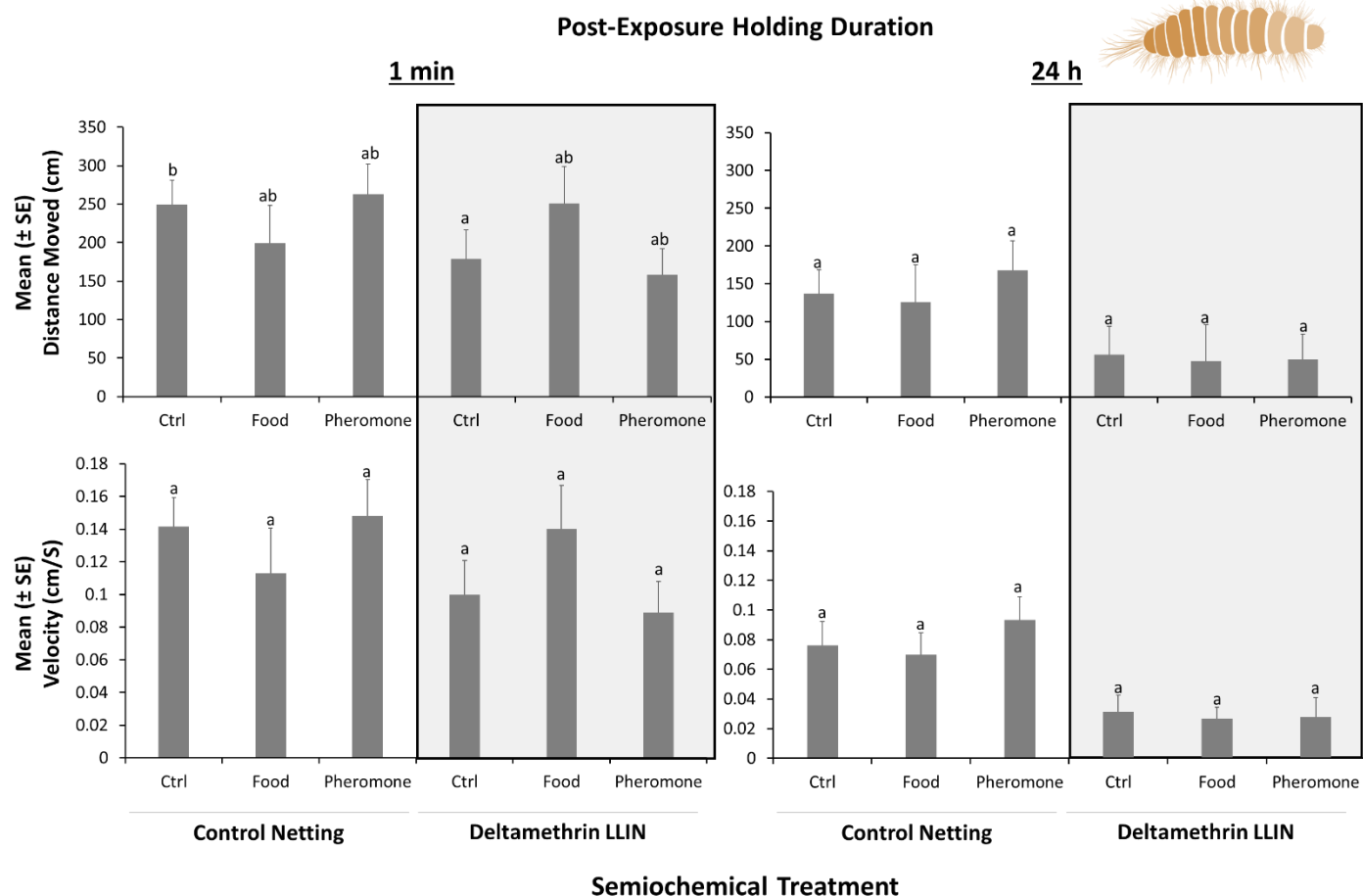


Figure 3.5 Distance moved ($\pm$ SE) or instantaneous velocity ($\pm$ SE) by the larval *T. inclusum* after exposure to control netting or to LLIN in the presence of semiochemicals

Distance moved ($\pm$ SE, cm) (top panels) or instantaneous velocity ($\pm$ SE, cm/s) (bottom panels) by larval *T. inclusum* after 1 min of exposure to control netting without insecticide or to 0.4% deltamethrin-incorporated long-lasting 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, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments.

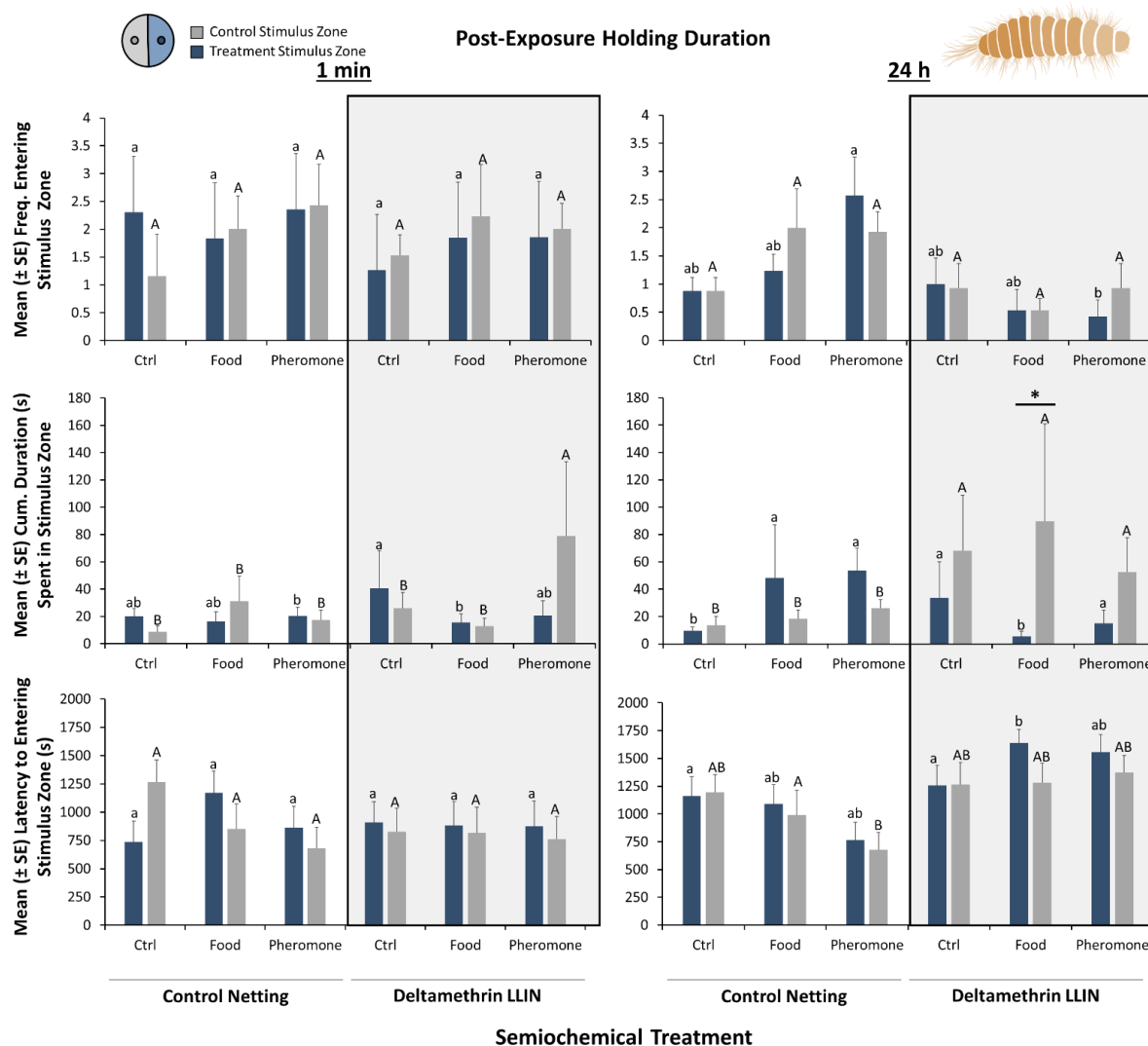


Figure 3.6 Frequency of entering ($\pm$ SE), cumulative duration spent in ($\pm$ SE) or latency to finding the treatment stimulus zone or control stimulus zone by larval *T. inclusum* after exposure to the control netting and LLIN in the presence of important semiochemicals. Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels) or latency to finding (bottom panels) the treatment stimulus zone (blue bars) or control stimulus zone (gray bars) by larval *T. inclusum* after 1-min exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting 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). Shaded grey area represents LLIN-exposed treatments.

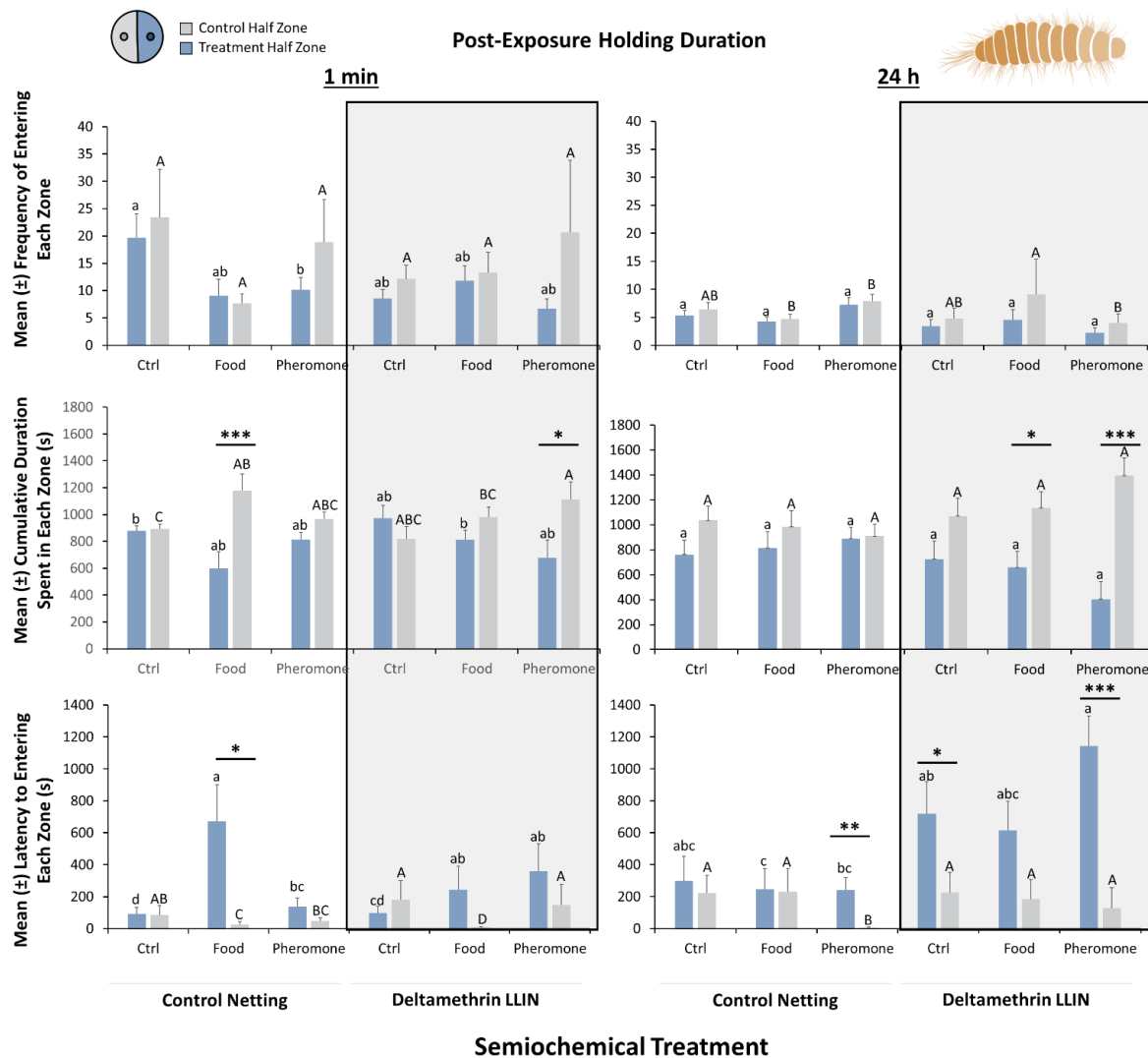


Figure 3.7 Frequency of entering ($\pm$ SE), cumulative duration spent in ($\pm$ SE) or latency to finding the treatment half or control half of the Petri dish by larval *T. inclusum* after exposure to control netting or LLIN in the presence of important semiochemicals

Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ 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 *T. inclusum* after 1-min exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting 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). Shaded grey area represents LLIN-exposed treatments.

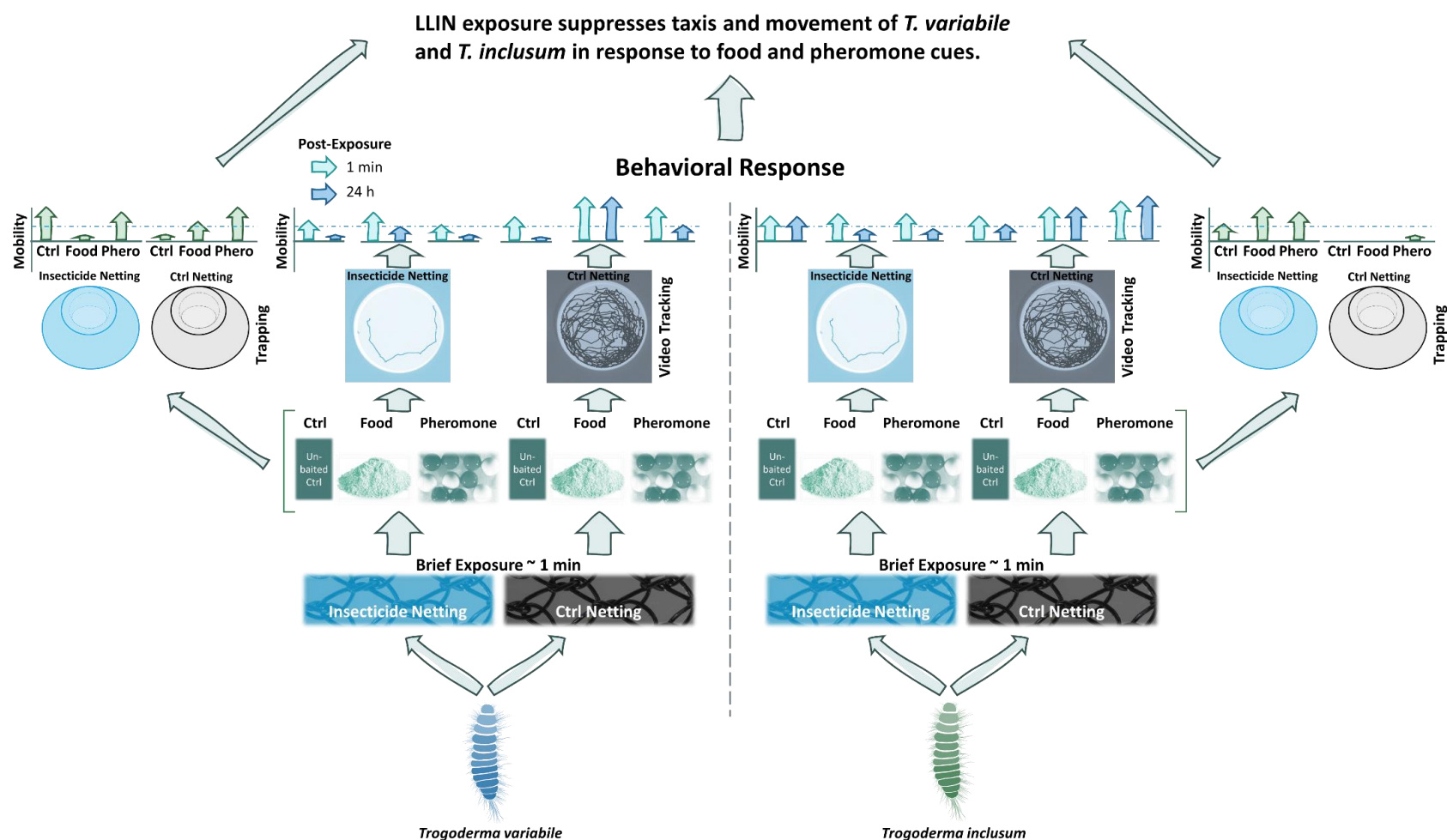


Figure 3.8 Synthesis schematic of experimental design, materials and methods, and main results from study testing larval *Trogoderma* spp. after exposure to control netting or LLIN in the presence of important semiochemicals
 Synthesis schematic of experimental design, materials and methods, main results, and take-home conclusions from manuscript testing larval *T. variable* and *T. inclusum* after exposure to control netting or LLIN 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.

Chapter 4 - Sex-linked differences in semiochemical-mediated movement by *Trogoderma variable* Ballion and *Trogoderma inclusum* LeConte (Coleoptera: Dermestidae) after exposure to long-lasting insecticide-incorporated netting

Submitted as: Ranabhat, S., M.J. Domingue, I. Stoll, G.V. Bingham, K.Y. Zhu, and W.R.

Morrison III. 2024. Sex-linked differences in semiochemical-mediated movement by *Trogoderma variable* Baillon and *Trogoderma inclusum* Le Conte (Coleoptera: Dermestidae) after exposure to long-lasting insecticide netting. Journal of Stored Products Research.

<https://doi.org/10.1016/j.jspr.2024.102415>

Abstract

Stored product insects are highly mobile and present in the landscape and can disperse by tracking odors of food and pheromones. A novel preventative integrated pest management (IPM) tactic to intercept dispersing insects of durable commodities (e.g., packaged goods, flour, bulk commodities), is a long-lasting insecticide-incorporated netting (LLIN) containing 0.4% deltamethrin. However, it is unknown how exposure to the deltamethrin-based LLIN (LLIN used thereafter) may affect olfaction and orientation to important semiochemicals by stored product insects. In this study, our aims were to evaluate whether exposure to LLIN affected male and female (or mixed sex) populations of *Trogoderma variable* and *T. inclusum* movement in the presence of important semiochemicals, including food kairomones and pheromones, including the major sex pheromone for *Trogoderma* spp. (e.g., (Z)-14-methyl-8-hexadecenal). We evaluated this with a video-tracking protocol and confirmed volatile emissions with headspace collection coupled with gas chromatography and mass spectrometry (GC/MS). Disruption in movement in this study is taken to mean a significant upregulation or downregulation in response compared to our netting without active ingredient (control). Distance moved increased 4–6-fold in *T. variable* after exposure to LLIN compared to control netting, while it was reduced by a third to a half in *T. inclusum*. Overall, out of 40 possible olfactory and taxis patterns which could be disrupted, exposure to LLIN interrupted 68–73% of these in *T. inclusum* and *T. variable*, respectively, compared to control netting-exposed individuals. Pheromonal stimuli were more important for males, but food and pheromones were equally important for females. Our research suggests the use of LLIN may enhance the effectiveness of other behaviorally-based management strategies when used as part of a comprehensive IPM program for these short-lived adults.

Keywords: warehouse beetle, larger cabinet beetle, Dermestidae, behavior, olfaction, IPM

Introduction

Insect movement is an ongoing challenge in integrated pest management (IPM) programs. The movement of insects allows individuals to find essential food resources, mating and egg-laying sites, and refugia (Jian, 2019). After harvest, one of the main purposes of food facilities is to separate commodities from the environment and prevent, or at least minimize the movement and dispersal of insects. However, insects that are highly mobile and present in the landscape (Jian, 2019; Scheff et al., 2021a) may still be able to readily immigrate into food facilities as they track plumes of food cues and pheromones from ongoing infestations (Morrison et al., 2021; Cardé, 2021). As a result, stored product pests can easily attack commodities at each linkage throughout the post-harvest supply chain, ranging from storage on-farm after harvest to the end consumer. Thus, it is crucial to understand stored product insect dispersal ecology and behavioral response to the odor cues present in food facilities to help to effectively manage these pests and their infestation.

A key component of IPM for food facilities is prevention of infestation (Phillips and Throne, 2010). A novel and successful, preventative IPM tactic to intercept immigrating insects used in a postharvest setting is the use of long-lasting insecticide-incorporated netting (LLIN) (Wilkins et al., 2021; Ranabhat et al., 2024). Both deltamethrin-based and alpha-cypermethrin-based LLIN have shown direct efficacy against 13 stored product insects (Agrafioti et al., 2021; Morrison et al., 2018; Paloukas et al., 2020; Rumbos et al., 2018; Scheff et al., 2021; Ranabhat et al., 2024). Other research has found that LLIN is effective against both phosphine-resistant and -susceptible red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) (Ranabhat et al., 2022). As a result, LLIN may be used to cover the doors, windows, vents, cracks or crevices, intake or

outtake manifolds on processing facilities (McKay et al., 2024), awnings on grain silos (Ranabhat et al., 2024), pallets in warehouses (Wilkins et al., 2021), or any other types of opening to intercept insects as they disperse in and around the food facilities. In addition, LLIN has been shown to reduce movement and dispersal of stored product insects by multiple-fold after exposure (Morrison et al., 2018; Wilkins et al., 2021), including adults and larvae (Wilkins et al., 2020). Recent work also found that exposure to LLIN reduced progeny production by stored product insects in subsequent generations (Gerken et al., 2021a). Importantly, prior work has found that multiple brief exposures with LLIN result in the same mortality and behavioral effects as a continuous longer dosing (Gerken et al., 2020b). Finally, research has also evaluated how brief exposures to both control netting and LLIN may affect upwind movement toward semiochemicals for *Trogoderma granarium* Everts (Coleoptera: Dermestidae) (Domingue et al., 2021), but further consideration of this topic in more depth is warranted.

Two economically important and cosmopolitan dermestids include the warehouse beetle, *Trogoderma variabile* Ballion, and the larger cabinet beetle, *Trogoderma inclusum* LeConte (Coleoptera: Dermestidae). Both of these species are external-feeding pests (e.g., those that feed on damaged or broken kernels) that infest a variety of products, including durable commodities at mills and processing plants (Ghimire et al., 2016; Partida and Strong, 1975), as well as animal products such as furs, fabrics, and preserved museum or insect specimens (Hagstrum et al., 2012). Both species are short-lived as adults (Partida and Strong, 1970) and have similar life histories. Female dermestids are usually larger than male dermestids in size (USDA, 1991). Males of both species have 6 or 7 segmented antennal clubs while females' antennae are 4 or 5 segmented (Bousquet, 1990).

The chemical ecology of adult *Trogoderma* spp. is relatively well-known. For example, there is a female-produced sex pheromone, (Z)-14-methyl-8-hexadecenal, that was identified as the major sex component for both *T. variabile* and *T. inclusum* (Cross et al., 1976). Males of *T. inclusum*, *Trogoderma simplex* Jayne (Coleoptera: Dermestidae), and *T. variabile* exhibited strong cross-attraction to female extracts of *T. inclusum*, *T. simplex*, *T. variabile*, *T. granarium*, and *Trogoderma grassmani* Beal (Coleoptera: Dermestidae) (Vick et al., 1970). Many *Trogoderma* spp. share the major component for their pheromone among species within the genus (Vick et al., 1970; Olson et al., 2013). However, the minor components vary among the species. The major component is necessary and sufficient to elicit substantial attraction by all *Trogoderma* species under consideration here. Peak pheromone release by *T. variabile* females and optimal male response was observed at dawn during the early photophase. On the other hand, female calling behavior and mating in *T. inclusum* primarily took place in the mid-photophase (Vick et al., 1973). Not many articles have evaluated whether these pheromones elicit arrestment, a process in which individuals slow movement and increase turning frequency, but Morrison et al. (2020) found that they do in *T. granarium* and *T. variabile*. Baits with food volatiles such as Storgard™ oil, wheat germ, and dermestid tablets, have all been used as attractants for dermestids (Morrison et al., 2020; Morrison et al., 2023).

Trogoderma spp. can fly in long dispersal flights to new habitats or within the same habitat (Campbell and Mullen 2004), but walking is critical for meso- and close-range orientation to pheromone cues. For example, in evaluating the chemo-orientation of *T. variabile*, males responded in less than a second to a puff of female sex pheromone with alert, antennae-raised posture, and frequent local walking search loops with a constant turn bias (Tobin and Bell, 1986). In a pheromone corridor with wind, male *T. variabile* oriented upwind by walking

relatively straight paths with side sweeping motions across the 10 cm corridor (Tobin and Bell, 1986). In the field, ground-deployed traps were found to capture many more *T. variabile* than traps deployed higher (Wright and Delvesi, 1994), suggesting they may be accessing the trap by walking. Thus, at close-range walking is essential for orientation to pheromonal cues by *Trogoderma* spp.

Dermestids are often more difficult to control than other stored product insects because they are more tolerant to the active ingredients that are currently available in stored product management approaches (Campbell and Arbogast, 2004; Scheff et al., 2016). For example, prior work found that exposure to the novel tactic, LLIN, reduced the movement and reproductive rate of one of the common dermestids in the US, *T. variabile*, although not as much as other stored product insects (Gerken et al., 2021; Wilkins et al., 2020). However, there is not much information on the orientation and olfaction of dermestids to semiochemicals after exposure to LLIN. It has been reported that continuous exposure to control netting resulted in a thigmotactic response by larvae of the khapra beetle, *T. granarium* that caused reduction in their movement (Domingue et al., 2021). In the same study, brief exposures to LLIN caused an increase in movement and a disruption in larval orientation toward odor sources. Further, Ranabhat et al. (2024) demonstrated that exposure to LLIN disrupted the olfaction and orientation of larval *T. variabile* and *T. inclusum* towards the important semiochemical. In exposing adult *T. variabile* to 3000 ppm deltamethrin incorporated polymer ZeroFly® storage bags, Kavallieratos et al. (2017) found all individuals were knocked down on the outside and inside surfaces of the bag after one hour. However, it is unknown what happens when adult dermestids are exposed to LLIN or if responses vary according to species compared to the control netting. Further, it is possible that male and female dermestids behave differently in response to the same stimuli, including

conspecific pheromones or food cues after exposure to LLIN, thus it is essential to assess the behavior of both sexes. Therefore, the aims in this study were to evaluate whether there were sex-based differences in exposure to LLIN by *T. variable* and *T. inclusum* through movement in the presence of important semiochemicals, including food kairomones and pheromones. We evaluated this with a video-tracking protocol and confirmed volatile emissions with headspace collection coupled with gas chromatography and mass spectrometry (GC/MS).

Materials and Methods

Insects

Four to six-week-old *T. variable* and *T. inclusum* were sexed or mixed-sex adults were used for the experiment to mimic natural populations at food facilities. *Trogoderma variable* and *T. inclusum* were obtained from the field in Kansas in 2016 and 2012, respectively. Both species were fed 300 g of ground dog food (SmartBlend, Lamb flavor, PurinaOne, Nestlé Inc., St. Louis, MO, USA) with oats and a moistened, crumpled paper towel on the surface in a 950 ml mason jar. Colonies were maintained at 27.5°C, 65% RH, and 14:10 (L:D) h photoperiod. For both species, adults were sexed based on body size and antennal segments as mentioned above and they were observed under the stereoscope microscope at between 10–100× magnification (SMZ18, Nikon, Tokyo, Japan).

Treatments

The LLIN in this study consisted of a polyethylene netting (2 × 2 mm mesh, Vestergaard, Inc., Lausanne, Switzerland) with 0.4% deltamethrin, or control netting without insecticide but otherwise identical in physical properties, including mesh size and material composition. These were used with the movement assay.

Food and pheromonal semiochemicals

We assessed the movement in the vicinity of important pheromone and food kairomones after exposure to the deltamethrin-based LLIN^{ter}) or control netting. Food stimuli included 0.01 g of whole, organic, unbleached flour (Heartland Mills, Marienthal, KS, USA), and pheromonal stimuli consisted of a broad spectrum, multi-species lure with the sex or aggregation pheromones for six stored product species (*Plodia-Trogoderma-Lasioderma* [PTL] lure, IL-108-10, Batch# 1288200321, Insects Limited, Westfield, IN), including *Trogoderma* spp. pheromone. We disassembled the lure and used a single bead out of the 15 beads present in each replicate, and affixed it in place so it did not move in a Petri dish using a 1 × 1 mm square of parafilm followed the similar procedure as in the study of Ranabhat et al. (2024). We used a fresh lure for each replication of testing.

Movement assay

Single-sex or mixed-sex adult *T. variabile* and *T. inclusum* were exposed to LLIN and their behaviors were compared to those exposed to control netting. Cohorts of 5 adults were exposed for 1 min to the control net or LLIN affixed to a 24 × 24 cm Petri dish in the laboratory. Effects on the movement of exposed adults in response to food cues (using 0.01 g of flour) or with conspecific sex pheromones as described above were assessed either immediately or after being held for 24 h in Petri dishes under the same environmental chamber conditions as the colonies but without supplemental food, and then assayed using the video-tracking combined with Ethovision video-tracking system software (v.14.5 Noldus Inc., Leesburg, VA, USA). Only alive or affected adults were tracked and placed in the center of arenas for experiments. We used the definitions in Ranabhat et al. (2022), but shortly alive adults were defined as moving with normal speed and activity and able to right themselves if flipped, while affected adults exhibited

sluggish or drunken movements, could not right themselves if flipped, and some or all of their limbs exhibited twitching. Dead adults showed no signs of movement. The network camera (GigE, Basler AG, Ahrensburg, Germany) was suspended 76 cm above and centered over six replicate 100 × 15 cm (D:H) Petri dish arenas held in place with a foamboard on an artist's light box (LPB3, Litup, Shenzhen, China). Arenas were lined with a piece of filter paper (85 mm D, Ahlstrom-Munksjo Filtration, LLC, Mt. Holly Springs, PA, USA) adhered with four pieces of double-sided tape (CHI4050, Permanent, Skillcraft, USA) placed equally on cardinal side of the arena. 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 (Figure 4.1). A hidden zone is one in which the system assumes that when a walking insect enters it, the insect disappears from view and the amount of time it spends within and frequenting the zone is tracked (Noldus et al., 2002). The advantage of a hidden zone is that it allows a stimulus to be placed within it without creating artifacts in tracking. A hidden zone can also double as a normal zone when the insect is visible within the boundaries of the zone, Ethovision assumes that it is on top of it and not inside the zone. In the case of the current study, the stimulus treatment zone was a hidden zone where either pheromone or food stimuli were placed so they would not be tracked with the Ethovision software, and the control stimulus zone was left empty. 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. A 30-min period was chosen to be comparable with prior data (Ranabhat et al., 2024). The control side of the arena remained empty. We included the response variables of total distance moved (cm) and instantaneous velocity (cm/s), because they are very well-studied

variables, these variables can be compared with the prior work, and they accurately describe overall patterns in movement that often translate to the field for stored product insects (Morrison et al., 2018; Wilkins et al., 2020; Wilkins et al., 2021). Other response variables such as 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 were included because they accurately depict aspects of arrestment and are generally understudied but important components of behavior. A total of 12–16 replicate beetles were run per combination for both species for each combination of sex or mixed population, species, and treatment.

Headspace collection

Headspace samples were collected to characterize the main component of the PTL lure relevant for this study. *Trogoderma* spp. sex pheromone was used to determine whether the emission rate of volatiles produced by the PTL lure was suitable for the experiment, and whether they were different from background odors in the laboratory. Four 500 mL-capacity headspace volatile chambers (10.2 × 12.7 cm D: H) (Volatile Collection Systems, Gainesville, FL, USA) were attached in parallel to four flow meters by polytetrafluoroethylene (PTFE), chemically inert tubing (5mm ID). Central air was scrubbed with an activated charcoal filter (Sigma Scientific, LLC, Micanopy, FL, USA) prior to be controlled to a flow rate of 1 L per min by flow meters. In each headspace chamber, ten beads of a disassembled PTL lure were added in three chambers and compared to a negative control consisting of empty headspace chambers to identify whether emissions were significantly different from background laboratory air. Headspace volatiles were collected over a 0.5, 3, and 24-h period using 7.62 cm-long volatile collection traps with 20 mg of Porapaq-Q (Volatile Collections Systems, Gainesville, FL, USA). The headspace volatiles were extracted from the volatile collection traps using 150 µl of dichloromethane into a 2-mL GC vial

with magnetic screwtop cap, with the solvent pushed through by inert N₂ gas. A total of 1 µl of tetradecane (190.5 ng at 99% purity) was added as an internal standard after extraction for quantification in each of the labeled vials using a microsyringe (Hamilton Company, Inc., Reno, NV, USA). All volatile collection traps were washed with 700 µl of dichloromethane in triplicate between uses. All GC vials were with PTFE tape and stored at -20°C until all replicates were run together on the GC-MS. There was a total of 6–8 replicates per treatment.

Gas chromatography coupled with mass spectrometry (GC/MS)

All headspace sample extracts were run on an Agilent 7890B gas chromatograph (GC) equipped with an Agilent Durabond HP-5 column (30 m length, 250 µm diameter, and 0.25 µm film thickness) with He as the carrier gas at a constant 1.2 mL/min flow and 39 cm/s velocity, which was coupled with an Agilent 5977B mass spectrometer (MS) single-quadrupole detector. The compounds were separated by injecting 1 µL of each sample under splitless in an inlet heated to 250°C. The program began at 30°C for 1 min followed by ramping by 10°C/min to 300°C over 27 min, and subsequently held for 0.5 min. After a solvent delay of 4 min, mass ranges between 50 and 550 atomic mass units were scanned. The preliminary assignment of compounds was obtained by comparing sample spectral data with the NIST 17 library through deconvolution (see below for more information).

Statistical analysis

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. Assumptions of normality and homogeneity in variances were checked by inspecting residuals and diagnostic

plots, and log transformation was used when appropriate. All analyses were run using R software (R Core Team, 2022), with $\alpha=0.05$, and Tukey HSD for multiple comparisons.

Disruption in movement in this study was defined as significant increase or decrease in response to LLIN compared to our control netting (without active ingredient). In order to evaluate this from typical olfactory and taxis patterns for *T. variabile* and *T. inclusum*, the ratio of behavioral response between the treatment zone and control zone was compared in the presence of either no stimuli, food, or pheromone after LLIN exposure was evaluated with a Chi-squared test for a significant deviance in pattern using the ratios for the same stimuli found after exposure to control netting. The *a priori* expectations were equal responses or no differences among the groups.

For the chemical data, the program Unknowns Analysis (Agilent, Santa Clara, CA, USA) was initially used to automatically integrate and obtain reference hits to the NIST17 library for any peak in a sample that was at least 2% the height of the largest peak. This was all aggregated into a single CSV file and outputted, then data analysis was further streamlined using the R package *uafR* to spread out data, align all compounds with a greater than 75% reference library match into a single table for all samples, and obtain emission values in units of internal standard (e.g., ng of emissions per h) (Stratton et al., 2024). Next, to determine whether PTL lure samples were significantly different from background laboratory air, a pairwise Bray-Curtis dissimilarity measure was calculated among all samples, and non-metric multidimensional scaling ordination was implemented to visualize differences. A total of 1000 permutations were used, and stress was <0.13 , indicating reasonable interpretation from the ordination was possible. In addition, an analysis of similarity (ANOSIM) was calculated to test for significance in the treatment, again with 1000 permutations. ANOSIM outputs an R statistic, which represent a direct measure of

similarity/dissimilarity, ranging from 0 (identical) to -1 or 1 (completely different). Finally, we extracted the values just for the cumulative emissions of (Z)-14-methyl-8-hexadecenal, which comprise the major pheromone component for *T. inclusum* and *T. variabile* (Cross et al. 1976), and look at rate of emission of a single bead from above over 3 h or 24 h. Visualizations were performed in *ggplot2* in R with 95% confidence interval bands calculated for the emission rate (Wickham et al., 2016).

Results

***Trogoderma variabile* movement**

No mortality was observed by *T. variabile* over the trial period. The semiochemical stimuli significantly affected female *T. variabile* velocity (Table 4.1, Figure 4.2). Specifically, females increased their velocity by 3.7 to 5.6-fold in response to food immediately after 1-min exposure to LLIN compared to control netting. However, males did not show a significant difference in the distance moved or velocity after exposure to LLIN or control netting although they displayed a similar pattern as females. Overall, the 4-way interaction among treatment, netting, sex, and post-exposure time did not significantly affect the distance and velocity moved (Table 4.1). For mixed-sex *T. variabile*, there was a much more pronounced effect of exposure to LLIN (Table 4.S1, Figure S4-1). In addition, the interaction between treatment and post-exposure holding duration significantly affected the distance moved. Exposure to LLIN significantly reduced the movement towards food and pheromone much more after 24 h compared to immediately after exposure by mixed-sex *T. variabile* (Table 4.S1, Figure S4-1). Overall, the pattern of movement for male and female *T. variabile* was significantly disrupted by exposure to LLIN in all of the four possible evaluated patterns (Figure 4.2, Panels 1–4), including for the distance moved and instantaneous velocity after 1 min (distance: $\chi^2 = 43.3$, df =

2, $P < 0.0001$; velocity: $\chi^2 = 43.3$, $df = 2$, $P < 0.0001$) and 24 h (distance: $\chi^2 = 98.6$, $df = 2$, $P < 0.0001$; velocity: $\chi^2 = 85.6$, $df = 2$, $P < 0.0001$).

Behavior of *T. variable*

Frequency of entering treatment, control stimulus and half zone

Semiochemical treatment, exposure to netting, sex, and post-exposure holding duration all had a significant effect on the frequency of entries into the stimulus zone or half zone by single-sex groups of *T. variable* (MANOVA, Table 4.2). Moreover, the two-way interaction between semiochemical treatment and netting exposure, and the three-way interaction including post-exposure holding duration significantly affected the frequency of entries into each zone. For example, there was a 95% reduction in frequency of entry to the stimulus treatment zone with pheromone after 24 h compared to the unbaited control after exposure to LLIN by female *T. variable*, but this was not the case immediately after exposure (Figure 4.3). Among female *T. variable*, exposure to LLIN significantly affected the frequency of entering the stimulus treatment zone containing pheromone after 24 hours post-exposure (Figure 4.3). The main effects of semiochemical treatment, exposure to netting, and sex affected the frequency of entries into the treatment half of the arena, but importantly there were no significant 2-, 3-, or 4-way interactions among the variables for female *T. variable* (Figure S4-2). The patterns of frequency of entry to stimuli by female *T. variable* was significantly disrupted after exposure to LLIN compared to the controls for the stimulus zone 24 h later ($\chi^2 = 45.0$, $df = 2$, $P < 0.0001$) and for each half of the arena after 1 min ($\chi^2 = 7.02$, $df = 2$, $P < 0.05$; Figure 4.3). For males, there were no significant differences in the frequencies of entering the stimulus zones immediately after exposure to LLIN in the presence of semiochemicals (Figure 4.4). Nevertheless, after a 24-hour post-exposure holding duration, male *T. variable* frequencies of entering the stimulus treatment

zone significantly increased after exposure to LLIN compared to control netting (Figure 4.4). The frequency of entering each treatment half of the arena was greater in the presence of food 24 h after exposure to control netting compared to when pheromone was present or when no stimulus was present, but this effect disappeared when males were exposed to LLIN (Figure S4-3). In addition, the pattern of frequency of entry to stimuli by male *T. variable* was significantly disrupted after exposure to LLIN compared to the controls for the stimulus zone after 1 min exposure ($\chi^2 = 37.1$, $df = 2$, $P < 0.0001$) and for each half of the arena after 1 min ($\chi^2 = 18.5$, $df = 2$, $P < 0.0001$) and 24 h ($\chi^2 = 72.3$, $df = 2$, $P < 0.0001$; Figure 4.4).

For mixed-sex groups of *T. variable*, the frequency of entering each zone was significantly affected by the main effects of post-exposure holding duration and netting (MANOVA, Table 4.S2). Additionally, the three-way interaction among semiochemical treatment, netting, and post-exposure holding duration significantly affected the frequency of entering the stimulus zone by mixed-sex *T. variable* (Table 4.S2, Figure S4-4). A total of 24-hour after LLIN exposure, the frequency of entering the stimulus zone containing pheromone was significantly reduced by about 4-fold in comparison to when mixed-sex *T. variable* were exposed to the control netting (Figure S4-4). After LLIN exposure at both 1 min and 24 h later, the frequencies of entries onto each half of the arena in mixed-sex groups by *T. variable* was decreased by 46–76% compared to control netting (Figure S4-5).

Cumulative duration spent in treatment, control stimulus and half zone

For single-sex groups of *T. variable*, the cumulative duration spent in each zone was significantly affected by all the model variables, including semiochemical treatment, exposure to netting, sex, and post-exposure holding duration (Table 4.3). The cumulative duration spent in each zone was significantly impacted by each two-way, three-way, and four-way interaction

among those variables (Table 4.3). For female *T. variable*, exposure to LLIN significantly reduced the cumulative duration spent in the treatment stimulus zone by 90–91% compared to the control netting when in the presence of the unbaited control and pheromone, as well as in the cumulative duration spent in the control stimulus zones by 89–94% after 1 min (Figure 4.3). By contrast, the cumulative duration spent on each half of the arena was uniform by female *T. variable* (Figure S4-2). The pattern of cumulative duration spent with stimuli by female *T. variable* was significantly disrupted after exposure to LLIN compared to the controls for the stimulus zone and each half of the arenas at 1 min (stimulus zone: $\chi^2 = 298$, $df = 2$, $P < 0.0001$; arena half: $\chi^2 = 15.6$, $df = 2$, $P < 0.001$) but only the stimulus zone 24 h later ($\chi^2 = 409$, $df = 2$, $P < 0.0001$). By contrast, male *T. variable* increased the cumulative duration spent in the stimulus treatment zone in response to pheromone stimuli by 14-fold after 1 min of exposure to LLIN compared to control netting (Figure 4.4). However, after 24 h, cumulative duration spent in stimulus control zones in the presence of pheromone was 108-fold greater after exposure to control netting compared to LLIN (Figure 4.4). Like females, the cumulative duration spent by male *T. variable* on each half of the arena was relatively equal (Figure S4-3). The pattern of cumulative duration spent with stimuli by male *T. variable* was significantly disrupted after exposure to LLIN compared to the controls for the stimulus zone at 1 min and 24 h later (1 min: $\chi^2 = 7.48$, $df = 2$, $P < 0.05$; 24 h: $\chi^2 = 8927$, $df = 2$, $P < 0.0001$) but only the patterns in each half of the arena 24 h later ($\chi^2 = 7.14$, $df = 2$, $P < 0.05$).

For mixed-sex groups of *T. variable*, the cumulative duration spent in the stimulus zone or half zone was significantly influenced by the main effects of post-exposure holding duration, semiochemical treatment, exposure to netting. The 4-way interaction between all factors significantly affected the cumulative duration spent in zones by mixed-sex *T. variable* (Table 3;

Figure S4-4). At 1 min after exposure to control netting or LLIN, there was roughly equal duration spent on each half of the arena by mixed-sex *T. variable*, but 24 h after, mixed-sex *T. variable* spent a greater amount of time on the control half than the treatment half of arenas in the presence of food and pheromone when exposed to LLIN compared to control netting (Figure S4-5).

Latency to treatment, control stimulus and half zone

For single-sex *T. variable*, the latency to finding the stimulus zone or each half of the arena was significantly affected by the semiochemical treatment, exposure to netting, and post-exposure holding duration (Table 4.4). Additionally, the two-way interactions of netting by sex or post-exposure holding duration significantly affected the latency to finding each zone (Table 4.4). The latency to finding the food and pheromone in the treatment stimulus zone by female *T. variable* was significantly greater than when the treatment zone had an unbaited control (Figure 4.3). There was a much larger delay in female *T. variable* finding food and pheromone halves of arenas after exposure to LLIN compared to control netting (Figure S4-2). The overall pattern of latency to finding stimuli by female *T. variable* was significantly disrupted after exposure to LLIN compared to the control for the stimulus zone and each half of the arenas at 1 min (stimulus zone: $\chi^2 = 12.8$, $df = 2$, $P < 0.001$; arena half: $\chi^2 = 634$, $df = 2$, $P < 0.0001$) and 24 h later (stimulus zone: $\chi^2 = 12.8$, $df = 2$, $P < 0.001$; arena half: $\chi^2 = 5.71$, $df = 2$, $P = 0.06$). Directly after exposure to LLIN, the latency to finding all treatments by male *T. variable* decreased compared to exposure to control netting, while latency to finding treatments in the stimulus zones stayed relatively constant 24 h afterward (Figure 4.4). Unlike females, male *T. variable* quickly found food in the treatment half of arenas after 1 min and 24 h when exposed to untreated netting compared to LLIN. The overall pattern of latency to finding stimuli by male

T. variable was significantly disrupted after exposure to LLIN compared to the control for the stimulus zone and each half of the arenas at 1 min (stimulus zone: $\chi^2 = 18.3$, $df = 2$, $P < 0.0001$; arena half: $\chi^2 = 21.2$, $df = 2$, $P < 0.0001$) but only each half of the arena at 24 h ($\chi^2 = 32.6$, $df = 2$, $P < 0.0001$).

For mixed-sex *T. variable*, the main effects of netting and post-exposure significantly affected the latency to finding the stimulus and each half of the arena. The two-way interaction between netting and post-exposure also significantly affected the latency to finding each zone (Table S4-4). The latency to finding the control stimulus zone was significantly increased after 1 min exposure to LLIN in the presence of food and pheromone (Figure S4-4). There was a slight decrease in the latency to finding the stimulus zones after exposure to control netting compared to 1 min after LLIN exposure by mixed-sex *T. variable* (Figure S4-4). Further, the latency to finding each half of the arena by mixed-sex *T. variable* was actually increased, especially for pheromone and food, 1 min after exposure to LLIN (Figure S4-5). In addition, the latency to finding the treatment stimulus half (containing pheromone) by mixed-sex *T. variable* was significantly increased 24 h after LLIN exposure compared to control netting exposure (Figure S4-5).

Overall, of 40 possible olfactory and taxis patterns that could be interrupted, exposure to LLIN interrupted 73% of these in *T. variable* compared to control netting exposed individuals. Of the 12 possible olfactory and taxis patterns for female *T. variable*, 67% were interrupted after exposure to LLIN compared to control individuals exposed to netting without insecticide. Similarly, of the 12 possible patterns for male *T. variable*, 75% of the patterns were significantly interrupted after brief exposure to LLIN compared to controls.

***Trogoderma inclusum* movement**

No mortality was observed in *T. inclusum* over the trial period. The two-way interaction between sex and post-exposure holding duration significantly affected the distance move by single-sex groups of *T. inclusum* (Table 4.1). Female *T. inclusum* moved 54% less after 24 h compared to 1 min, while male *T. inclusum* only moved 28% less (Figure 4.5). The 4-way interaction among treatment, netting, sex, and post-exposure holding duration did not significantly affect the distance moved and velocity of single-sex *T. inclusum* (Table 4.1). Twenty-four hours after exposure to LLIN, the distance moved by the male *T. inclusum* in response to the unbaited control or pheromone was significantly increased by 13- and 5-fold respectively compared to the control netting (Figure 4.5). For mixed-sex groups of *T. inclusum*, exposure to the LLIN significantly affected the distance moved by conspecifics (Table 4.S1, Figure S4-6). Finally, for mixed-sex *T. inclusum*, the interactions among the main effects, including treatment, netting, and post-exposure did not significantly affect the distance or velocity moved (Table 4.S1). Overall, the pattern of movement for male and female *T. inclusum* was significantly disrupted in half of the four possible evaluated patterns (Figure 4.2), including 24 h after exposure to LLIN compared to the controls for the distance moved and instantaneous velocity (distance: $\chi^2 = 12.9$, $df = 2$, $P < 0.001$; velocity: $\chi^2 = 17.4$, $df = 2$, $P < 0.001$).

Behavior of *T. inclusum*

Frequency of entering treatment, control stimulus and half zone

For single-sex groups of *T. inclusum*, the frequency of entering each zone was significantly affected by all main effects of the factors included in the model (e.g., semiochemical treatment, exposure to netting, sex, and post-exposure holding duration). However, the four-way interaction among these factors also significantly affected the frequencies

of entering each zone (Table 4.2). For female *T. inclusum*, there were significant differences in the frequency of entering the stimulus zones immediately after exposure to LLIN in response to food or pheromone compared to the control. After 24 h, there were 14-fold more entries into the stimulus zone with pheromone by females after exposure to LLIN than by those exposed to control netting (Figure 4.6). Further, the movement by female *T. inclusum* was generally increased, because the frequency of entering control or treatment halves of the arena increased by 5.1–5.9-fold after exposure to LLIN compared to control netting after 24 h (Figure S4-7). Also, female *T. inclusum* increased the frequency of their entries into each half by 2.7–4.8-fold after exposure to LLIN compared to control netting (Figure S4-7). The overall patterns of frequency of entry to stimuli by female *T. inclusum* was significantly disrupted after exposure to LLIN compared to the controls for the stimulus zone ($\chi^2 = 6.37$, $df = 2$, $P < 0.05$; Figure 4.6) and each half of the arena ($\chi^2 = 6.35$, $df = 2$, $P < 0.05$; Figure S7) 24 h later. For male *T. inclusum*, the frequency of entering to stimulus zone containing pheromone was slightly higher (but not significant) when exposed to LLIN compared to control netting and the effect was similar after 24 h (Figure 4.7). The frequency of male *T. inclusum* entering the treatment stimulus zone significantly increased by 2.4-fold in the presence of pheromone after 1 min of exposure to LLIN compared to control netting, while male number of entries to the treatment half of the arena increased by 9.3-fold when pheromone was present 24 h after exposure to LLIN compared to control netting (Figure 4.7, Figure S4-8). The patterns of frequency of entry to stimuli by male *T. inclusum* was significantly disrupted after exposure to LLIN compared to the controls for the stimulus zone ($\chi^2 = 47.4$, $df = 2$, $P < 0.0001$; Figure 4.7) and each half of the arena ($\chi^2 = 46.6$, $df = 2$, $P < 0.0001$; Figure S4-8) 24 h later.

For mixed-sex groups of *T. inclusum*, the semiochemical treatment, exposure to netting, and post-exposure holding duration significantly affected the frequency of entering each zone (Table 4.S2). In addition, the two-way interaction of netting exposure by semiochemical treatment or post-exposure holding duration also significantly affected the frequency of entering each zone. For the stimulus zones, all the main factors significantly affected the frequencies of entry (Table 4.S2, Figure S4-9). Twenty-four hours after exposure to LLIN, the frequency of entering both the treatment and control stimulus zone was significantly reduced (Figure S4-9). Further, there was a significant reduction in the frequency of entering the treatment stimulus zone containing food immediately after exposure to LLIN by mixed-sex *T. inclusum*. Twenty-four hours later, the frequency of mixed-sex *T. inclusum* entering the stimulus zone containing food and pheromone was reduced by 16- and 6-fold, respectively.

Cumulative duration spent in treatment, control stimulus and half zone

For single-sex *T. inclusum*, the cumulative duration spent in the stimulus zone or half zone was significantly affected by all main effects in the model (MANOVA, Table 4.3). However, the four-way interaction among all the main factors also significantly affected the cumulative duration spent in each zone (Table 4.3). For female *T. inclusum*, there was a significant increase by 5.9-fold in the cumulative duration spent in the stimulus zone containing pheromone immediately after exposure to LLIN compared to those exposed to control netting (Figure 4.6). After 24, there was a significant increase in the cumulative duration spent in the control stimulus zone in response to pheromone stimuli after exposure to LLIN compared to control netting (Figure 4.6). For female *T. inclusum*, immediately after exposure to LLIN and 24 h later, the cumulative duration spent in the treatment half zone and control half zone in the presence of pheromones was significantly different compared to control netting (Figure S4-7).

The pattern of cumulative duration spent with stimuli by female *T. inclusum* was significantly disrupted after exposure to LLIN compared to the controls for the stimulus zone and each half of the arenas at 24 h later (stimulus zone: $\chi^2 = 73.4$, $df = 2$, $P < 0.0001$; arena half: $\chi^2 = 8.02$, $df = 2$, $P < 0.05$; Figure 4.6) but only each half of the arenas after 1 min ($\chi^2 = 21.7$, $df = 2$, $P < 0.0001$; Figure S4-7). For male *T. inclusum*, after 24 h post-exposure holding duration to LLIN, the cumulative duration spent in the treatment stimulus zone containing pheromone stimuli was significantly increased by 16-fold, when exposed to LLIN compared to the control netting (Figure 4.7). Male *T. inclusum* were on the treatment half much more often directly after exposure to control netting compared to LLIN, where conspecifics were more equally distributed between the treatment and control halves of the arena (Figure S4-8). Overall, the pattern of cumulative duration spent with stimuli by male *T. inclusum* was significantly disrupted after exposure to LLIN compared to the controls for the stimulus zone at 1 min ($\chi^2 = 14.9$, $df = 2$, $P < 0.001$) and 24 h later ($\chi^2 = 48.8$, $df = 2$, $P < 0.0001$; Figure 4.7 and Figure S4-8).

Overall, for mixed-sex groups of *T. inclusum*, the cumulative duration spent in each zone was significantly affected by the two-way interaction of netting and post-exposure holding duration (Table S4-3). Twenty-four hours after exposure, the cumulative duration spent in the stimulus zone containing food or pheromone was significantly reduced (Figure S4-9). However, there was a significant increase in the cumulative duration spent in the control stimulus zone in response to food and pheromone stimuli by 59- and 8-fold after 24 h post-exposure holding duration to LLIN as compared to control netting (Figure S4-9). Likewise, 24 h after exposure to LLIN, there was a large jump in the cumulative duration spent on control halves of arenas by *T. inclusum* compared to those exposed to control netting (Figure S4-10).

Latency to treatment, control stimulus, and half zone

Semiochemical treatment, exposure to netting, and post-exposure exposure holding duration, as well as the two-way interaction of netting by semiochemical treatment or post-exposure holding duration, all significantly affected the latency to finding each zone by single-sex groups of *T. inclusum* (Table 4.4). However, there was no significant four-way interaction among the main factors for the latency to finding the semiochemical treatment in the stimulus or half zone by single-sex *T. inclusum*. Directly after exposure to LLIN, there was a significant 56% reduction in the latency to finding the stimulus treatment zone when it contained pheromone stimuli for female *T. inclusum* (Figure 4.6), compared to only a 27% reduction when females were exposed to the control netting. There were no significant differences in the latency to finding the semiochemical stimuli in each half of the arena by female *T. inclusum* (Figure S4-7). Overall, the pattern of latency to finding each stimulus by female *T. inclusum* was significantly disrupted after exposure to LLIN compared to the controls for the stimulus zones ($\chi^2 = 15.1$, $df = 2$, $P < 0.001$; Figure 4.6) and each half of the arenas ($\chi^2 = 54.2$, $df = 2$, $P < 0.0001$) at 1 min as well as for each half arena after 24 h ($\chi^2 = 70.9$, $df = 2$, $P < 0.0001$; Figure S4-7). Male *T. inclusum* also experienced a reduction of 63% in the latency to finding the stimulus zone when it had pheromone after exposure to LLIN compared to control netting after 24 hours (Figure 4.7). There were no significant differences in the latency to finding the semiochemical stimuli in each half of the arena by male *T. inclusum* (Figure S4-8). The pattern of latency to finding each stimulus by male *T. inclusum* was significantly disrupted after exposure to LLIN compared to the controls for each half of the arenas at 1 min ($\chi^2 = 419$, $df = 2$, $P < 0.0001$; Figure 4.7) as well as each arena half ($\chi^2 = 38.8$, $df = 2$, $P < 0.0001$) and stimulus zone ($\chi^2 = 11.6$, $df = 2$, $P < 0.01$) after 24 h.

For mixed-sex groups *T. inclusum*, the main factors of netting and post-exposure holding duration significantly affected the latency to finding the semiochemical treatment in each zone (Table S4-4, Figure S4-9 & 4.S10). There was no significant three-way interaction among the main factors of semiochemical treatment, netting, and post-exposure holding duration on for the latency to finding the treatment in each zone. However, 24 hours after exposure to LLIN, the latency to finding the treatment in each half zone significantly increased by 28–44% (Figure S4-10).

Overall, of 40 possible olfactory and taxis patterns that could be interrupted, exposure to LLIN disrupted 68% of these in *T. inclusum* compared to control netting exposed individuals. Of the 12 possible olfactory and taxis patterns for female and 12 for male *T. inclusum*, 67% and 67% of the patterns, respectively, were disrupted after exposure to LLIN compared to control individuals.

Headspace Collection

There was a significant difference in the composition of headspace spiked with the pheromone lure compared to our unbaited control (ANOSIM: $R = 0.768$; Perm = 1000; $P < 0.001$; Figure 4.8). In addition, the duration of collection had a significant effect on the cumulative emissions of (Z)-14-methyl-8-hexadecenal (ANOSIM: $R = 0.167$; Perm = 1000; $P < 0.01$; Figure 4.9). On average, $13.5 \text{ ng} \cdot \text{h}^{-1} \cdot \text{lure}^{-1}$ of (Z)-14-methyl-8-hexadecenal was emitted over 3 h by 10 beads. Over the study period in the movement assay, 0.675 ng of *Trogoderma* pheromone was estimated to be emitted by a single bead.

Discussion

Here, we evaluated the behavioral response of *T. variabile* and *T. inclusum*, two important and cosmopolitan dermestids (Hagstrum and Subramanyam 2006), and found in both cases, that the distance moved, velocity, and general movement (e.g., frequencies of entry) was

increased after brief contact with deltamethrin-based LLIN both immediately after exposure and 24 h later for single-sex populations. This effect was more pronounced immediately after exposure. However, we found when the opposite sex was present, as in mixed-sex populations, movement (e.g., distance moved and velocity) decreased but only after contact with LLIN. This suggests that the opposite sex may elicit arrestment and that in a realistic setting where both sexes are present, the ability of dermestid adults to locate food and mates and move towards them may be impaired. By contrast, in single-sex populations, LLIN increased movement in stored product insect adults, likely as a result of hyperactivity in the nervous system from pyrethroid uptake given the mode of action.

Importantly, we repeatedly observed patterns of olfaction and taxis disrupted after contact with LLIN by both male and female *T. variabile* and *T. inclusum*. In some cases, changes elicited behavior that favored each stimulus, and in other cases, it favored control zones. However, behavioral response was markedly changed by both species in the majority of cases after exposure to LLIN. Practically, this may mean that individuals cannot respond as reliably to food cues and pheromones at food facilities after exposure to LLIN. This would make conspecific and heterospecific ability to find commodities and mates more tenuous, and thus less likely to succeed. Contrary to prior studies which only observed a thigmotactic response by *T. granarium* to netting (Domingue et al., 2021), for example, we found disruption of olfaction above and beyond response to the physical presence of netting alone. Exposure to LLIN can impair locating a novel food patch by stored product insects (Morrison et al., 2018; Wilkins et al., 2021). While several studies (Paloukas et al., 2020; Agrafioti et al., 2021; Scheff et al., 2021b; Agrafioti et al., 2023; Galán et al., 2024) have found that there is robust mortality when stored product insects are exposed to LLIN, Gerken et al. (2021a) also found *T. variabile*

exposed to LLIN had a lower net reproductive output, suggesting other life history parameters may also be affected by netting exposure beyond mortality and behavior. Our study adds to the growing body of literature, suggesting that exposure to LLIN may interrupt normal olfactory and orientation processes.

However, we did observe some species-specific patterns of response to the LLIN. For example, netting affected orientation to olfactory cues preferentially for *T. inclusum* more so than *T. variable*, though both were affected to some extent. The frequency of entries into the smaller stimulus zone around the food and pheromone was suppressed for both the 1 and 24 h periods in *T. inclusum*, whereas increased response to both was exhibited by *T. variable* by 24 h. Other work has assessed movement of *T. variable* after exposure to LLIN on Ethovision, but not in the presence of olfactory stimuli (Wilkins et al., 2020b). Those authors found movement was reduced by about half for *T. variable* after a 1 min exposure to LLIN. Less is known about how *T. inclusum* typically responds to netting. If LLIN is deployed at a food facility and either species of dermestid contacts the netting, it is possible that they would not subsequently be able to locate food or pheromone sources, potentially hampering dispersal to commodities and/or mate-finding.

There were also sex-linked changes in patterns of orientation to olfactory stimuli after exposure to LLIN. For example, males were more likely to spend a greater duration of time in the smaller stimulus zone near the pheromone emissions for both species, though this was most pronounced for *T. inclusum*. Pheromones appeared to be more important to males than they were for females of both species, who oriented to both food and pheromones equally. Other sex-linked behaviors by *T. inclusum* and *T. variable* that were not included in this study were, but which have been documented are: female calling, which peaks at 10:00 (Cross et al. 1977), and differing amounts of VOCs emitted (half the amount of methoxy-phenyl-oxime in females than

males; Alshuwaili 2020). Most of the work to-date with newer kairomones and pheromones has occurred with larval dermestids. For example, food cues were more important to young *T. variabile* larvae, but old *T. variabile* larvae positively responded to both food cues and pheromones (Morrison et al., 2020). Laboratory trapping assays in the US and Greece found that young *T. variabile* larvae did not show a preference among multiple baits, while older *T. variabile* larvae preferred baits with both food and pheromone cues (Sakka et al., 2023). Trapping field work in Greece has found there is not a strong preference by dermestids among multiple baits with kairomones and/or pheromones together or alone (Morrison et al., 2023).

We confirmed that appropriate *Trogoderma* spp. sex pheromones, (Z)-14-methyl-hexadecenal were emitted from the multi-species lure and were not present in the negative control, even when in close proximity to the multi-species lure. (Z)-14-methyl-8-hexadecenal was identified as the major component in both *T. inclusum* and *T. variabile* sex pheromone emissions, while (E)-14-methyl-8-hexadecenal was identified as the major component of *T. glabrum*'s emissions (Cross et al., 1976). The quarantine threat *T. granarium*, uses both components in a 92Z:8E ratio. While we did not collect food odors, food odors may emit substantial volatiles and affect stored product insect behavior (Quellhorst et al., 2024). An important reason to evaluate populations of dermestids on a sex-by-sex approach is because female dermestids, including *T. variabile*, will call during the day, which can strongly influence the behavior of males (Cross et al., 1977), and could alter the outcome of an experiment. As a result, isolating each sex and testing them separately helped us account for this potentially confounding variable, namely sex. Nonetheless, we did include a mixed-sex population, because at a food facility, there will be a mixture of females and males admixed. Thus, this latter treatment may be more realistic.

Under field conditions, mark-released-and-recaptured *T. variable* were found to have flown an average of 75 m, but up to 508 m outside a food facility (Campbell and Mullen, 2004). At close-range, walking is important for *Trogoderma* to locate pheromone sources, and will make characteristic approaches in pheromone corridors (Tobin and Bell, 1986). As a result, this insect can definitely be thought of as an immigration risk to food facilities. There have sometimes been poor results by *T. variable* to common commercial traps such as the Dome trap and Box trap (Sakka et al. 2023). Domingue et al. (2020) found frozen extracts of *Trogoderma* spp. containing oleic acid were repellent to conspecifics. Moreover, trapping with prior captures of dead *Trogoderma* spp. revealed disruption of typical patterns in monitoring traps (Gourgouta et al. 2021). In addition, our study suggests the patterns of olfaction was disturbed after exposure to LLIN for both sexed and mixed- sex *T. variable* and *T. inclusum*. This presents another benefit of using LLIN in and around food facilities. Future work should evaluate the flight capacity of *Trogoderma* spp. (e.g., Ruiz et al., 2022; Abshire et al., 2024). For example, the stored product moth *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) was found to fly an average of 24–34 km in 24 h, and its flight capacity was affected by the presence of food and pheromonal stimuli (Abshire et al., 2024). Overall, our study suggests both sex- and species-specific patterns of behavioral response after exposure to LLIN in the presence of semiochemicals. This adds to our knowledge about the sublethal effects of LLIN as it intersects with the chemical ecology of stored product insects.

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Figures:

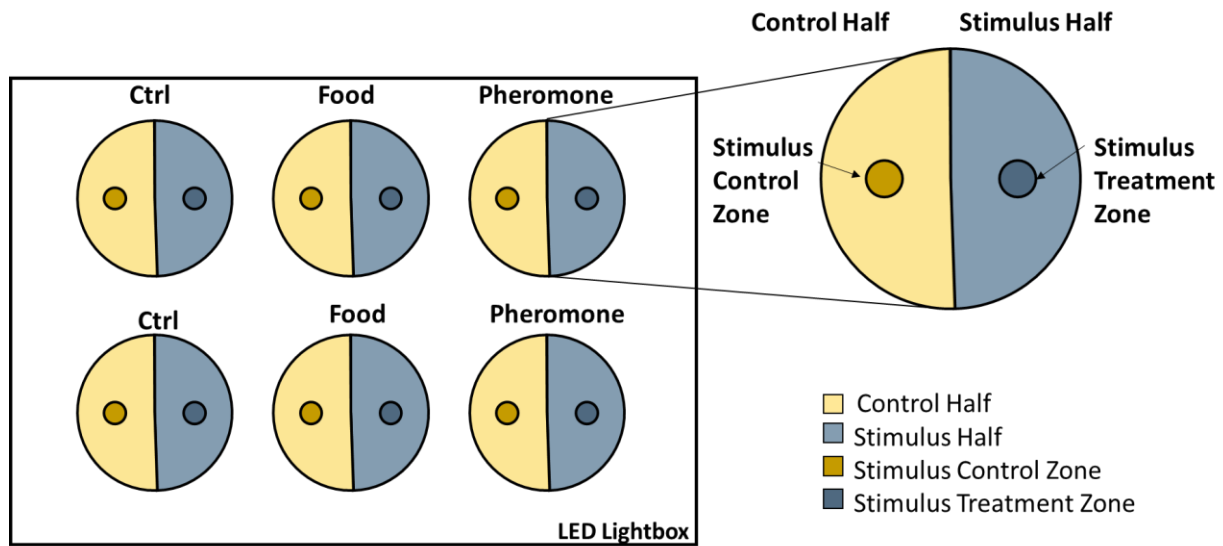


Figure 4.1 Experimental set-up for Ethovision

Experimental set-up for Ethovision. Frequency of entry, cumulative duration and latency of entering each zone was tracked by the Ethovision over a 30- min period.

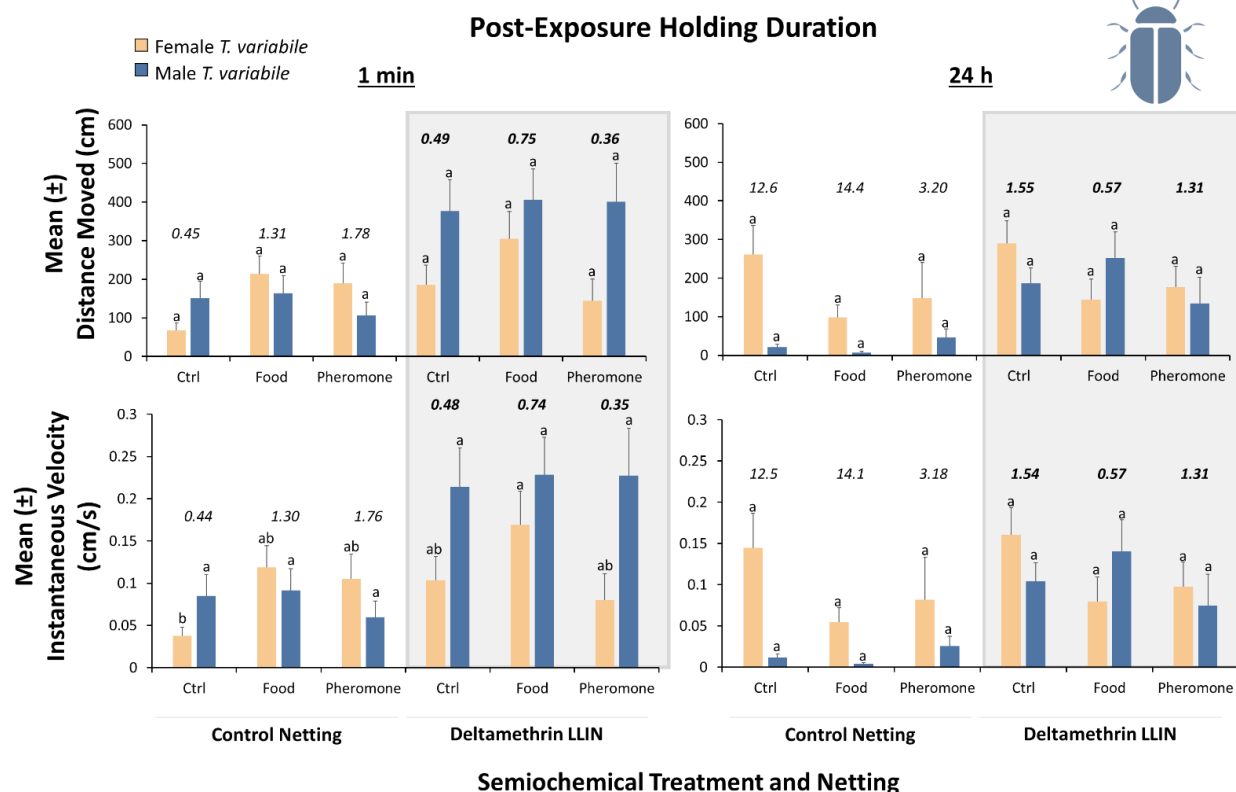


Figure 4.2 Distance moved ($\pm$ SE) or instantaneous velocity moved ($\pm$ SE) by female and male *T. variabile* after exposure to control netting or LLIN in the presence of important semiochemicals

Distance moved ($\pm$ SE, cm) (top panels) or instantaneous velocity moved ($\pm$ SE, cm/s) (bottom panels) by female (light orange) and male (dark blue bars) *T. variabile* after 1 min of exposure to control netting without insecticide or 0.4% deltamethrin-based long-lasting insecticide-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24 h later (right). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Italicized numbers over bars represent ratio of female:male response for each stimulus, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

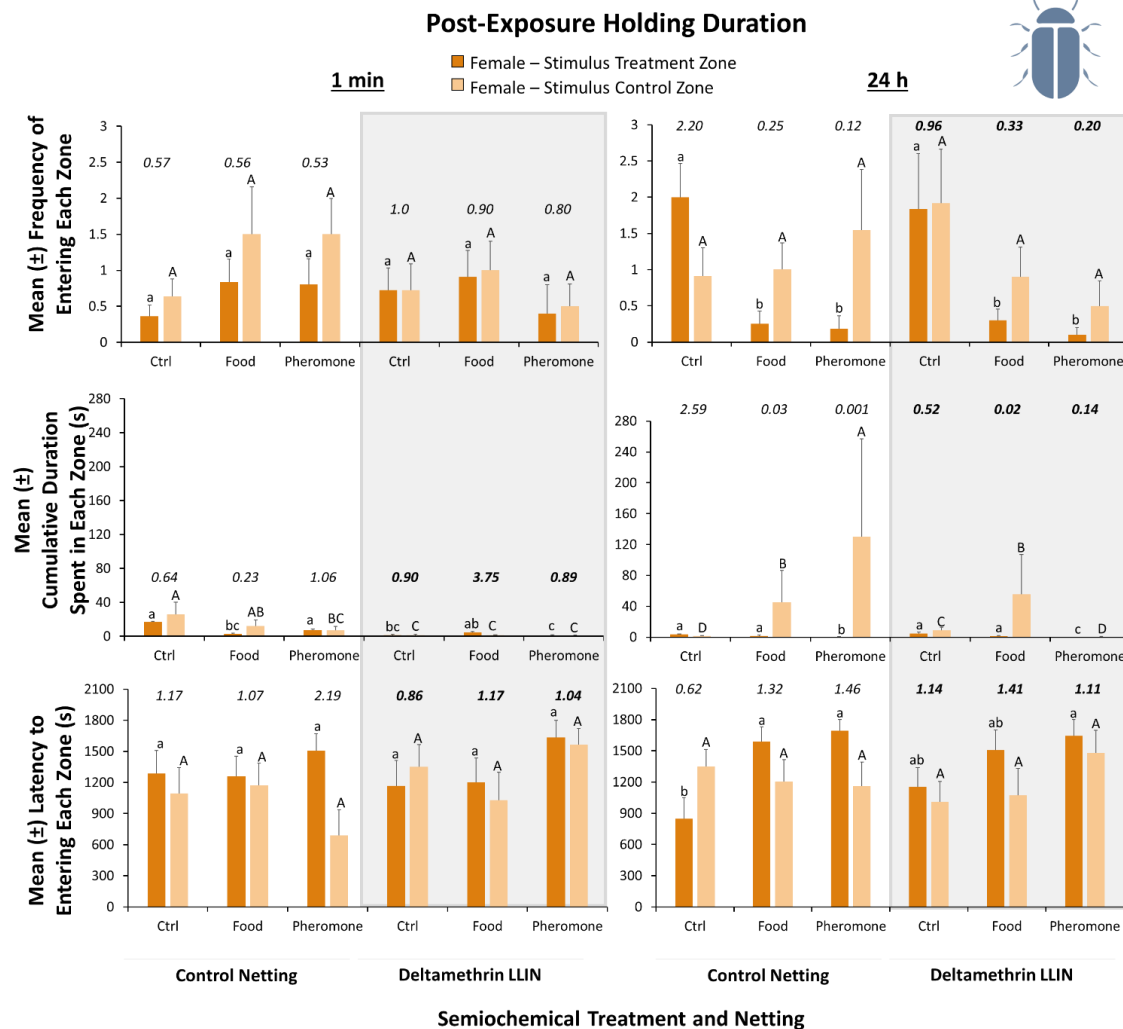


Figure 4.3 Frequency of entering ($\pm$ SE), cumulative duration spent in ($\pm$ SE) or latency to finding the treatment stimulus zone or control stimulus zone by female *T. variable* after exposure to control netting or LLIN in the presence of important semiochemicals

Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels) or latency to finding (bottom panels) the treatment stimulus zone (dark orange) or control stimulus zone (light orange) by female *T. variable* after 1-min exposure to control netting without insecticide or 0.4% deltamethrin-based long-lasting insecticide-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control stimulus zones, while lower case letters represent multiple comparisons among treatments in treatment stimulus zones. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italicized numbers over bars represent ratio of female *T. variable* behavioral response in the stimulus treatment:control zone, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

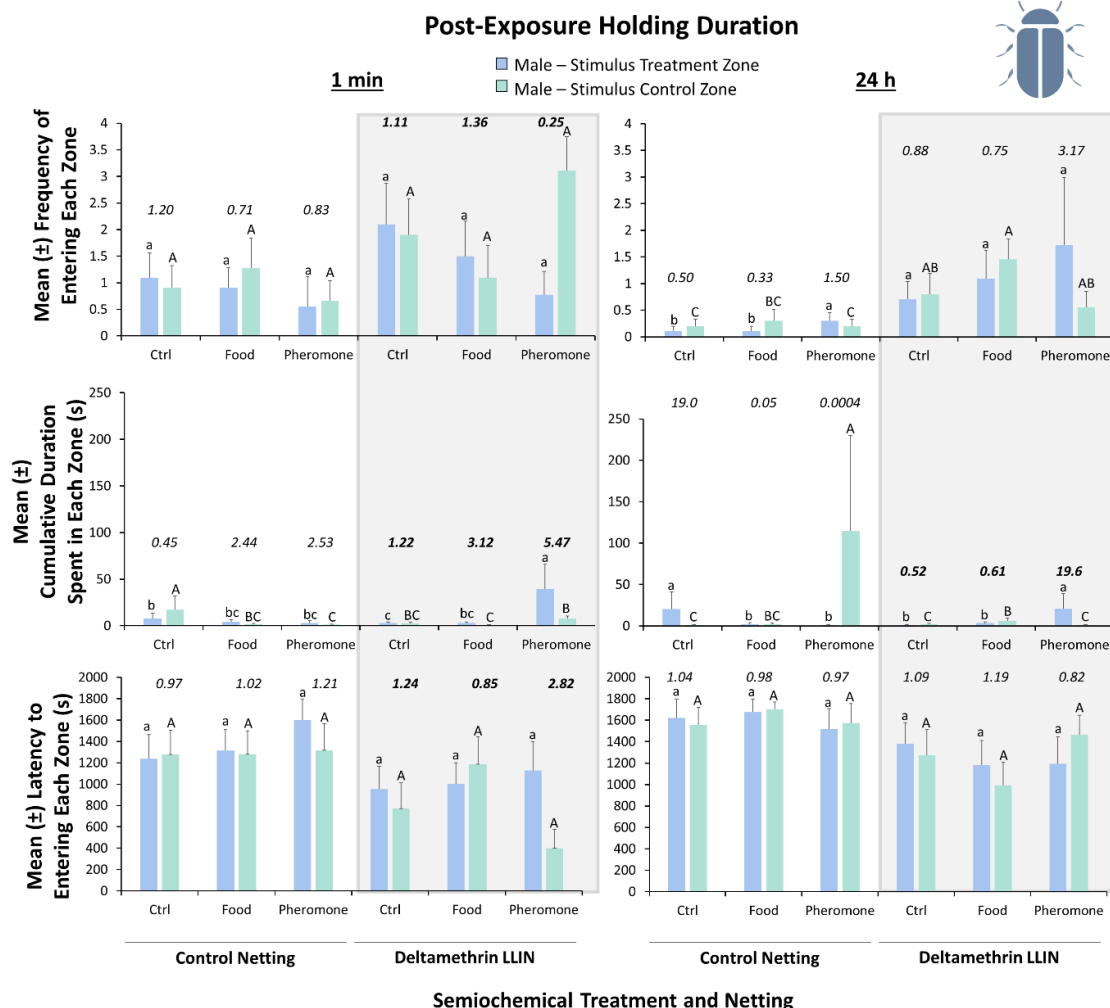


Figure 4.4 Frequency of entering ($\pm$ SE), cumulative duration spent in ($\pm$ SE), or latency to finding the treatment stimulus zone or control stimulus zone by male *T. variable* after exposure to control netting LLIN in the presence of important semiochemicals

Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels), or latency to finding (bottom panels) the treatment stimulus zone (light blue) or control stimulus zone (light green) by male *T. variable* after exposure to control netting without insecticide or 0.4% deltamethrin-based long-lasting insecticide-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control stimulus zones, while lower case letters represent multiple comparisons among treatments in treatment stimulus zones. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italicized numbers over bars represent ratio of male *T. variable* behavioral response in the stimulus treatment:control zone, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

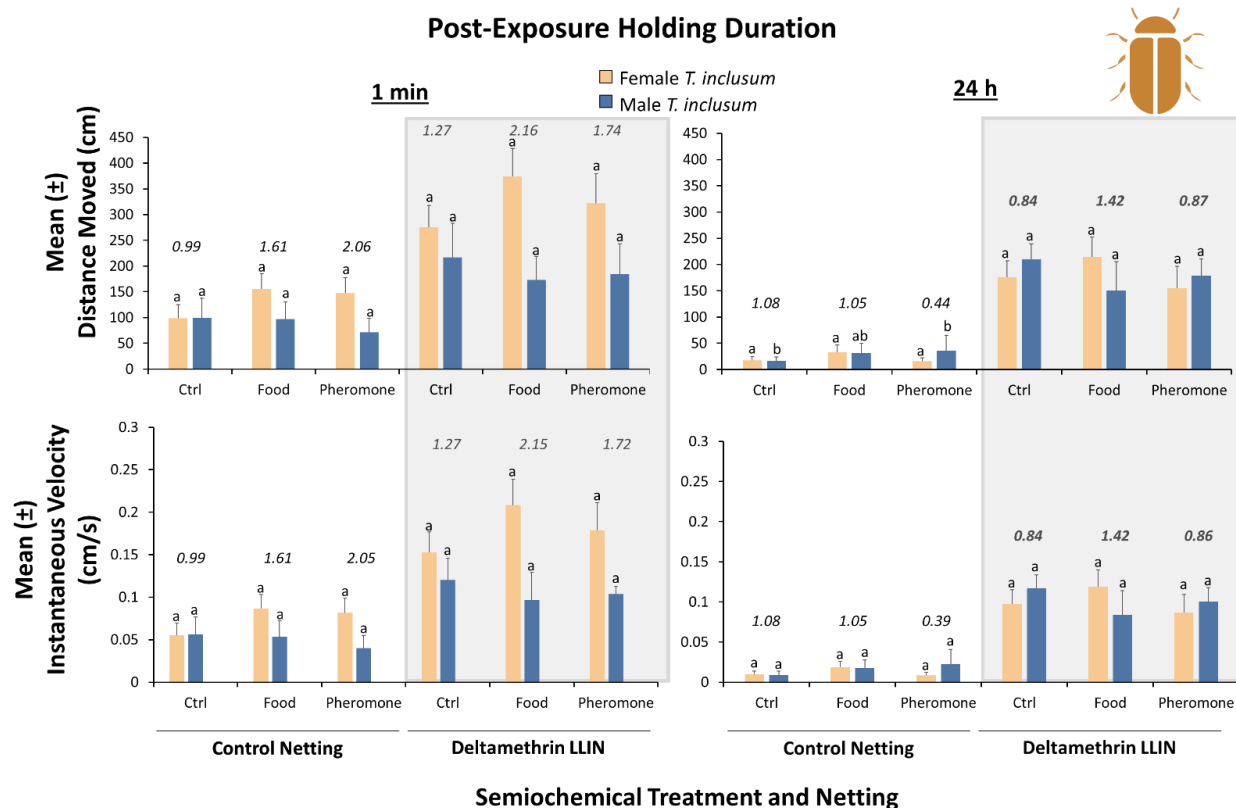


Figure 4.5 Distance moved ($\pm$ SE) or instantaneous velocity moved ($\pm$ SE) by female and male *T. inclusum* after exposure to control netting or LLIN in the presence of important semiochemicals

Distance moved ($\pm$ SE, cm) (top panels) or instantaneous velocity moved ($\pm$ SE, cm/s) (bottom panels) by female (light orange) and male (dark blue bars) *T. inclusum* after 1 min of exposure to control netting without insecticide or 0.4% deltamethrin-based long-lasting insecticide-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24 h later (right). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Italicized numbers over bars represent ratio of female:male response for each stimulus, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

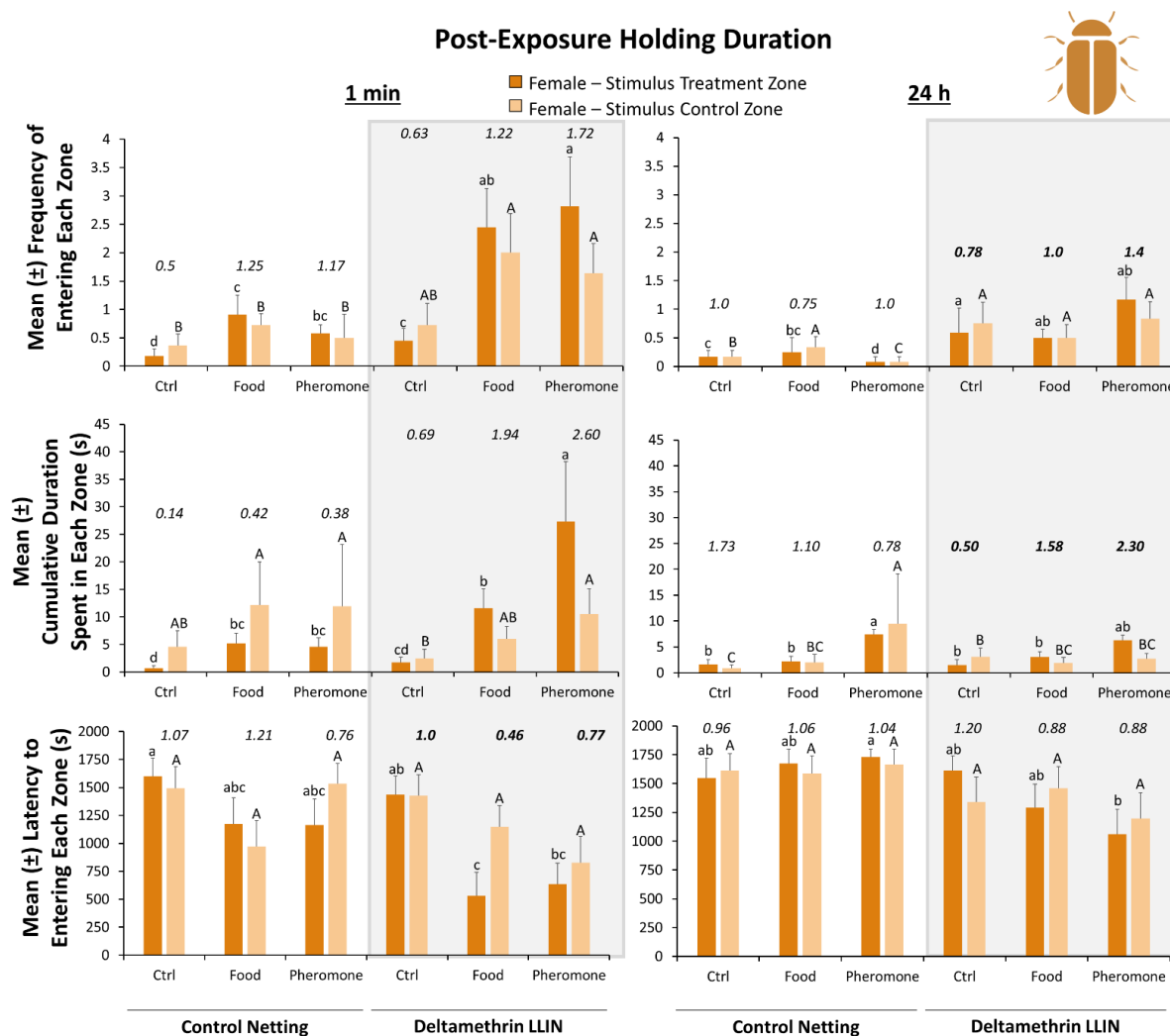


Figure 4.6 Frequency of entering ($\pm$ SE), cumulative duration spent in ($\pm$ SE) or latency to finding the treatment stimulus zone or control stimulus zone by female *T. inclusum* after exposure to control netting or LLIN in the presence of important semiochemicals

Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels), or latency to finding (bottom panels) the treatment stimulus zone (dark orange) or control stimulus zone (light orange) by female *T. inclusum* after exposure to control netting without insecticide or 0.4% deltamethrin-based long-lasting insecticide-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control stimulus zones, while lower case letters represent multiple comparisons among treatments in treatment stimulus zones. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italicized numbers over bars represent ratio of female *T.inclusum* behavioral response in the stimulus treatment:control zone, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

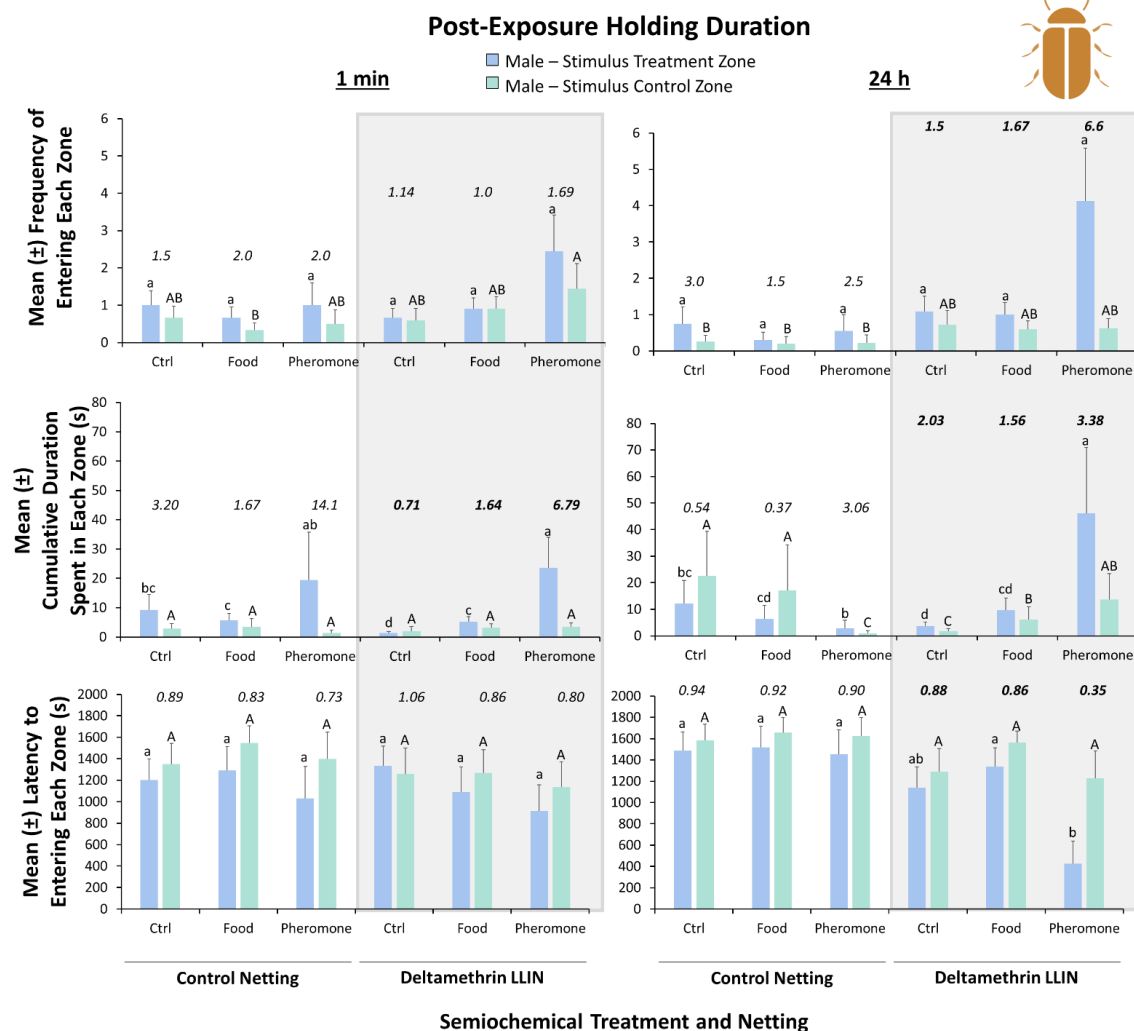


Figure 4.7 Frequency of entering ($\pm$ SE), cumulative duration spent in ($\pm$ SE), or latency to finding the treatment stimulus zone or control stimulus zone by male *T. inclusum* after exposure to control netting LLIN in the presence of important semiochemicals

Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels), or latency to finding (bottom panels) the treatment stimulus zone (light blue) or control stimulus zone (light green) by male *T. inclusum* after exposure to control netting without insecticide or 0.4% deltamethrin-based long-lasting insecticide-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control stimulus zones, while lower case letters represent multiple comparisons among treatments in treatment stimulus zones. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italicized numbers over bars represent ratio of male *T. inclusum* behavioral response in the stimulus treatment:control zone, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

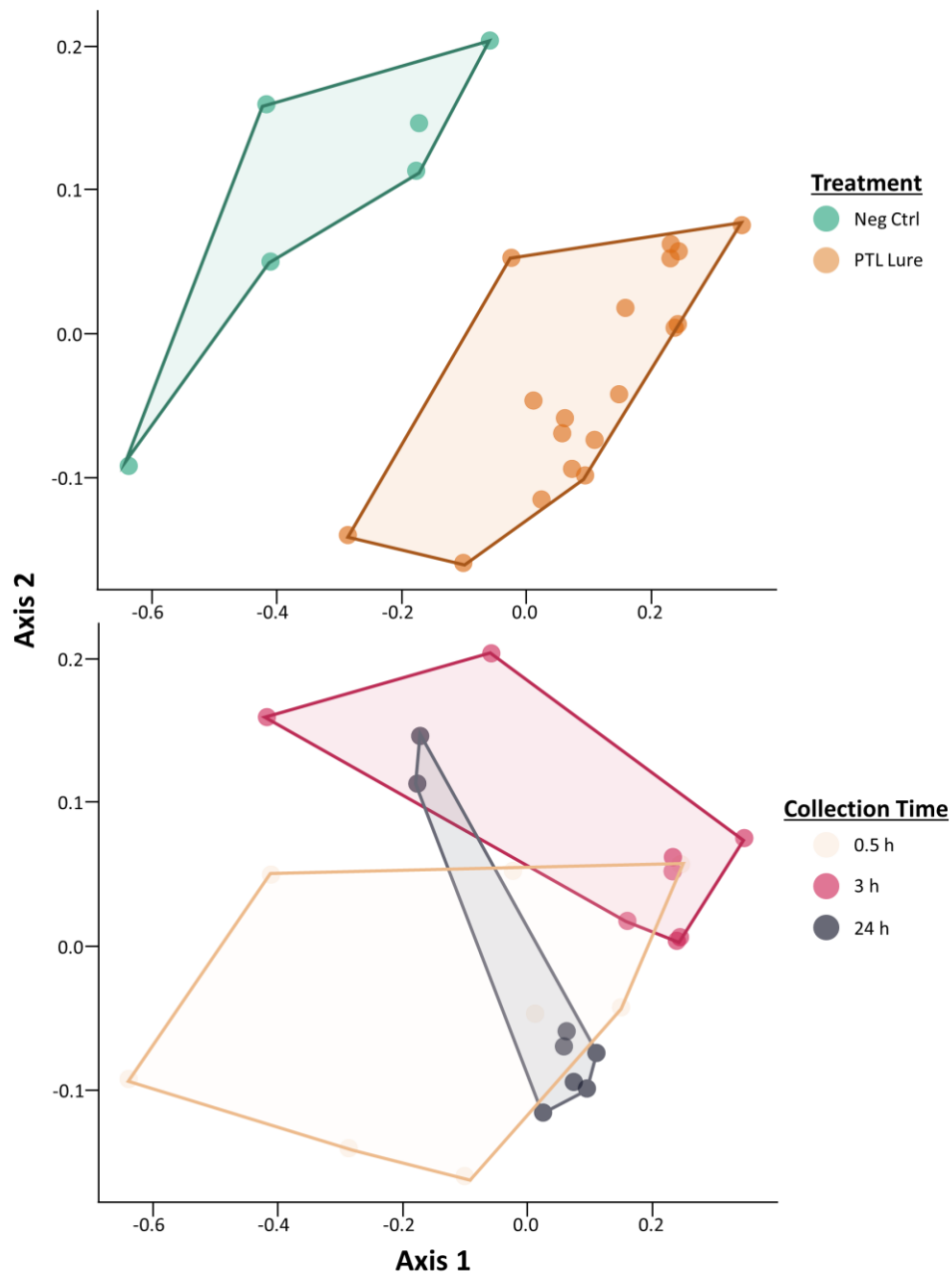


Figure 4.8 Non-metric multi-dimensional scaling ordination based on pairwise Bray-Curtis dissimilarity index calculated among samples in a pairwise fashion highlighting differences by treatment or by collection time of lure

Non-metric multi-dimensional scaling ordination based on pairwise Bray-Curtis dissimilarity index calculated among samples in a pairwise fashion highlighting differences by treatment (top panel) or by collection time of lure (bottom panel). Emissions from the pheromone lure were significantly different than background air in the laboratory (ANOSIM, $\alpha = 0.05$). Stress is less than 0.08.

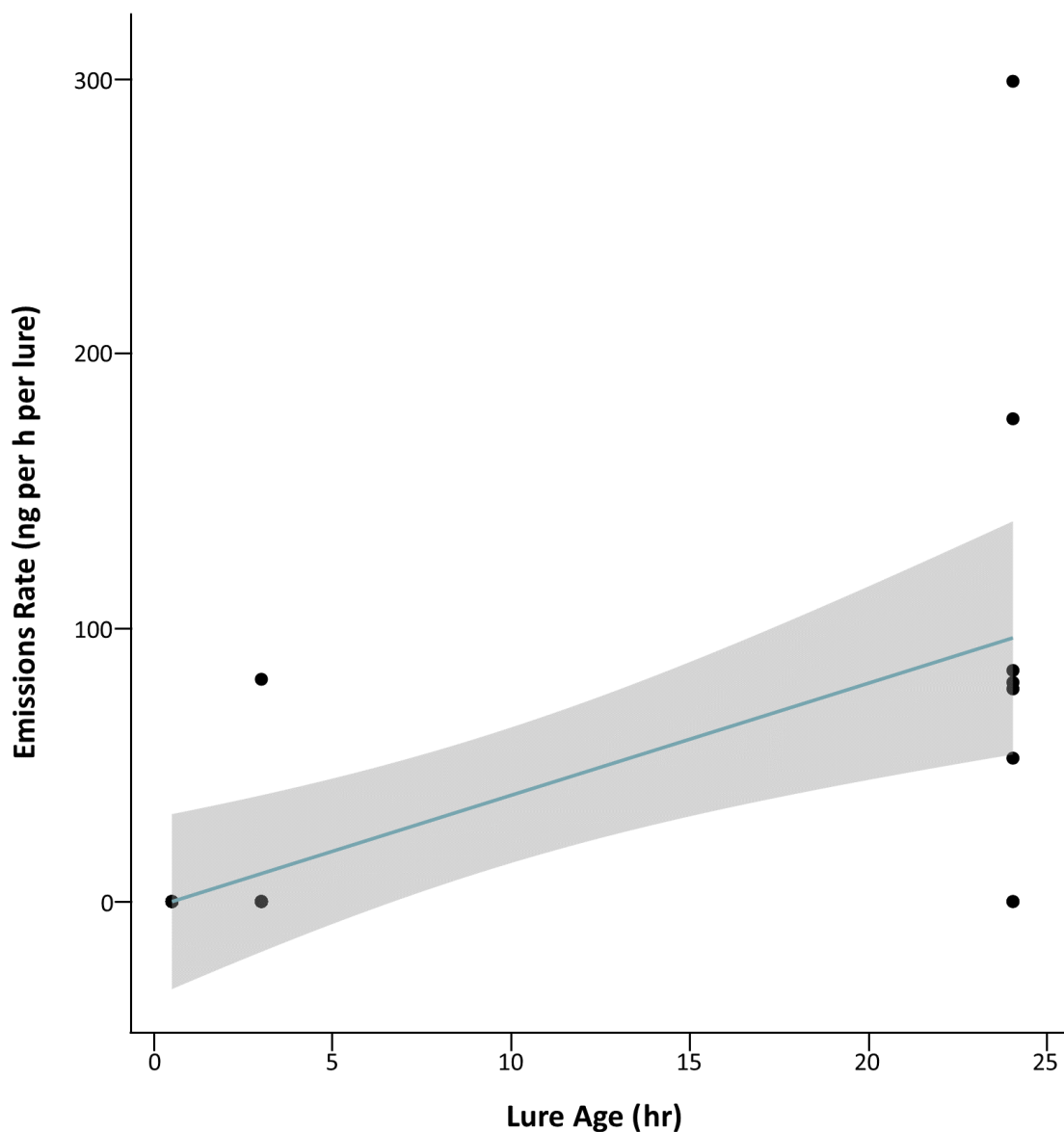


Figure 4.9 Emissions rate of (Z)-14-methyl-8-hexadecenal (*Trogoderma* spp. pheromone; in $\text{ng} \cdot \text{h}^{-1} \cdot \text{lure}^{-1}$) from 0–24 hr in the laboratory

Emissions rate of (Z)-14-methyl-8-hexadecenal (*Trogoderma* spp. pheromone; in $\text{ng} \cdot \text{h}^{-1} \cdot \text{lure}^{-1}$) from 0–24 hr in the laboratory, with a line and 95% confidence intervals fitted based on restricted maximum likelihood (REML). On average, $13.5 \text{ ng} \cdot \text{h}^{-1} \cdot \text{lure}^{-1}$ of (Z)-14-methyl-8-hexadecenal is emitted over 3 h. Over the study period, 6.75 ng of *Trogoderma* pheromone are estimated to be emitted.

Table:

Table 4.1 Summary of statistical model results for the log of the distance moved and velocity by single-sex dermestids after exposure to 0.4% deltamethrin-based long-lasting insecticide-incorporated netting in response to semiochemicals over a 30-min period.

Variable	Distance Moved (ANOVA)			Velocity (ANOVA)		
	df	F	P	df	F	P
<i>Single-sex T. variabile</i>						
treatment	2	0.40	0.67	2	2.93	0.06
netting	1	1.90	0.17	1	0.81	0.37
sex	1	0.19	0.67	1	1.90	0.17
postexposure	1	1.10	0.29	1	10.5	0.01
treatment:netting	2	0.37	0.69	2	1.74	0.18
treatment:sex	2	0.68	0.51	2	1.63	0.20
netting:sex	1	0.39	0.53	1	0.13	0.72
treatment:postexposure	2	1.18	0.31	2	3.32	0.04
netting:postexposure	1	2.28	0.13	1	2.71	0.10
sex:postexposure	1	1.15	0.28	1	5.97	0.02
treatment:netting:sex	2	0.11	0.90	2	0.36	0.70
treatment:netting:postexposure	2	1.21	0.30	2	0.46	0.63
treatment:sex:postexposure	2	0.70	0.50	2	1.94	0.15
netting:sex:postexposure	1	0.06	0.81	1	0.30	0.59
treatment:netting:sex:postexposure	2	0.73	0.49	2	1.32	0.27
Residuals	229			229		
<i>Single-sex T. inclusum</i>						
treatment	2	0.16	0.85	2	0.33	0.72
sex	1	0.28	0.60	1	0.02	0.89
netting	1	0.32	0.57	1	0.37	0.54
postexposure	1	2.41	0.12	1	0.13	0.72
treatment:sex	2	0.18	0.83	2	0.61	0.55
treatment:netting	2	0.15	0.86	2	0.29	0.75
sex:netting	1	0.85	0.36	1	1.98	0.16
treatment:postexposure	2	0.15	0.86	2	0.19	0.83
sex:postexposure	1	8.12	0.01	1	0.39	0.53
netting:postexposure	1	0.50	0.48	1	0.00	1.00
treatment:sex:netting	2	0.34	0.71	2	0.33	0.72
treatment:sex:postexposure	2	0.27	0.76	2	0.05	0.96
treatment:netting:postexposure	2	0.56	0.57	2	0.18	0.83
sex:netting:postexposure	1	1.59	0.21	1	2.77	0.10
treatment:sex:netting:postexposure	2	0.28	0.76	2	0.58	0.56
Residuals	237			237		

Table 4.2 Summary of statistical model results for the log of frequency of entering into zone/kernel zone by sexed dermestids after exposure to 0.4% deltamethrin-based long-lasting insecticide-incorporated netting in response to important semiochemicals over a 30 -min period.

Variable	MANOVA			Sequential ANOVAs							
	df	F		Freq. ¹ of Stimulus Trt Zone		Freq. of Half Trt. Zone		Freq. of Stimulus Ctrl Zone		Freq. of Half Ctrl. Zone	
		F	P	F	P	F	P	F	P	F	P
Single-sex <i>T. variabile</i>											
treatment	2	4.71	0.01	5.05	0.01	3.90	0.02	0.94	0.39	2.84	0.06
netting	1	3.58	0.01	3.42	0.07	4.77	0.03	1.92	0.17	10.4	0.001
sex	1	8.36	0.0001	0.03	0.87	31.9	0.0001	1.00	0.32	9.30	0.01
postexposure	1	2.74	0.05	0.30	0.59	0.68	0.41	1.74	0.19	9.67	0.01
treatment:netting	2	3.69	0.000	1.57	0.21	2.06	0.13	3.25	0.04	3.96	0.02
treatment:sex	2	1.60	0.18	0.89	0.41	1.04	0.35	0.37	0.69	1.76	0.17
netting:sex	1	0.80	0.52	0.56	0.46	2.80	0.10	0.04	0.83	0.04	0.84
treatment:postexposure	2	1.58	0.18	2.19	0.11	0.77	0.46	1.90	0.15	0.11	0.89
netting:postexposure	1	1.84	0.12	0.64	0.42	0.13	0.72	6.26	0.01	0.03	0.87
sex:postexposure	1	2.50	0.05	2.01	0.16	2.31	0.13	4.73	0.03	0.02	0.88
treatment:netting:sex	2	2.01	0.09	2.73	0.07	2.29	0.10	0.10	0.90	0.33	0.72
treatment:netting:postexposure	2	2.55	0.04	4.59	0.01	1.88	0.15	0.07	0.93	0.52	0.59
treatment:sex:postexposure	2	1.71	0.15	2.65	0.07	0.65	0.52	0.96	0.39	1.61	0.20
netting:sex:postexposure	1	0.82	0.51	1.24	0.27	0.02	0.88	1.54	0.22	0.49	0.49
treatment:netting:sex:postexposure	2	1.44	0.22	0.18	0.83	0.03	0.97	0.28	0.75	2.26	0.11
Residuals	229										
Single-sex <i>T. inclusum</i>											
treatment	2	7.83	0.0001	6.61	0.01	7.02	0.01	3.92	0.02	0.83	0.44
netting	1	34.1	0.0001	37.0	0.0001	24.1	0.0001	58.1	0.0001	63.6	0.0001
sex	1	11.6	0.0001	19.6	0.0001	6.46	0.01	0.71	0.40	25.0	0.0001

postexposure	1	18.3	0.0001	2.70	0.10	14.2	0.01	42.7	0.0001	29.6	0.0001
treatment:netting	2	1.71	0.15	0.12	0.89	1.49	0.23	2.81	0.06	2.94	0.05
treatment:sex	2	6.55	0.0001	0.74	0.48	2.77	0.06	10.6	0.0001	0.10	0.90
netting:sex	1	5.87	0.002	8.78	0.01	9.94	0.01	4.02	0.05	1.23	0.27
treatment:postexposure	2	5.42	0.002	9.07	0.001	0.39	0.67	0.87	0.42	0.63	0.53
netting:postexposure	1	14.0	0.0001	0.00	0.98	0.09	0.76	45.2	0.0001	12.4	0.001
sex:postexposure	1	5.95	0.001	3.49	0.06	18.0	0.0001	1.24	0.27	0.00	1.00
treatment:netting:sex	2	1.86	0.12	0.01	0.99	2.59	0.08	3.45	0.05	1.32	0.27
treatment:netting:postexposure	2	2.16	0.07	3.77	0.05	0.01	0.99	2.57	0.08	0.56	0.57
treatment:sex:postexposure	2	9.43	0.0001	12.9	0.0001	4.56	0.01	2.84	0.06	0.11	0.90
netting:sex:postexposure	1	1.03	0.39	0.77	0.38	2.77	0.09	0.03	0.85	0.13	0.71
treatment:netting:sex:postexposure	2	6.74	0.0001	2.95	0.05	3.97	0.05	9.28	0.001	0.39	0.68
Residuals	237										

¹ Abbreviations: freq. – frequency, trt – treatment, ctrl – control

Table 4.3 Summary of statistical model results for the log of cumulative duration spent in zone/kernel zone by single-sex dermestids after exposure to 0.4% deltamethrin-based long-lasting insecticide-incorporated netting in response to important semiochemicals over a 30-min period.

Variable	MANOVA			Sequential ANOVAs							
	df	F	P	Cum. ¹ Duration in Stimulus Trt Zone		Cum. Duration in Half Trt Zone		Cum.Duration in Stimulus Ctrl Zone		Cum. Duration in Half Ctrl Zone	
				F	P	F	P	F	P	F	P
Single-sex T. variabile											
treatment	2	25.9	0.0001	38.1	0.0001	0.56	0.57	23.9	0.0001	0.54	0.58
netting	1	19.8	0.0001	0.26	0.61	6.27	0.01	69.0	0.0001	5.27	0.02
sex	1	25.8	0.0001	62.4	0.0001	2.11	0.15	38.8	0.0001	2.46	0.12
postexposure	1	19.4	0.0001	11.2	0.001	4.36	0.04	61.6	0.0001	4.41	0.04
treatment:netting	2	2.63	0.05	1.05	0.35	0.19	0.83	4.30	0.01	0.20	0.82
treatment:sex	2	2.33	0.06	0.45	0.64	0.44	0.64	3.85	0.05	0.45	0.64
netting:sex	1	1.03	0.39	0.17	0.68	0.67	0.41	0.87	0.35	0.38	0.54
treatment:postexposure	2	9.44	0.0001	1.25	0.29	2.74	0.07	16.3	0.0001	2.71	0.07
netting:postexposure	1	5.49	0.001	0.11	0.73	1.37	0.24	20.1	0.0001	1.43	0.23
sex:postexposure	1	3.06	0.02	2.43	0.12	10.5	0.01	0.47	0.50	10.1	0.01
treatment:netting:sex	2	5.45	0.001	1.19	0.31	1.18	0.31	9.58	0.001	1.34	0.26
treatment:netting:postexposure	2	15.1	0.0001	1.80	0.17	0.08	0.92	29.2	0.0001	0.13	0.88
treatment:sex:postexposure	2	4.90	0.001	4.29	0.01	1.67	0.19	8.21	0.001	1.65	0.19
netting:sex:postexposure	1	5.63	0.001	0.19	0.66	0.83	0.36	20.9	0.0001	0.62	0.43
treatment:netting:sex:postexposure	2	11.5	0.0001	2.34	0.10	0.27	0.77	21.1	0.0001	0.14	0.87
Residuals	229										
Single Sex T. inclusum											
treatment	2	45.1	0.0001	83.2	0.0001	0.41	0.67	3.34	0.04	0.52	0.59
netting	1	18.4	0.0001	8.61	0.01	15.9	0.0001	49.5	0.0001	20.1	0.0001
sex	1	7.67	0.0001	23.4	0.0001	3.39	0.07	2.81	0.09	5.08	0.03

postexposure	1	6.67	0.0001	1.67	0.20	16.22	0.0001	8.34	0.01	18.9	0.0001
treatment:netting	2	1.88	0.11	1.96	0.14	1.90	0.15	0.35	0.70	2.01	0.14
treatment:sex	2	2.40	0.05	3.48	0.05	0.18	0.83	1.17	0.31	0.09	0.92
netting:sex	1	5.21	0.001	16.2	0.0001	0.10	0.76	0.00	0.98	0.03	0.87
treatment:postexposure	2	3.61	0.01	2.89	0.06	0.96	0.38	1.04	0.35	1.87	0.16
netting:postexposure	1	1.18	0.32	3.92	0.05	0.81	0.37	0.14	0.71	0.74	0.39
sex:postexposure	1	23.8	0.0001	71.8	0.0001	7.10	0.01	7.09	0.01	8.59	0.01
treatment:netting:sex	2	3.16	0.01	4.92	0.01	0.26	0.77	1.45	0.24	0.09	0.92
treatment:netting:postexposure	2	0.74	0.57	0.55	0.58	0.45	0.64	0.37	0.69	0.48	0.62
treatment:sex:postexposure	2	8.95	0.0001	17.2	0.0001	0.54	0.59	0.83	0.44	0.52	0.60
netting:sex:postexposure	1	0.74	0.56	1.74	0.19	0.02	0.88	0.31	0.58	0.11	0.74
treatment:netting:sex:postexposure	2	3.95	0.01	1.12	0.33	0.14	0.87	6.63	0.01	0.15	0.86
Residuals	237										

¹Abbreviations: Cum. – Cumulative, Trt – Treatment, Ctrl – Control

Table 4.4 Summary of statistical model results for the latency to zone/kernel zone by single-sex dermestids after exposure to 0.4% deltamethrin-based long-lasting insecticide-incorporated netting in response to important semiochemicals over a 30 -min period.

Variable	MANOVA			Sequential ANOVAs							
	df	F	P	Lat. to Stimulus Trt Zone		Lat. to Half Trt Zone		Lat. to Stimulus Ctrl Zone		Lat. to Half Ctrl Zone	
				F	P	F	P	F	P	F	P
Single Sex <i>T. variabile</i>											
treatment	2	2.85	0.02	4.21	0.02	2.19	0.11	0.01	0.99	0.23	0.79
netting	1	4.66	0.00	3.99	0.05	6.46	0.01	2.48	0.12	5.31	0.02
sex	1	0.95	0.44	0.44	0.51	0.03	0.86	0.48	0.49	2.17	0.14
postexposure	1	13.2	0.00	2.79	0.10	29.4	0.00	5.57	0.02	6.93	0.01
treatment:netting	2	0.80	0.53	0.31	0.73	0.62	0.54	1.39	0.25	0.48	0.62
treatment:sex	2	2.17	0.07	2.54	0.08	3.26	0.04	0.41	0.67	0.56	0.57
netting:sex	1	3.93	0.00	4.97	0.03	3.54	0.06	9.64	0.01	0.78	0.38
treatment:postexposure	2	2.04	0.09	0.93	0.39	1.10	0.33	1.35	0.26	2.17	0.12
netting:postexposure	1	5.05	0.00	0.04	0.84	14.5	0.00	0.65	0.42	0.13	0.72
sex:postexposure	1	2.00	0.10	1.00	0.32	3.88	0.05	3.48	0.06	0.84	0.36
treatment:netting:sex	2	1.45	0.22	0.03	0.97	0.05	0.96	2.08	0.13	0.27	0.76
treatment:netting:postexposure	2	0.38	0.82	0.40	0.67	0.42	0.66	0.49	0.61	0.12	0.89
treatment:sex:postexposure	2	1.23	0.30	2.13	0.12	1.14	0.32	0.49	0.61	0.45	0.64
netting:sex:postexposure	1	0.59	0.67	0.06	0.80	0.27	0.60	1.98	0.16	0.00	0.96
treatment:netting:sex:postexposure	2	1.64	0.17	0.38	0.68	0.75	0.48	2.84	0.06	1.28	0.28
Residuals	229										
Single Sex <i>T. inclusum</i>											
treatment	2	3.53	0.01	6.10	0.01	0.15	0.86	0.50	0.61	0.52	0.60
netting	1	15.4	0.0001	15.7	0.0001	17.8	0.0001	9.13	0.01	13.16	0.001
sex	1	1.84	0.12	2.13	0.15	0.00	0.99	0.28	0.60	3.73	0.05

postexposure	1	16.0	0.0001	9.35	0.01	32.28	0.0001	6.63	0.01	6.00	0.02
treatment:netting	2	2.60	0.04	3.41	0.05	0.28	0.76	2.15	0.12	0.08	0.92
treatment:sex	2	1.72	0.15	1.99	0.14	1.63	0.20	1.30	0.27	0.09	0.91
netting:sex	1	0.20	0.94	0.32	0.57	0.09	0.76	0.00	0.99	0.09	0.76
treatment:postexposure	2	1.53	0.19	1.89	0.15	0.01	0.99	0.93	0.40	0.22	0.80
netting:postexposure	1	6.47	0.0001	0.90	0.34	13.3	0.001	0.17	0.68	2.51	0.11
sex:postexposure	1	2.07	0.09	3.27	0.07	1.07	0.30	0.26	0.61	2.57	0.11
treatment:netting:sex	2	0.59	0.67	0.47	0.62	0.31	0.74	0.70	0.50	0.28	0.76
treatment:netting:postexposure	2	1.26	0.29	1.15	0.32	0.49	0.61	0.28	0.76	0.82	0.44
treatment:sex:postexposure	2	1.13	0.34	0.93	0.40	0.79	0.45	0.53	0.59	0.40	0.67
netting:sex:postexposure	1	0.86	0.49	3.17	0.08	0.34	0.56	0.03	0.87	0.26	0.61
treatment:netting:sex:postexposure	2	0.61	0.66	0.27	0.77	0.30	0.74	0.59	0.56	0.05	0.95
Residuals	237										

¹Abbreviations: Lat. – Latency, Trt – Treatment, Ctrl – Control

Chapter 5 - Leveraging long-lasting insecticide-incorporated netting to improve fumigation efficacy against stored product insects

Submitted as: Ranabhat, S., D. Brabec, M. Lilich, D.S. Scheff, G.V. Bingham, K.Y. Zhu, and W.R. Morrison III. 2024. Leveraging insecticide-treated netting to improve fumigation efficacy for the protection of bulk storage of commodities. Pest Management Science.

<https://doi.org/10.1002/ps.8423>

Abstract

BACKGROUND: Long-lasting insecticide-incorporated netting (LLIN) has successfully been used to impair mobility and prevent infestation of stored grain by stored product beetles.

Understanding how to integrate LLIN with existing integrated pest management (IPM) tactics, such as phosphine fumigation, can further enhance IPM programs.

RESULTS: We used three 110 metric tons (MT) capacity grain bins, and in each, 60 perforated buckets (e.g., miniature silos) were filled with 500 g of uninfested wheat. Miniature silos were protected by LLIN (0.3% α -cypermethrin, Carifend®, BASF), positive control (without insecticide), or negative control (no netting). Half of each treatment was randomly assigned to phosphine fumigation treatment, while the remainder were not fumigated. Monthly samples of 100 g of grain from four silos in one of four blocks from three-grain bins were taken between June and October both in 2022 and 2023. We determined whether phosphine fumigation could be reduced with the use of LLIN over the season. Overall, we found that silos protected with LLIN showed insect dispersal and progeny production that was reduced by 83–99% and 89–99%, respectively, compared to insecticide-free netting and no-netting controls. Additionally, damage in silos was reduced by 37–99% compared to controls. Importantly, the total number of fumigations could be reduced by 68–91% by using LLIN compared to controls.

CONCLUSION: Our study demonstrates that LLIN is consistently effective for existing pest management tactics such as phosphine fumigation in bulk storage structures.

Keywords: stored product insect, infestation, fumigation, insecticide netting, integrated pest management

Introduction

Bulk grain storage is an important segment of the postharvest supply chain. Over 24 billion bushels of grain are stored every year in the United States (US),¹ and the grain storage system is quite diverse, including on-farm storage, country and terminal elevators, transportation-related storage, and end-user storage. In the US, during bulk storage, stored product insects can readily attack these commodities, causing huge economic losses in quality and quantity, and amounting to five percent of the total costs of storage.¹ Globally, postharvest losses by the stored product pests can reach over \$100 billion annually² or 10–30% of harvested crops.³ Thus, it is crucial to have effective pest management to prevent these losses and help alleviate food insecurity.

Pest management in bulk storage heavily relies on fumigation after harvest. Methyl bromide (CH_3Br) and phosphine (PH_3) have been used as common fumigants, but methyl bromide has been phased out for bulk grain storage due to its deleterious role in depleting the ozone layer and currently restricted to quarantine pre-shipment treatments for select commodities or a triggered emergency response.⁴ Phosphine remains the main fumigant for the protection of stored bulk grains, and to a much lesser extent, the more expensive alternative sulfuryl fluoride (SO_2F_2),⁵ which is more typically used for structural fumigations. However, there are increasing challenges to using phosphine as a fumigant. For instance, 10 stored product insect species and 73% of 980 populations are reported to be resistant to this fumigant globally, and resistance has been increasing since the 1970s.^{6,7} The emergence of insecticide resistance is a problem as 96% of grain storage operations fumigate their grain for insects in the US.⁸ Further, there has been increasing demand for low or no insecticide inputs throughout the post-harvest supply chain.⁹ As a result, there is a push to diversify integrated pest management (IPM) programs for stored products.

However, a central challenge of IPM is the movement and dispersal of insects into food facilities.¹⁰ Insects can move into food facilities or migrate between fumigated and unfumigated parts of food facilities either through anthropogenic-mediated or insect-mediated means.^{11–13} All facilities have multiple entry points, such as doors, windows, and loading docks, that allow insects to enter from the nearby landscape. For example, many structures are not sealed from the landscape,¹⁴ and contain vents, loose fitting doors and/or openings, and other routes of entry for insects. Therefore, implementing strategies to intercept immigrating insects before they reach commodities in bulk storage, processing facilities, or storage warehouses, could serve as an initial hurdle against infestations. The use of long-lasting insecticide-incorporated netting (LLIN) is one such tactic that has proven adept at intercepting immigrating insects after harvest.^{15,16}

LLIN is effective against various stored product pests, plus this tactic has been shown to have both direct lethal^{17–20} and sublethal effects on multiple species of stored product pests.^{15,21–23} LLIN containing the active ingredients (a.i) 0.4% deltamethrin and 0.34% α -cypermethrin have shown direct efficacy against various life stages of multiple stored product insect species.^{15,17,19,24} In laboratory trials, LLIN exposure can reduce stored product insect progeny production in subsequent generations by half.²² In a pilot-scale warehouse, LLIN exposure reduced lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), warehouse beetle, *Trogoderma variabile* Baillon (Coleoptera: Dermestidae), and red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) dispersal by 93–95% and progeny production in commodities by 99%.¹⁶ Further, LLIN was found equally effective against phosphine-resistant and -susceptible strains of *R. dominica* and *T. castaneum*.²³ Finally, LLIN was projected to contribute to the long-term suppression of *T. castaneum* populations based on empirically derived and biologically relevant parameters.²⁵ Hence, LLIN may serve as an ideal additional

tool for protecting bulk storage in IPM programs to help in intercepting insects as they disperse in and around food facilities or preventing their entry into commodities.¹⁶

However, it is unknown whether LLIN can fruitfully be used in combination with fumigation to synergize efficacy of this existing pest management tactic at food facilities. In this study, our aim was to assess whether the use of LLIN can be combined with fumigation for bulk grain storage in bins and whether their combined use may increase the effectiveness of fumigation, while reducing the requirement to fumigate in the first place. We hypothesized that using LLIN in a bulk storage environment would decrease the need to fumigate, while increasing the efficacy of the remaining fumigations. It may increase the efficacy of the remaining fumigations by extending the period required between needed fumigations for stored product insect populations to build up to threshold. In addition, we hypothesized LLIN-protected commodities would have less damage, lower insect dispersal, and lower progeny production. We evaluated the individual or combined use of fumigation, netting without insecticide, and LLIN to protect bulk stored wheat in 110-MT capacity grain bins in a field-level, replicated study over two years.

Materials and Methods

Experimental insects

The field strains of *T. castaneum*, *R. dominica* and the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), were used in this study. For all species, 4- to 8-week-old adults were used. Cultures of strains of *T. castaneum* (collected from eastern Kansas), *R. dominica* (from Pottawatomie County, KS), and *S. oryzae* (from eastern KS) have been maintained in the laboratory since 2012, 2019, and 2012 respectively at the USDA Center for Grain Animal Health Research in Manhattan, KS. All of these strains have been shown to be susceptible to

commercially relevant concentrations of phosphine.²⁶ *Tribolium castaneum* was reared on a mixture of 95% unbleached, organic flour and 5% brewer's yeast, while *R. dominica* and *S. oryzae* were reared on tempered organic whole wheat. The colonies were maintained at 25–27.5°C, 65% RH, and 14:10 or 16:8 (L:D) h photoperiod.

LLIN treatments

In each grain bin, a total of 60, 7.57L (2 gal.) capacity buckets (hereafter, miniature silos, or silos) were each filled with 500 g of clean wheat (20% cracked grain, containing 10.8% grain moisture) and placed on the floor of a grain bin. Each of the miniature silos had holes drilled every ~7 cm around the circumference of the base at a height of 1.3 cm above the bottom and a 12.7 × 12.7 cm square cut out of the lid. The entire outer surface of the miniature silos was roughened up with sandpaper to provide an easy climbing substrate for insects. Twenty miniature silos employed Carifend® insecticide-incorporated netting (0.34% alpha-cypermethrin at 163.2 mg/m² active ingredient (a.i.), 40 deniers, 100 holes/cm²; BASF, Ludwigshafen, Germany) covering the holes and gaps attached inside with a hot glue gun. We used two different controls, including the positive control with 20 miniature silos using control netting (physical identical to LLIN without insecticide; Casa Collection, Mesh White, 1721-9668; Jo-Ann's, Hudson, OH, USA), and the negative control with 20 miniature silos without using any netting.

A total of three different 110-MT capacity grain bins (6.7 × 4.3 m D: H) were used in Manhattan, KS. Each grain bin was divided into quarters and in each, we randomly placed 15 miniature silos with 5 silos of each treatment represented in each quadrant. On a monthly basis from June to October 2022 and the experiment was completely replicated from June to October 2023, while unmanaged stored product insect populations were supplemented by additional

releases of insects. In each quarter of the grain bin, a release point for insects was randomly chosen during each month, and 25 each of adults *T. castaneum*, *R. dominica*, and *S. oryzae* were released. Thus, a total of 300 mixed-sex adults of *T. castaneum*, *R. dominica*, and *S. oryzae* were released each month in each grain bin. Dataloggers (UX100-011A Hobo Temp/RH logger, Onset, Bourne, MA, USA) were also placed inside the grain of a miniature silos in each quadrant of the grain bin and were set up to record the temperature and RH every 10 min from June to September. Dataloggers were placed on the top of grain inside miniature silos (hereafter, inside grain). Two additional data loggers were also placed on the north- and south-facing walls of the grain bins at a height of 150 cm (hereafter, inside bin).

Sampling procedure

Monthly samples of 100 g of grain were taken from July to October in 2022 and 2023 and coincided with additional releases of insects as described above. During each monthly sample, 100 g were taken from four miniature silos belonging to each treatment (e.g., BASF Carifend® LLIN, positive control, and negative control), which were scooped in plastic containers (5 × 11 cm D: H) to a pre-measured fill line demarcating 100 g. Samples were added to pre-labeled Ziplock bags and immediately brought back to the laboratory. Samples were sieved using two sieves (#10 sieve, 2.0 × 2.0 mm mesh, W.S. Tyler, Mentor, OH; and #20 sieve, 0.841 × 0.841 mm mesh; W.S. Tyler, Cleveland, OH) and the types of insect species dispersing into the grain, the number of individuals belonging to each species, and their life stages were recorded. Adults were identified to species, while immature stages of external-infesting insects were grouped together as larvae or pupae. Eggs were excluded from analysis. The health condition of each adult was classified as alive, affected, or dead (according to Morrison et al.¹⁵). Briefly, alive was moving around normally without impairment, while those affected showed erratic movements,

twitching of extremities, and/or were not able to right themselves after gentle prodding with a paintbrush and, those classified as dead were completely immobile. Grain was held in individual containers (5 × 11 cm D: H) for an additional 6 weeks after removing all the dispersing adults in the sample at 27.5°C, 65% RH, and 16:8 L:D in an environmental chamber (Percival, Perry, IA, USA) to determine progeny production. Grain quality measures was also assessed at initial collection, including the number of insect-damaged kernels (IDK), weight of damaged and undamaged grain, and mold rating (using the procedure developed in Winkle et al.²⁷). We used the definition of IDK from Quellhorst et al.²⁸ as any kernel which showed signs of chewing and/or exit holes by insects.

Phosphine fumigation

Prior to the study, half of the miniature silos in a bin were randomly assigned to a possible phosphine fumigation management treatment if above the insect infestation threshold, whereas half remained unfumigated regardless of insect pressure. For the fumigation management treatment, fumigation was triggered when the number of IDK and/or the number of insects found in the equivalent of 100 g was at the Federal Grain Inspection Service (FGIS) specified tolerances of two live insects, or conservatively 16 IDKs, which is half the tolerance for when a lot is considered “sample grade” or unfit for human consumption by USDA.

Once insect infestation levels triggered a fumigation event, the affected miniature silos were moved to a dedicated 110-MT capacity fumigation-only grain bin. The fumigation treatments were performed in 55-gallon (~208 L) barrels which were filled with 9 miniature silos (at maximum) containing sample wheat. Miniature silos were carefully and individually hand-swaddled with a clear plastic bag to ensure no loss of grain in transit between experimental

location and fumigation bin. Aluminum phosphide pellets (Detia Degesch AG, Laudenbach, Germany) were used. For each barrel, two pellets (0.6 g per pellet) were placed onto a disposable plate holding a damp paper towel. A plate was set inside each barrel and the barrels were covered with lids that contained rubber gaskets and a clamping ring which was used to hold the lid tightly to the top of the barrel. The lids had several ports which were used for gas sampling, input-air, and exhaust-air during ventilation. The phosphine concentration inside each sealed barrel reached ~1000–1200 ppm after 8 hours and was maintained for approximately 4 days. The average concentration * time (CT) product values were > 100,000 ppm*hr, which is considered a very strong fumigation and able to kill weakly phosphine resistant species.^{29,30} The phosphine concentration and temperature were monitored in each barrel with wifi phosphine sensors (Centaur Analytics, Ventura, CA). The wifi sensors collected data every 1–3 hours over the 4-day fumigation period. A control sensor was included that was located directly adjacent to the barrels to ensure there was no leakage. In addition, phosphine concentration measurements were taken approximately daily with a hand-held meter (X-am 5000 multi-gas detector, Dräger, Lübeck, Germany). The Dräger hand-held meter was used as a calibration reference for the wi-fi phosphine sensors. After the 4-day fumigation treatment, each barrel was carefully vented by flushing with fresh air for ~20 min to purge the phosphine gas from each barrel and directed out through a small chimney pipe. Then, the lid was removed, and the experimental buckets were unloaded. To calculate the C*T Product, phosphine hourly or every 3 h concentrations were obtained from the Centaur sensor, corrected with a Dräger measurement, then summed over 4 d for the length of fumigation to obtain the final value.

Insect drop test

A testing arena was constructed using a $34.6 \times 21 \times 12.4$ cm (L $\times$ W $\times$ H) plastic container (Sterilite®, Sterilite Corporation, Townsend, MA) with a 9 cm hole drilled in the center of the container. The hole was covered on the bottom of the container with Carifend® LLIN (Figure 5.1A). The netting was affixed to the bottom of container using adhesive caulking (DAP Kwik Seal, DAP Products Inc., Baltimore, MD) and a plastic funnel was attached below the netting (Figure 5.1B). The top 3-5 cm of the container was coated with Fluon® (polytetrafluoroethylene, Sigma-Aldrich Co., St. Louis, MO) and the lid was fitted with 3 cm hole covered with a fine mesh screen to prevent insect escape and allow for airflow. The container was placed above a 0.57 L jar filled with 12-13 cm of insect diet. The testing apparatus was placed above the bucket and the jar containing insect diet was placed inside the bucket. The bucket was as a secondary container to prevent insect escape during the testing period (Figure 5.1C).

Insects used in this study were obtained from pesticide susceptible lab strains maintained at the USDA Center for Grain Animal Health Research in Manhattan, KS. For all species, 2-3-week-old adults and larvae were used. Adult species used were *T. castaneum*, the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), the larger cabinet beetle, *Trogoderma inclusum* LeConte (Coleoptera: Dermestidae), the warehouse beetle, *Trogoderma variabile* Ballion (Coleoptera: Dermestidae), *R. dominica*, *S. oryzae* (L.), the maize weevil, *Sitophilus zaeamais* Motschulsky (Coleoptera: Curculionidae), the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Ptinidae), the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), and the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae). Larval species used were *T. castaneum*, *T. confusum*,

T. inclusum, *T. variabile*, and *O. surinamensis*. Two- to three-week-old larvae were used for all the tested species in this experiment.

Fifty adults or larvae of each species were placed inside one of three testing containers for ~24 h at ambient laboratory temperatures. There was only one species tested per container at a time. After 24 h, the number of insects that passed through the netting and found in the diet below the container were recorded. Insects that were partially through the netting or clinging on the underside of the net, were considered as passed through. Each drop test was repeated twice on separate days and with separate insect species for a total of six independent replicates.

Statistical analysis

A repeated measure, linear mixed model was used to assess insect dispersal, progeny production, and grain damage with quadrant in grain bin used as a nested random variable. Separate models were made for each year and species. Netting type (BASF, positive control, negative control) and fumigation (yes or no) were used as fixed, explanatory variables. Assumptions of normality and homogeneity in variances were checked using residuals and histograms (counts of each response by classes of response size were visualized), and to correct issues, log-transformation was used. The Holm method of multiple comparisons was used. All analyses were run using R software³¹ (v. 4.3.1) and tests were considered significant at $\alpha = 0.05$. The package ggplot2 was used to produce all graphs.³²

A generalized linear model was used to evaluate the time course of fumigation. Overdispersion was a problem, so a quasi-poisson distribution was used with a log-link function. Type of sensor (phosphine or control) was used as a fixed, explanatory variable. There were separate overall models for each year, then upon a significant effect of month, separate models

were run for each month. The Holm method of multiple comparisons was used. The concentration * time (CT) product was used to evaluate the success of fumigation.

A repeated measure, linear mixed model was used to assess the effects of temperature and RH on success of fumigation and of LLIN in bulk storage. Time within each grain bin was used as a repeated measure and coded as a random variable. There were separate overall models for each year, then upon a significant effect of month, separate models were run for each month. Location of sensor (inside grain or inside bin) was used as a fixed, explanatory variable. Assumptions of normality and homogeneity in variances were checked using residuals and histograms, and to correct issues, log-transformation was used. The Holm method of multiple comparisons was used.

Results

Insect dispersal into bulk storage protected by LLIN

Overall, in 2022, miniature silos with LLIN (0.34% alpha-cypermethrin) had significantly less insect infestation or dispersal compared to the silos with untreated netting or no netting, regardless of fumigation (Figure 5.2). There was a significant main effect of the netting only on the overall insect dispersal (Table 5.1). On average, there was a 95–99% reduction in the number of stored product insects dispersing into LLIN-protected grain compared to the positive control or negative control. Over four months, a total of only 25 insects dispersed into the miniature silos protected by LLIN, whereas the miniature silos protect by only netting or nothing had a total of 645 and 2347 insects, respectively. Similar results were found with *T. castaneum* and *R. dominica* in LLIN-protected miniature silos (Table 5.1, Figure 5.2) and none to very few of *S. oryzae* was observed during the study (thus analysis was not performed). The dispersal of larvae was significantly reduced when both LLIN and netting only were used as preventive measures

(Figure 5.2, bottom left panel). LLIN reduced the dispersal of larvae by 27-fold compared to netting only. There was no significant difference between fumigated and unfumigated treatment when miniature silos were protected by LLIN.

In 2023, we observed consistent results with 2022. The overall insect dispersal was significantly reduced in LLIN-protected miniature silos by 83–99% regardless of the fumigation status (Figure 5.3). This translated to a sum total of only 66 stored product insects dispersing to LLIN-protected miniature silos, whereas 473 and 1563 made it to the netting only and negative control, respectively. This pattern was similar for *T. castaneum* and *R. dominica*. There was no significant interaction between the netting and fumigation for the overall dispersal of insects as well as for the dispersal of *T. castaneum*, *R. dominica*, and overall larvae (Table 5.1, Figure 5.3). In every case, LLIN-protected miniature silos had fewer insects than the other treatments, and fumigated treatments had fewer insects than unfumigated treatments.

Progeny production in bulk storage protected by LLIN

In 2022, miniature silos with LLIN had significantly fewer progeny compared to silos with untreated netting or no netting, regardless of fumigation. In fact, progeny production in LLIN-protected miniature silos was reduced by 89–99% compared to untreated netting and the negative control (Figure 5.4, Top left panel). In total over four months, only 56 progenies were produced in LLIN-protected miniature silos, but by contrast, 960 and 2660 progeny were produced in miniature silos protected by untreated netting or no netting, respectively. Fumigation also significantly affected the overall progeny production with an overall average reduction of 43% in progeny in fumigated compared to unfumigated miniature silos. However, there was no significant interaction between the netting and fumigation on the overall progeny production (Table 5.1). Similar results were observed for the adult progeny produced by *T. castaneum*, *R.*

dominica, and overall larvae produced (Table 5.1, Figure 5.4). For *S. oryzae*, no progeny was observed during the study (thus analysis was not performed)

We observed a consistent pattern of results in 2023 for progeny production from the monthly samples reared after six weeks. LLIN-protected miniature silos experienced progeny production that was reduced by 91–99% compared to the untreated netting and negative control (Figure 5.5). Unfumigated miniature silos protected with LLIN resulted in progeny production that was reduced by 80% after 6 weeks compared to unfumigated silos. LLIN-protected grain that was fumigated had the lowest progeny production for *T. castaneum*, *R. dominica*, total larvae, and overall progeny. However, the interaction between the netting and fumigation was not significant (Table 5.1)

Damage in bulk storage protected by LLIN

In 2022, we found both the type of netting and fumigation had a significant effect on IDK and the amount of damaged grain (Table 5.2; Figure 5.6). In particular, we found LLIN-protected silos contained 79–99% fewer IDK and 92–99% less damaged grain than silos with untreated netting or no netting (Figure 5.6). The average IDK in LLIN-protected grain did not exceed 0.04–2 kernels over the course of the season. Overall, LLIN significantly reduced the IDK by 24-fold compared to the negative control even without fumigation.

In 2023, we found similar patterns to those found in 2022. For example, the type of netting significantly affected the IDK and amount of damaged grain. Indeed, the number of IDK and percent damaged grain was reduced by 50–96% and 37–92% in LLIN-protected grain compared to positive control and negative control (Figure 5.7). There was not a single month when the number of IDK exceeded 9–11 kernels, remaining below the FGIS-tolerance. Fumigation had a significant effect on the percent weight of damaged grain but not on IDK

(Table 5.2). In particular, fumigation reduced the weight of damaged grain by 82% compared to unfumigated miniature silos. Finally, we did not find a significant effect of the interaction between netting and fumigation on IDK and the percent weight of damaged grain (Table 5.2).

Fumigation efficacy and reduction

For each year, fumigation successfully reached a high concentration (Table 5.3, Figure 5.8 & 5.9). The CT values for fumigation ranged from 95634–98912 ppm · h in 2022 and 96741–104,414 ppm · h in 2023. Fumigation successfully happened over 96 h, and it was confirmed by continuous wireless monitoring (Figure 5.8 & 5.9). Further, the temperature during the fumigation was within an acceptable range for phosphine efficacy from 18.5 to 45 °C in 2022 & 13–31°C in 2023 (Figure 5.8 & 5.9). In 2022 in the fumigated quadrants, there were a total of 10–20 fumigations of miniature silos in the positive control and negative control compared to only two fumigations in the LLIN-protected silos (Figure 5.10). In 2023, there were a total of 16–23 fumigations of miniature silos in the controls compared to only four fumigations of LLIN-protected silos. This represents a reduction in fumigation of LLIN-protected silos of 68–91% (Figure 5.10).

Abiotic conditions in grain bins

In 2022, the highest temperature was observed in August month in both the inside grain (inside bucket, 49.4°C) and inside bin (52°C) and the highest % RH was observed in June for both the inside grain (79.4 %) and inside bin (98.4 %) (Table 5.4 & Table 5.5, Figure S5-1). Similarly, in 2023, the highest temperature was observed in August month inside the grain (48.7°C) and inside the bin (61.2°C). However, the highest % RH inside the grain was observed in July (76.1%) and inside the bin was observed in August (93.5%) (Table 5.4 & Table 5.5, Figure S5-2)

Insect drop results

Out of ten different adult species tested, only two species consistently passed through the netting, *C. ferrugineus* and *O. surinamensis*. *Trogoderma inclusum* had two individuals that passed through the netting (Table 5.6). The immature stages for our five species tested, had greater success traversing the netting material. All species, except *T. variabile*, were able to pass through the netting (Table 5.6). These results demonstrate that there are differences among species on the ability to pass through the netting. However, it should be noted that we did not test all larval instars among our five species. We only used 2-to-3 week-old larvae this experiment. Thus, young *T. variabile* larvae could potentially pass through the netting if in close proximity.

Discussion

This is the first study to evaluate whether the use of LLIN can practically reduce fumigation in bulk storage of commodities. We found that on average, with the use of netting, total triggered fumigations could be reduced by 68–91% relative to the other treatments over two years. Our study shows using LLIN in food facilities would reduce the need for fumigations, which could help preserve the efficacy of phosphine for treating severe infestations. This could help food facility managers act as appropriate stewards of the remaining fumigants to preserve their efficacy and use them only when needed. Phosphine resistance has rapidly become a problem with the increasing use of phosphine since the 1970s and with the phase-out of methyl bromide,⁷ and it is possible that a more measured approach to its use could preserve it for when it is truly needed.

Even under conditions that caused significant insect damage to grain in control treatments, there was an 83–99% reduction in stored product insects dispersing into bulk wheat protected by LLIN compared to the positive and negative control. Dispersal into the grain in this

case likely depended in part on pest population size, pest mobility, encounter frequency, and ability to gain entrance to grain buckets. Higher dispersal into the grain is likely to be positively correlated with all of these variables. However, the presence of LLIN is likely to restrict the ability to gain entrance to a smaller number of individuals from select species compared to the other treatments in the field study. Indeed, despite individuals apparently flying and attempting to gain access to the grain from above, this often resulted in piles of dead red flour beetle and other stored product insects on top of insecticide nets on the buckets. Thus, the alpha-cypermethrin appeared to be partially a physical as well as a chemical barrier. In a previous study, exposure to LLIN resulted in an 89–93% reduction in the number of stored product insects dispersing to a food commodity in a pilot-scale warehouse ¹⁶. Most of the individuals released near where LLIN was deployed were affected or dead after 72 h. In addition, there was a 98% reduction in dispersal to a food source 175 cm away by *T. castaneum* after exposure to LLIN compared to control netting ¹⁵. Here, though, insecticide netting was deployed in a situation where grain was directly opposite of the insects inside the miniature silos, and some could theoretically pass through to an abundant food source; it is telling that there was so little dispersal by stored product insects in the LLIN-protected miniature silos. Thus, the outstanding performance showed that LLIN is an effective tactic that can act in the background as a strong physical and chemical barrier against multiple species of stored product insects that may enhance existing holistic IPM programs at food facilities.

The insects that were able to make it through the LLIN were not able to produce many progenies compared to the positive or negative control. This showed that alpha-cypermethrin LLIN was an effective kill mechanism and/or as a result of its sublethal effects, even after briefly passing through to reach the grain bulk. Likewise, Wilkins et al.¹⁶ demonstrated that while

interception traps baited with a lure attracted over 3,000 insects over two years in two states, there was a 99% reduction in progeny production when deltamethrin-based LLIN was used compared to control netting without insecticide.¹⁶ In addition, other work also showed exposure to LLIN more strongly decreased fecundity than survivorship by four-fold in *T. castaneum*.²⁵ In the current study, we found that progeny production decreased by 91–99% in LLIN protected grain compared to our other treatments. This was true even for unfumigated buckets, indicating that LLIN may act as an additional defense against the buildup of insect populations in bulk storage, thereby averting downgrades in quality as commodities are stored and make their way through the postharvest supply chain.

Importantly, we found that the resulting percentage damage produced by stored product insects was reduced by 37–99% compared to the other treatments in this study. This was significant considering damage was unacceptably high in the negative and positive control, even with fumigation, suggesting high insect pressure. By comparison, a previous study showed that progeny production of *R. dominica*, *S. oryzae* and *S. zeamais* was reduced by 68–77% with the use of Diacon® IGR *Plus* (e.g., active deltamethrin + methoprene IGR) and Gravista® (e.g., deltamethrin + synergist + methoprene IGR) as grain protectants immediately after application to wheat, corn and brown rice.³³ By contrast, when alpha-cypermethrin (Bestox 5 EC) was used as a standalone grain protectant, it only reduced progeny production of six common stored product species by 26–40% compared to an untreated control.³⁴ Thus, compared to other tactics, LLIN appears to competitively reduce progeny production in bulk storage, and it could do so without leaving any insecticide residues on grain bulks because it does not necessarily need to be in direct contact with the grain, which is increasingly important to consumers.

We found that alpha-cypermethrin LLIN was effective over a broad range of abiotic conditions ranging from 10-59°C and 10–93 % RH over two years. This corresponds with prior work by Scheff et al.³⁵ indicating that some LLIN is effective for over a year. In addition, it also corroborates work performed by McKay et al.³⁶ showing the LLIN is robust in effectiveness over six months at a commercial rice milling operation, even with food dust deposition. Even though prior work has observed higher recovery by *T. castaneum* after exposure to LLIN at higher RH,³⁷ this apparently was not a problem in hot and humid summer Kansas. Even though Agrafioti et al.²⁰ reported considerable delayed mortality for six stored product insects after exposure to alpha-cypermethrin LLIN, this apparently did not affect its efficacy in bulk storage, and indeed we observed many dead insects directly adjacent to insecticide netting-protected grain buckets. Overall, despite the harsh conditions, the LLIN successfully protected the grain through the Kansas summers.

Fumigations in this study were highly successful, evidenced by high CT products. Surprisingly, fumigation resulted in the greatest reduction of damage and progeny production in the negative control, but the difference was minimal between fumigated and non-fumigated grain buckets protected by alpha-cypermethrin LLIN. This suggests that there may be less need to fumigate bulk storage protected by LLIN. Indeed, this is borne out by the total number of potential fumigations during the season, with just a fraction for the grain protected by LLIN. We presented the hourly CT SE, which will range from zero at the beginning of the fumigation to the max CT at the height of the fumigation, and contribute to large variability in this measure as fumigation progresses. While the CT product in our study was high, ranging from 90,000 to 100,000+,²⁹ the consequence of low CT products are fumigation failures, and this can result in population rebound leading to the rise of insecticide resistance. The wireless phosphine sensing

system employed in our work has proven to be a reliable system for continuously tracking phosphine concentrations over extended time periods in bulk storage.¹⁴ While fumigation was successful at protecting grain with no other control measures, it appeared superfluous for grain protected with LLIN.

Each miniature silo had an equal and ample amount of clearance around its diameter, allowing free movement by stored product insects. In addition, the position of each treatment was randomized in each block in this study. Likewise, every grain silo had a similar amount of headspace volume above the miniature silos, and was oriented in the landscape similarly. These features controlled for positional effects of landscape, behavioral orientation, and flight of insects. Anecdotally but quite frequently, insects were observed on the LLIN on the tops of miniature silos all without signs of movement after they had presumably succumbed to exposure to alpha-cypermethrin. The results of the drop test in our study offer a snapshot in time on the capability of the netting to function as a physical barrier to many species of stored product insects, especially the highly mobile adult stages. *Tribolium castaneum*, *R. dominica*, and *S. oryzae* adults are generally unable to pass through the LLIN, but larvae of *T. castaneum* can pass through the openings in a 24 h period. However, if there is damage to the netting via a rip, tear, stretching, to increase the size of an opening, these species could pass through (Scheff, USDA-ARS, 2024, personal communication). Many stored product insect have been documented chewing through packaging and other materials,^{38,39} even Petri dishes (Quellhorst, Kansas State University, 2024, personal communication). However, it is the case with several stored product insects that if the female cannot pass through an opening, for example a package or netting, the female will deposit her eggs through or near the opening which will allow for the larvae to crawl into the space when they hatch from the egg shell.⁴⁰ This ability of the female to deposit eggs

through or around an opening, could account for the insect species observed in the LLIN buckets. In applying the LLIN by hand to the miniature silos, there was natural variation in the seal, which may also partially account for the range in reduction in fumigations for the LLIN treatment compared to the controls. In the context of a food facility, special care should be made by technical personnel when sealing a facility to ensure there are no imperfections through which insects could immigrate.

Ideally, food facilities should be implementing multi-hurdle IPM programs to intercept and prevent pest infestation of commodities. As part of these holistic programs, the deployment of LLIN appears appropriate for protection of bulk storage of commodities from stored product insects. Prior research has also shown that LLIN is also effective at combatting phosphine-resistant populations of insects,²³ which have been proven to be widespread in the US.⁴¹ Even more optimal would be if food facility managers could rotate the type of active ingredient in the LLIN deployed at the food facility to stave off potential development of pyrethroid resistance. The use of LLIN should be considered as a part of a larger program and not as a single solution. However, it is a promising new tactic that can not only preserve the efficacy of our remaining fumigants but the quality of our commodities as well.

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Tables:

Table 5.1 Summary of generalized linear model results for the dispersal and progeny production of stored product insects from miniature silos located in three 110-MT capacity grain bins from June to September 2022 and 2023 at the Center for Grain and Animal Health Research in Manhattan, KS over a two-year period of time.

Variable	<u>Response</u>								
	<i>Total</i>			<i>T. castaneum</i>		<i>R. dominica</i>		<i>Larvae</i> ¹	
	df	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Dispersal									
<i>2022</i>									
Netting	2	8.4	0.01	12.8	0.002	12.7	0.002	78.6	0.0001
Fumigation	1	0.0	1.0	0.2	0.6	5.6	0.02	0.4	0.5
Interaction	2	0.3	0.9	1.8	0.4	1.8	0.4	1.1	0.6
<i>2023</i>									
Netting	2	41.3	0.0001	19.9	0.0001	28.8	0.0001	23.5	0.0001
Fumigation	1	19.5	0.0001	8.7	0.003	22	0.0001	5.8	0.02
Interaction	2	0.3	0.8	0.6	0.7	0.9	0.6	1.2	0.5
Progeny Production									
<i>2022</i>									
Netting	2	76.8	0.0001	52.4	0.0001	31.0	0.0001	16.1	0.0003
Fumigation	1	7.02	0.01	11.2	0.001	1.00	0.3	0.36	0.6
Interaction	2	0.83	0.7	1.20	0.5	0.08	1.0	0.67	0.7
<i>2023</i>									
Netting	2	44.3	0.0001	27.9	0.0001	36.8	0.0001	27.1	0.0001
Fumigation	1	18.8	0.0001	7.24	0.0001	27.1	0.0001	2.14	0.1
Interaction	2	0.56	0.8	2.39	0.3	3.45	0.2	2.86	0.2

¹ All species' larval stages.

Table 5.2 Summary of generalized linear model results for the damaged grain caused by stored product insects per 100g samples from miniature silos located in three 110-MT capacity grain bins from June to September 2022 and 2023 at the Center for Grain and Animal Health Research in Manhattan, KS over a two-year period of time.

Variable	Response				
	%damaged grain			IDK	
	df	χ^2	P	χ^2	P
2022					
Netting	2	26.1	0.0001	52.9	0.0001
Fumigation	1	15.7	0.0001	3.75	0.1
Interaction	2	0.96	0.6	1.55	0.5
2023					
Netting	2	33.2	0.0001	36.6	0.0001
Fumigation	1	16.4	0.0001	17.5	0.0001
Interaction	2	2.49	0.3	2.52	0.3

Table 5.3 Summary of average and high phosphine (PH₃) concentration and accumulated Ct products for the fumigation for the samples from miniature silos located in three 110-MT capacity grain bins from July to September 2022 and 2023 at the Center for Grain and Animal Health Research in Manhattan, KS over two years.

Years	Average PH ₃ (ppm)	PH ₃ SE	High PH ₃ (ppm)	Mean Ct	Ct SE
2022					
July	860.6	21.35	1228.8	98912.2	50465.4
August	987.0	25.05	1332.8	96728.4	49351.2
September	944.1	20.75	1338.3	95633.7	48792.7
2023					
July	842.3	76.8	1290.1	104414.4	53272.7
August	727.6	88.49	1341.9	104763.7	53450.9
September	989.2	504.7	1059.2	96741.3	5133.9

Ct Product: Concentration ppm * time (ppm*hr)

Table 5.4 Summary of abiotic variables including temperature (°C) and relative humidity (%) from miniature silos located in three 110-MT capacity grain bins from June to September 2022 and 2023 at the Center for Grain and Animal Health Research in Manhattan, KS over two years.

Years	Inside grain							Inside bin						
	N	Average Temp	SE	Max Temp	Average RH	SE	Max RH	N	Average Temp	SE	Max Temp	Average RH	SE	Max RH
2022														
<i>June</i>	53100	29.5	0.03	45.0	53.2	0.04	79.4	28380	29.7	0.04	47.8	55.8	0.11	98.4
<i>July</i>	58032	31.0	0.02	46.0	51.4	0.04	77.2	31248	31.0	0.04	49.6	54.4	0.10	90.5
<i>August</i>	58032	30.8	0.02	49.4	50.0	0.03	69.7	31248	30.8	0.04	52.0	52.2	0.10	88.5
<i>September</i>	38896	26.4	0.03	45.2	45.7	0.04	69.7	12986	25.8	0.07	51.3	47.8	0.14	88.5
2023														
<i>June</i>	31080	29.3	0.04	45.6	45.5	0.04	69.98	18648	30.7	0.07	55.0	46.3	0.14	89.0
<i>July</i>	44640	30.4	0.03	48.7	51.8	0.04	76.17	26784	31.5	0.06	58.0	53.7	0.13	90.2
<i>August</i>	36734	31.1	0.03	48.7	49.9	0.05	74.52	26784	32.5	0.06	61.2	50.3	0.13	93.5
<i>September</i>	30239	26.9	0.03	44.3	44.2	0.05	68.64	25920	28.1	0.07	58.8	46.3	0.13	84.7

Table 5.5 Summary of repeated measures general linear mixed model results for the abiotic variables from miniature silos located in three 110-MT capacity grain bins from June to September in 2022 and 2023 at the Center for Grain and Animal Health Research in Manhattan, KS over a two-year period of time.

Variable	Temperature			%RH	
	Location of sensor			Location of sensor	
	df	χ^2	P	χ^2	P
<i>2022</i>					
<i>Overall</i>	1	25.49	0.0001	10668	0.0001
June	1	238.05	0.0001	2544.5	0.0001
July	1	50.35	0.0001	5055	0.0001
August	1	11.53	0.0001	1235	0.0001
September	1	1350.70	0.0001	1235	0.0001
<i>2023</i>					
<i>Overall</i>	1	1603.80	0.0001	769.43	0.0001
June	1	425.45	0.0001	47.259	0.0001
July	1	438.25	0.0001	332.35	0.0001
August	1	449.10	0.0001	104.34	0.0001
September	1	310.98	0.0001	368.71	0.0001

Table 5.6 Mean percentage of adult and larval individuals observed on top or below the netting after 24 h drop test.

% Top of netting	Species	% Bottom of netting
100.0	<i>T. variabile</i>	0.0
100.0	<i>T. confusum</i>	0.0
100.0	<i>T. castaneum</i>	0.0
100.0	<i>S. zeamais</i>	0.0
100.0	<i>S. oryzae</i>	0.0
100.0	<i>R. dominica</i>	0.0
100.0	<i>L. serricorne</i>	0.0
99.3	<i>T. inclusum</i>	0.7
35.0	<i>C. ferrugineus</i>	65.1
27.7	<i>O. surinamensis</i>	72.3
% Top of netting	Species	% Bottom of netting
100.0	<i>T. variabile</i>	0.0
84.0	<i>T. inclusum</i>	16.0
77.3	<i>O. surinamensis</i>	22.7
68.4	<i>T. castaneum</i>	31.6
58.1	<i>T. confusum</i>	41.9

Figures:

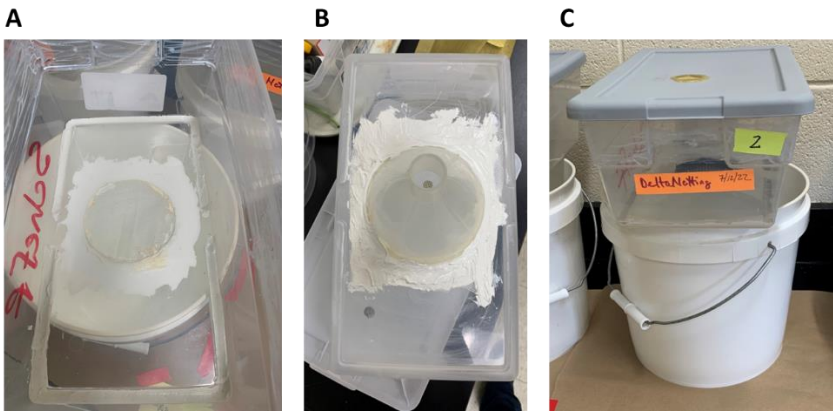


Figure 5.1 Insect drop test arena

Drop test arena with a 9 cm hole drilled in the center of the container and covered with netting, viewed A) from the top; B) bottom; and C) entire apparatus.

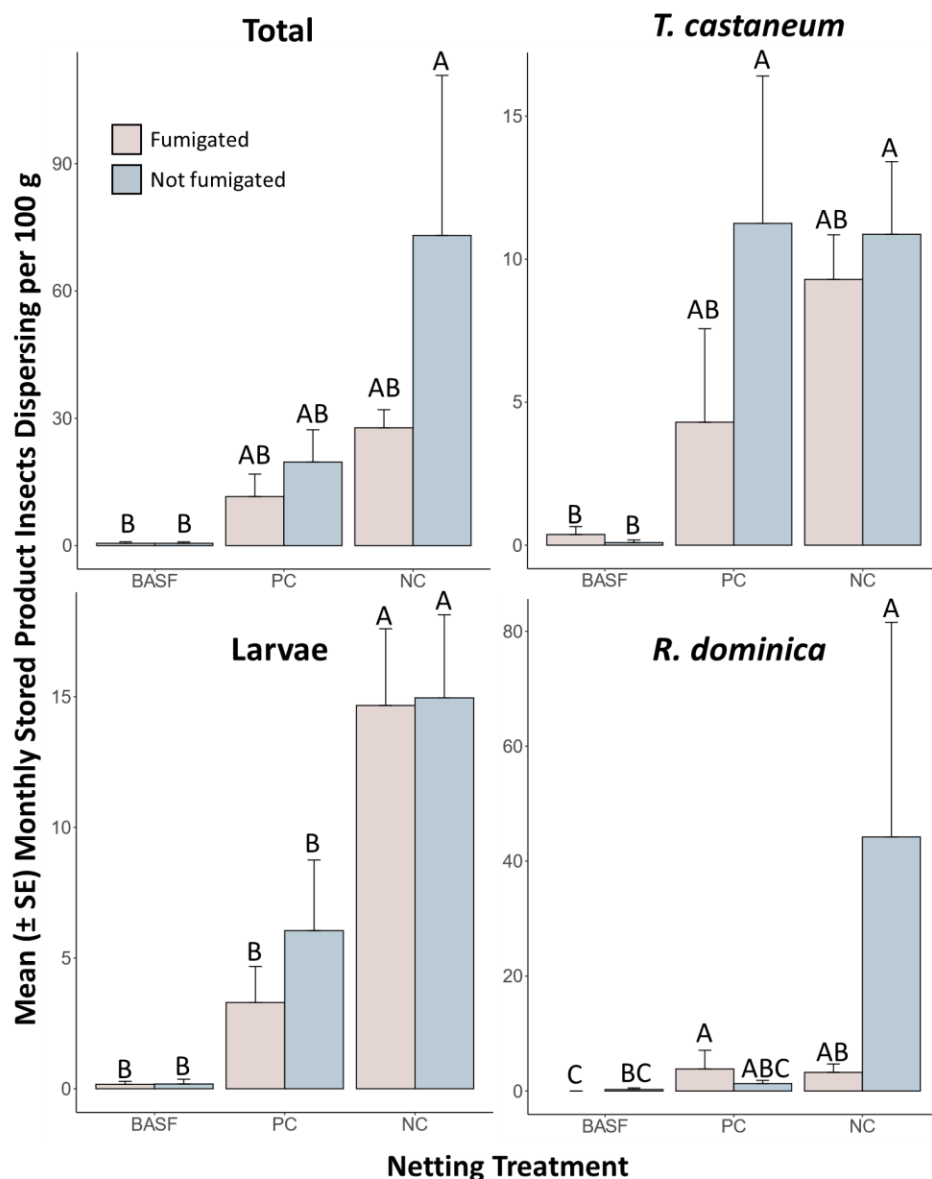


Figure 5.2 Mean ($\pm$ SE) initial dispersal of stored product insect life stages per 100 g during monthly samples collected from June to September 2022 in miniature silos

Mean ($\pm$ SE) initial dispersal of stored product insect life stages. Results include, total (top left), *T. castaneum* (top right), larvae (bottom left), and *R. dominica* (bottom right) per 100 g during monthly samples collected from June to September 2022 in miniature silos protected by Carifend® insecticide-incorporated netting (0.34% alpha-cypermethrin, BASF), control netting without insecticide (positive control or PC) or a negative control (NC) without netting. Bars with shared letters are not significantly different from each other (Holm method, $\alpha = 0.05$). Blue bars were not fumigated, while the red bars were fumigated based on the FGIS tolerances. Different total axis values used to adequately show patterns. Larvae are combined larvae of all external-infesting insects.

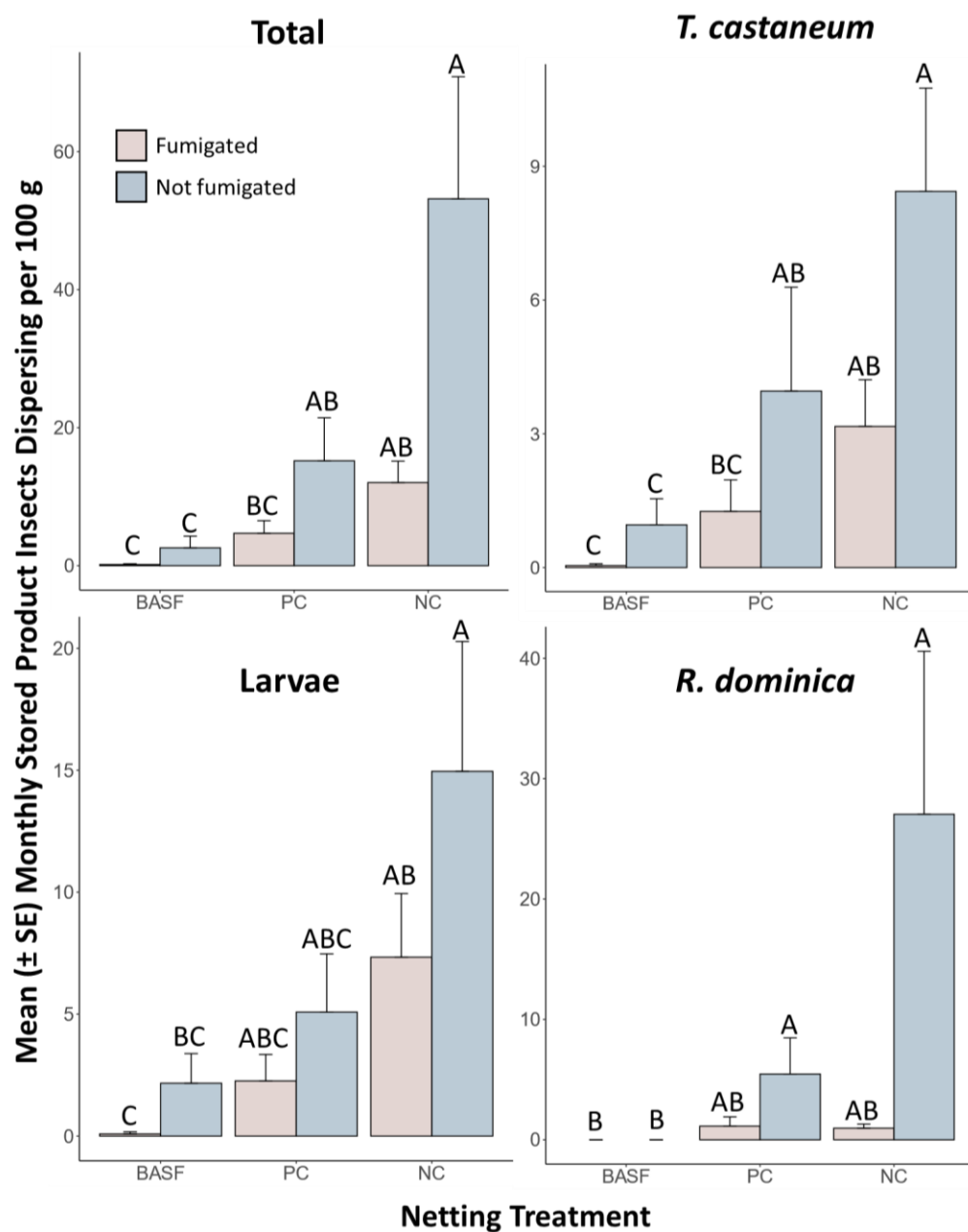


Figure 5.3 Mean ($\pm$ SE) initial dispersal of stored product insect life stages per 100 g during monthly samples collected from June to September 2023 in miniature silos

Mean ($\pm$ SE) initial dispersal of stored product insect life stages. Results include, total (top left), *T. castaneum* (top right), larvae (bottom left), and *R. dominica* (bottom right) per 100 g during monthly samples collected from June to September 2023 in miniature silos protected by Carifend® insecticide-incorporated netting (0.34% alpha-cypermethrin, BASF), control netting without insecticide (positive control or PC) or a negative control (NC) without netting. Bars with shared letters are not significantly different from each other (Holm method, $\alpha = 0.05$). Blue bars were not fumigated, while the red bars were fumigated based on the FGIS tolerances. Different total axis values used to adequately show patterns. Larvae are combined larvae of all external-infesting insects.

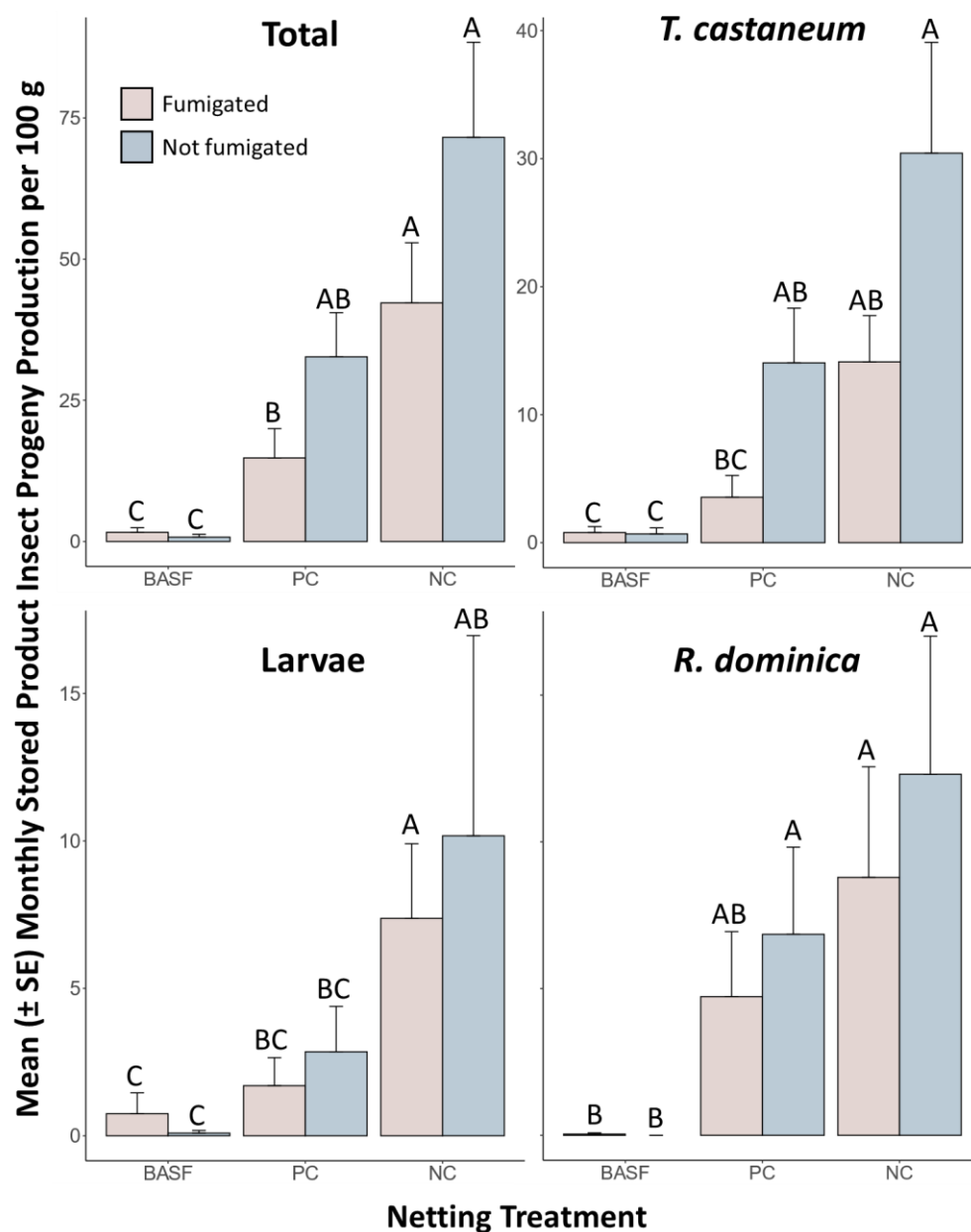


Figure 5.4 Mean ($\pm$ SE) progeny production of stored product insect life stages per 100 g during monthly samples (reared out after 6 wk) collected from June to September 2022
Mean ($\pm$ SE) progeny production of stored product insect life stages. Results include, total (top left), *T. castaneum* (top right), larvae (bottom left), and *R. dominica* (bottom right) per 100 g during monthly samples (reared out after 6 wk) collected from June to September 2022 in miniature silos protected by Carifend® insecticide-incorporated netting (0.34% alpha-cypermethrin, BASF), control netting without insecticide (positive control or PC) or a negative control (NC) without netting. Bars with shared letters are not significantly different from each other (Holm method, $\alpha = 0.05$). Blue bars were not fumigated, while the red bars were fumigated based on the FGIS tolerances. Different total axis values used to adequately show patterns. Larvae are combined larvae of all external-infesting insects.

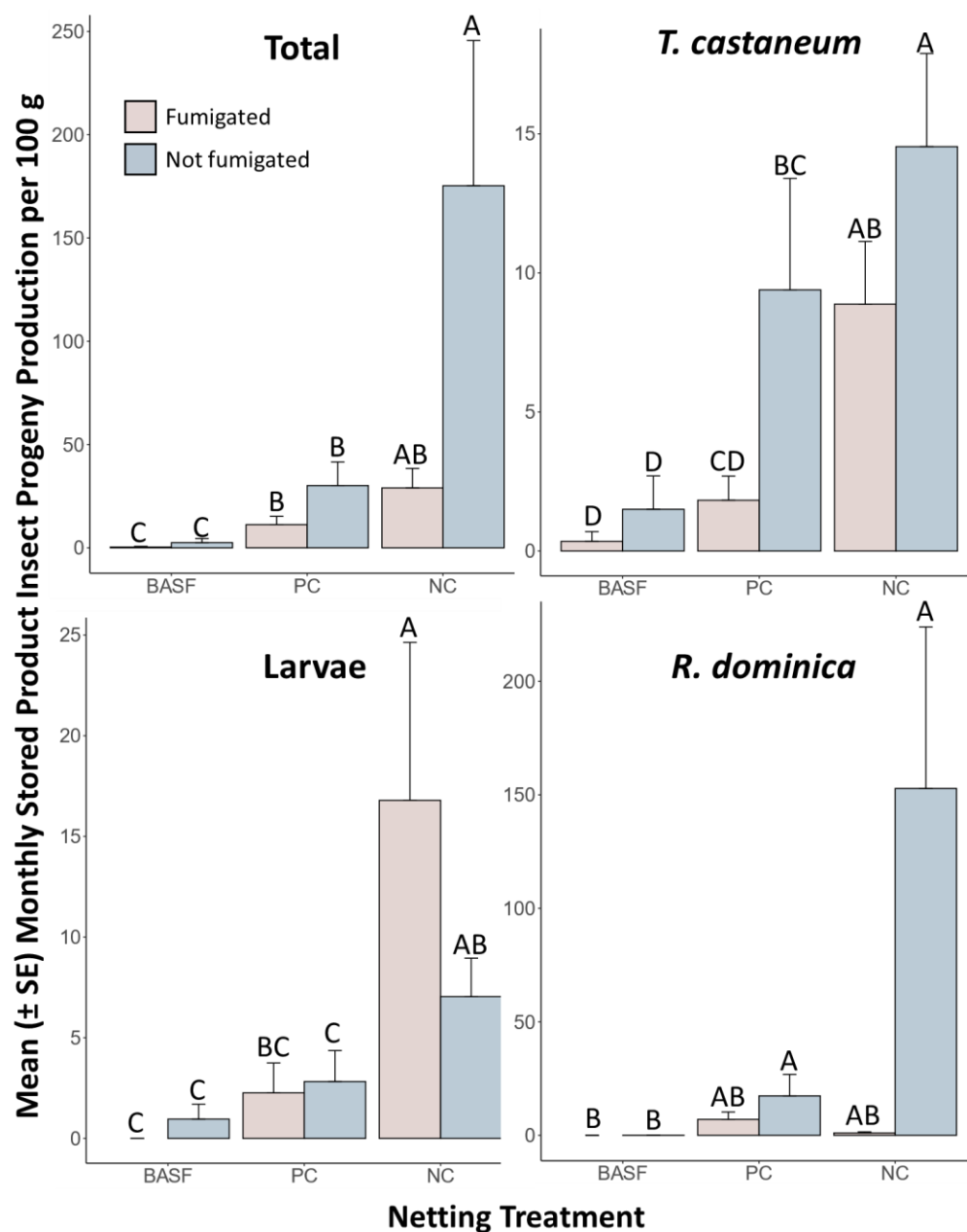


Figure 5.5 Mean ($\pm$ SE) progeny production of stored product insect life stages per 100 g during monthly samples (reared out after 6 wk) collected from June to September 2023

Mean ($\pm$ SE) progeny production of stored product insect life stages. Results include, total (top left), *T. castaneum* (top right), larvae (bottom left), and *R. dominica* (bottom right) per 100 g during monthly samples (reared out after 6 wk) collected from June to September 2023 in miniature silos protected by Carifend® insecticide-incorporated netting (0.34% alpha-cypermethrin, BASF), control netting without insecticide (positive control or PC) or a negative control (NC) without netting. Bars with shared letters are not significantly different from each other (Holm method, $\alpha = 0.05$). Blue bars were not fumigated, while the red bars were fumigated based on the FGIS tolerances. Different total axis values used to adequately show patterns. Larvae are combined larvae of all external-infesting insects.

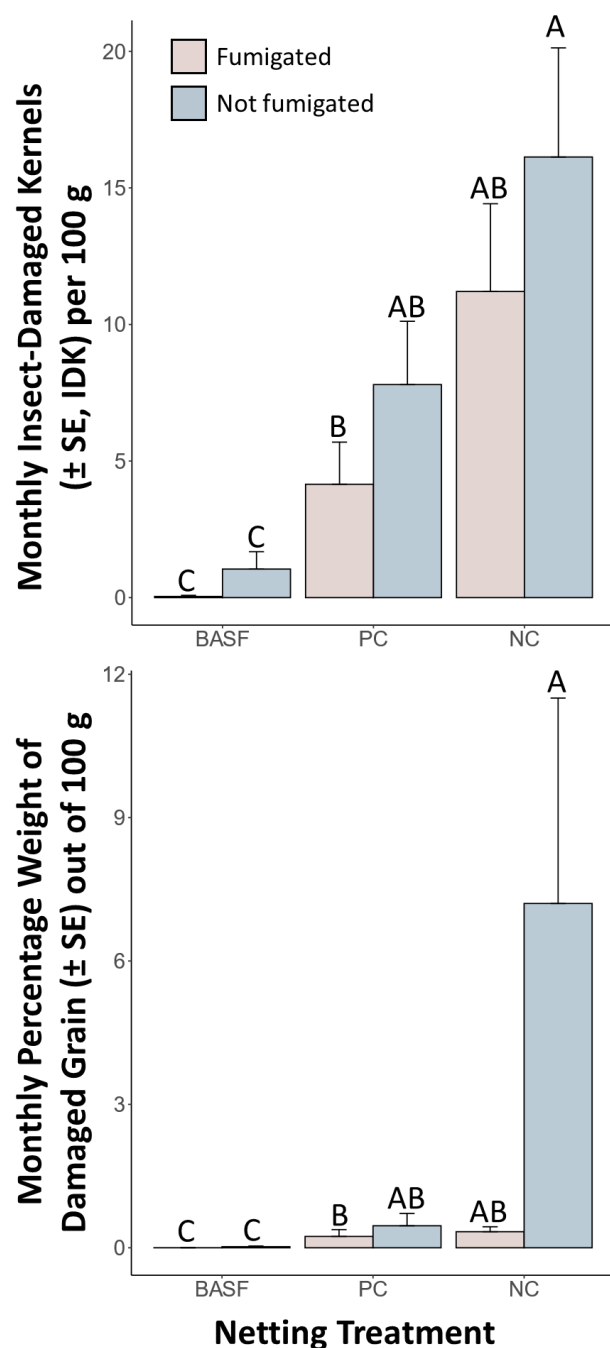


Figure 5.6 Monthly damage measures by stored product insects in 2022

Monthly damage measures by stored product insects. Quality measures include insect-damaged kernels ($\pm$ SE, IDK) (top panel) and percentage weight of damaged grain ($\pm$ SE) (bottom panel) out of 100 g of wheat collected from June to September 2022 from the miniature silos either protected by Carifend® insecticide-incorporated netting (0.34% alpha-cypermethrin, BASF) or control netting without insecticide (Positive Control or PC) or negative control (NC) without netting. Bars with shared letters are not significantly different from each other (Holm method, $\alpha = 0.05$). Blue bars were not fumigated, while the red bars were fumigated based on the FGIS tolerances.

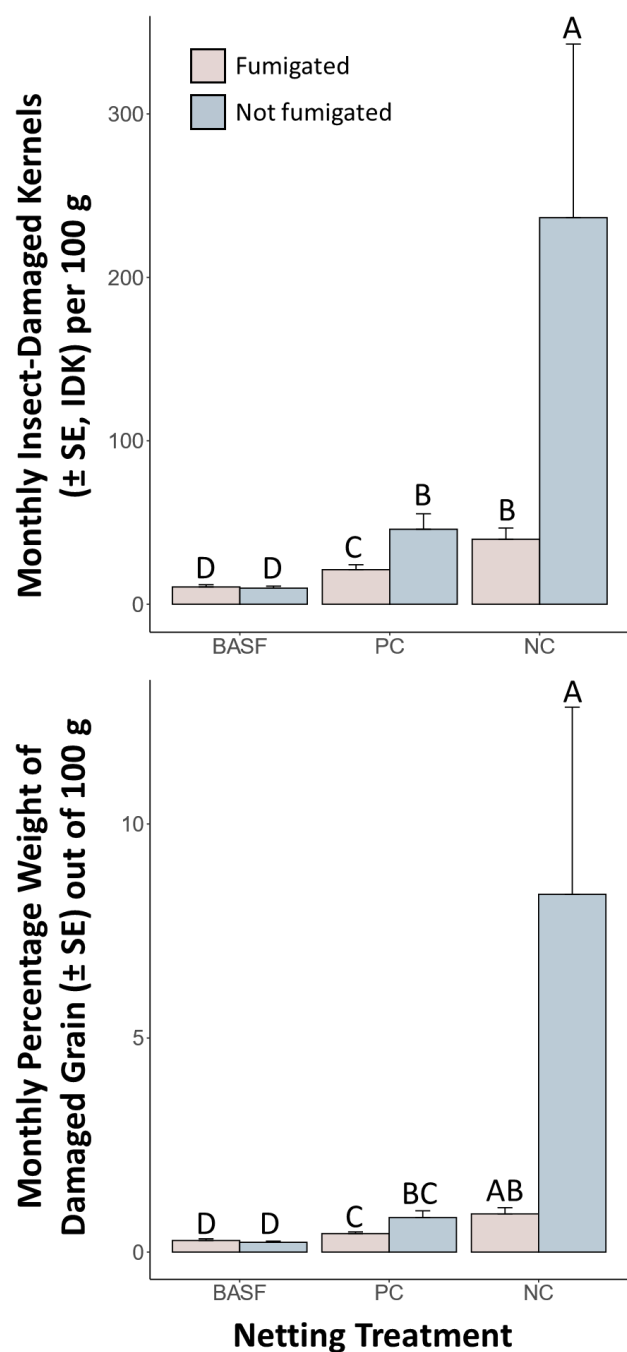


Figure 5.7 Monthly damage measures by stored product insects in 2023

Monthly damage measures by stored product insects. Quality measures include insect-damaged kernels ($\pm$ SE, IDK) (top panel) and percentage weight of damaged grain ($\pm$ SE) (bottom panel) out of 100 g of wheat collected from June to September 2023 from the miniature silos either protected by Carifend® insecticide-incorporated netting (0.34% alpha-cypermethrin, BASF) or control netting without insecticide (Positive Control or PC) or negative control (NC) without netting. Bars with shared letters are not significantly different from each other (Holm method, $\alpha = 0.05$). Blue bars were not fumigated, while the red bars were fumigated based on the FGIS tolerances.

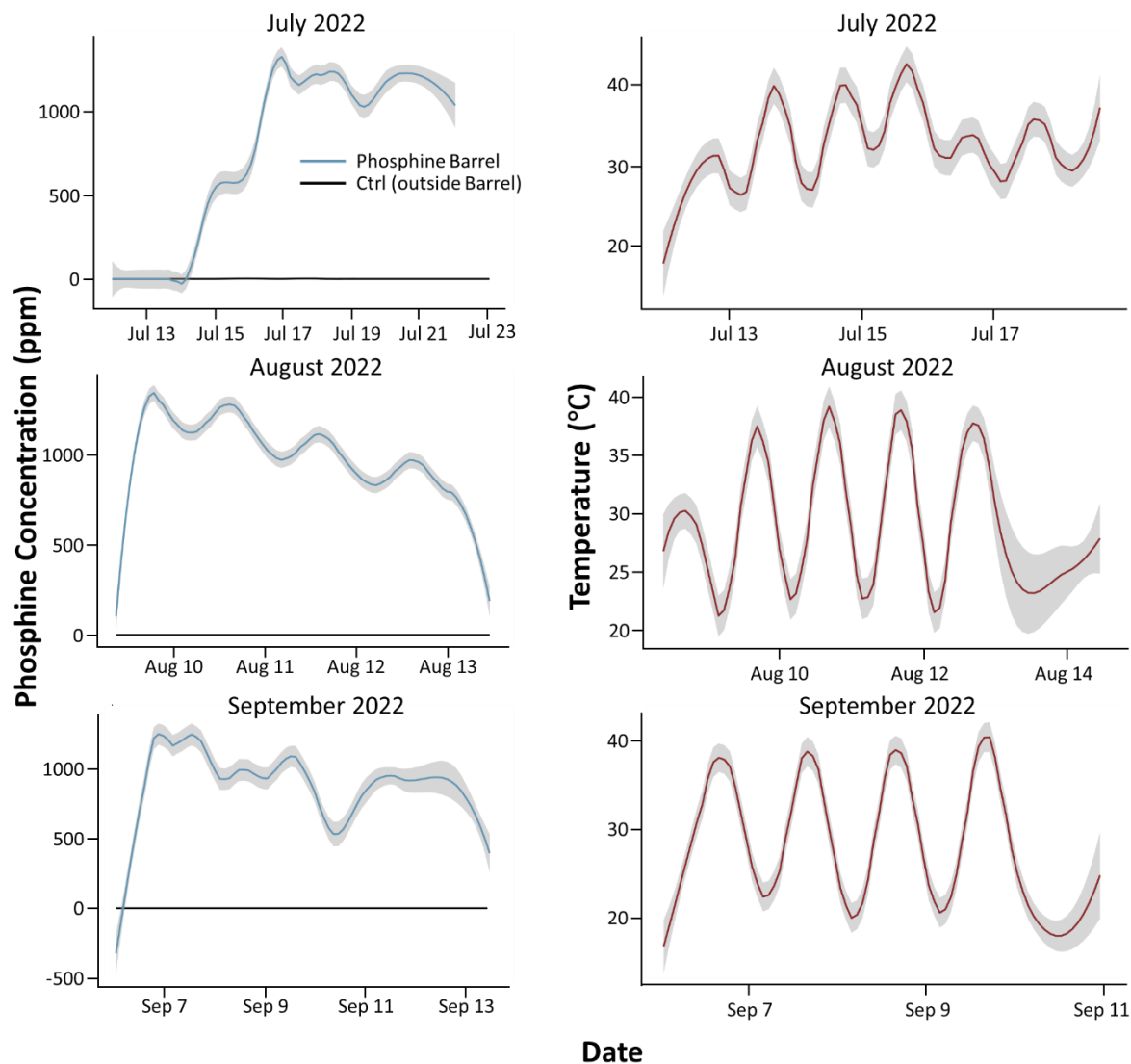


Figure 5.8 Hourly phosphine concentrations (ppm) and temperature (°C) during monthly fumigations in 2022

Hourly phosphine concentrations (ppm) (left column) and temperature (°C) (right column) during monthly fumigations. Fumigations were monitored in replicate barrels in real-time with a wireless Centaur sensor over the 4-day fumigation period from July to September 2022 of the wheat samples. Fumigation was triggered when the number of insect-damaged kernels (IDK) and/or the number of insects found in the equivalent of 100 g was at half of the Federal Grain Inspection Service (FGIS) specified tolerances for sample grade. Gray areas represent $\pm 95\%$ confidence bands. In cases where both phosphine and control sensors read zero, both are still recording for the full sampling period.

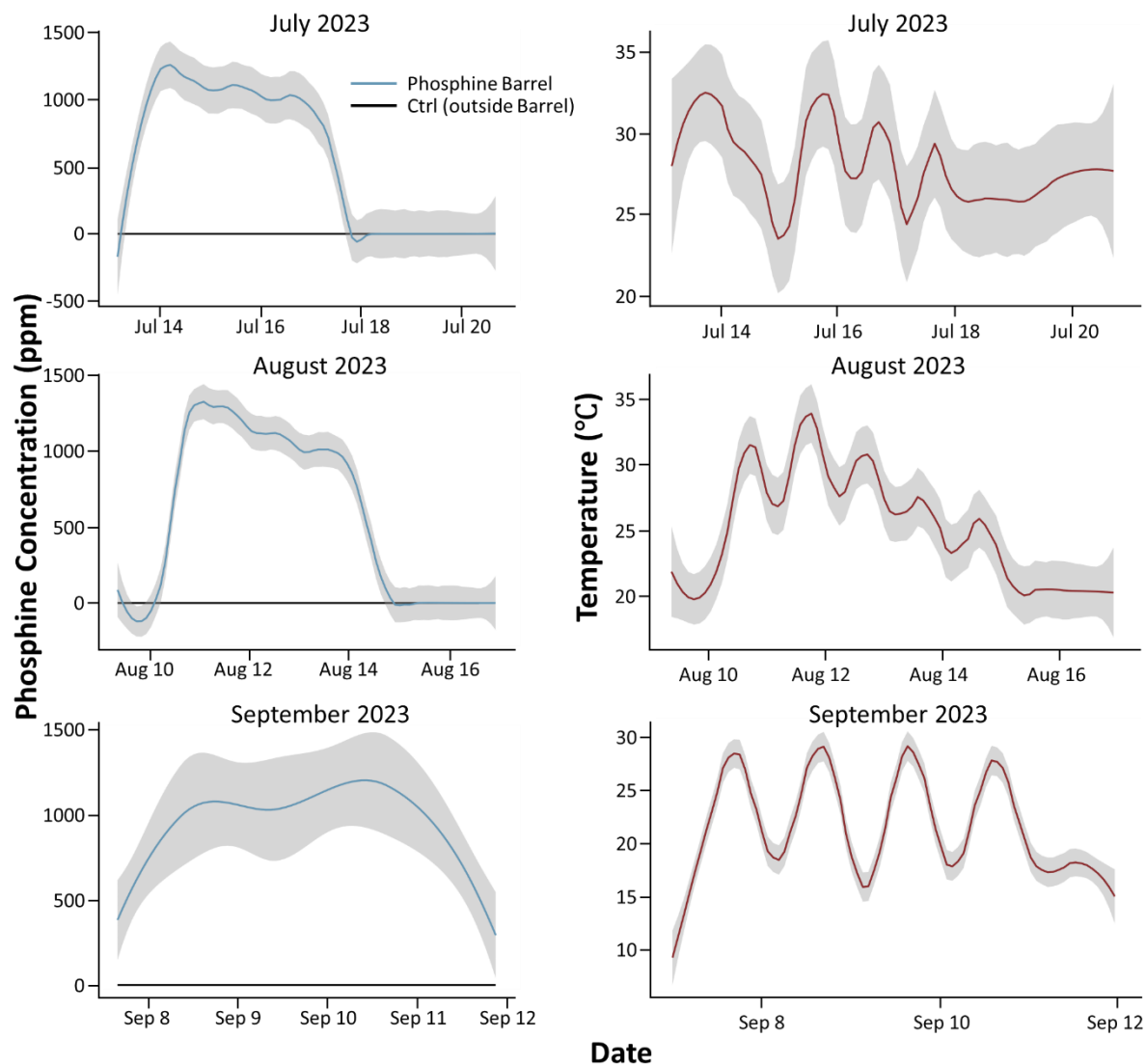


Figure 5.9 Hourly phosphine concentrations (ppm) and temperature (°C) during monthly fumigations in 2023

Hourly phosphine concentrations (ppm) (left column) and temperature (°C) (right column) during monthly fumigations. Fumigations were monitored in replicate barrels in real-time with a wireless Centaur sensor over the 4-day fumigation period from July to September 2023 of the wheat samples. Fumigation was triggered when the number of insect-damaged kernels (IDK) and/or the number of insects found in the equivalent of 100 g was at half of the Federal Grain Inspection Service (FGIS) specified tolerances for sample grade. Gray areas represent $\pm 95\%$ confidence bands. In cases where both phosphine and control sensors read zero, both are still recording for the full sampling period.

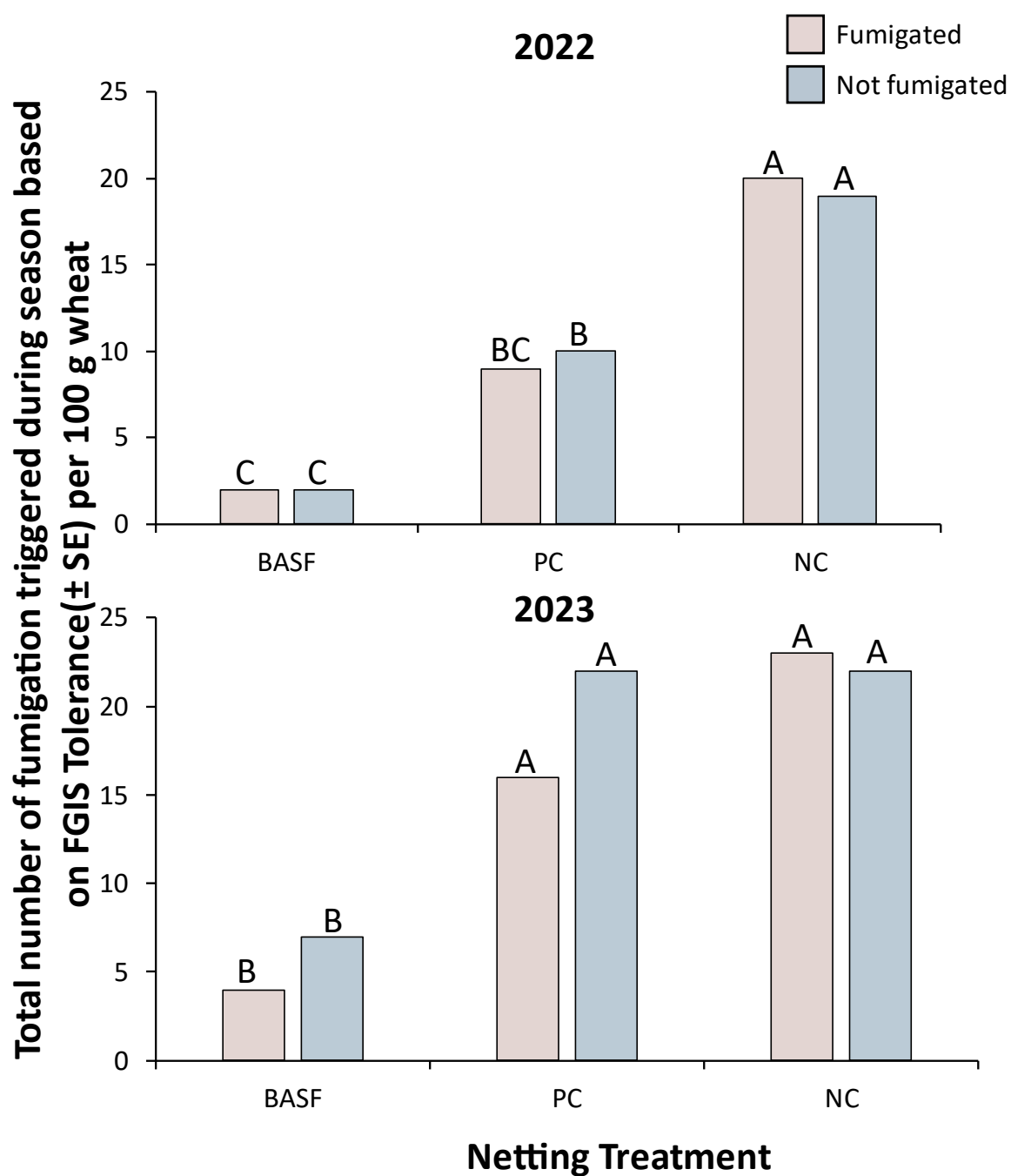


Figure 5.10 Total number of fumigations triggered

Total number of fumigations triggered. Fumigations were triggered based on Federal Grain Inspection Service (FGIS) specified tolerances for 100 g wheat sample for year 2022 (top panel) and 2023 (bottom panel). Bars with shared letters are not significantly different from each other (χ^2 - test, $\alpha = 0.05$). Blue bars were not fumigated, while the red bars were fumigated based on the FGIS tolerances.

Chapter 6 - Pairing residual contact insecticide use with long-lasting insecticide-incorporated netting to reduce dispersal and damage by stored product insects

Submitted as: Ranabhat, S., J. Abshire, J. Castaldi, D.S. Scheff, G.V. Bingham, T. McKay, K.Y. Zhu, and W.R. Morrison III. Pairing residual contact insecticide use with long-lasting insecticide-incorporated netting to reduce dispersal and damage by stored product insects.

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Abstract

1. Long-lasting insecticide-incorporated netting (LLIN) can intercept insects and has shown promise for pest management before and after harvest in agriculture. Understanding how to incorporate LLIN into existing integrated pest management (IPM) programs, with other commonly used tactics like residual contact insecticides, can significantly enhance IPM programs at food facilities.
2. In this study, we assessed whether LLIN (0.34% alpha-cypermethrin, BASF) may enhance the effects of residual contact insecticides such as Centanyl™ EC (Central Garden & Pet Co.), active ingredient (a.i.) deltamethrin and Evergreen® (McLaughlin Gormley King Co.), a.i. natural pyrethrins against *Tribolium castaneum* and *Rhyzopertha dominica*. We recorded their dispersal and progeny production in treatments with LLIN alone, deltamethrin or pyrethrins alone, LLIN + insecticide, or no treatment (positive control).
3. We demonstrated the use of both LLIN and residual insecticide with either deltamethrin or pyrethrins reduced adult insect dispersal to the food sources compared to the control, but only increased control of progeny production marginally by 8-19%.
4. LLIN alone was highly effective in reducing progeny production of *T. castaneum* by 40% compared to the positive control.
5. Importantly, pyrethrins did not cause significant direct mortality or prevent progeny production when used alone against *T. castaneum*. However, the combined use of pyrethrins with LLIN showed an enhanced efficacy against the insect.

6. Together, the combined use of LLIN and the residual contact insecticides evaluated in our study may have some benefits over using residual contact insecticide alone to manage stored product insects.

Keywords: stored product insect, infestation, insecticide netting, residual contact insecticide, behavior, integrated pest management

Introduction

The postharvest supply chain is an important and economically valuable component of agriculture, which has the potential to contribute to global food security. Stored grains such as corn, soybean, and wheat represent a value of over \$139 billion in the US alone (USDA-NASS 2023). However, when these crops are harvested and move along the supply chain, including on- and off-farm storage, processing, transportation, export, marketing, and end up in the pantry of consumers, stored product insects can easily attack these products at each link, causing large qualitative and quantitative economic losses. Annually, producers lose about 10–30% of their harvested crops after harvest, which adds up to losses of over \$100 billion worth of food products globally (Hagstrum & Subramanyam, 2006; Wacker, 2018). Therefore, it is vital to have effective pest management programs that enhance food security.

Chemical controls such as fumigation and residual contact insecticides are the most prevalent pest management strategies in the postharvest setting (Arthur & Subramanyam, 2012). Traditionally, methyl bromide and phosphine served as the primary fumigants for fumigation, but methyl bromide has been mostly phased out of use due to its deleterious effect on the ozone layer (Fields & White, 2002). Phosphine fumigants have their drawbacks such as increasing insect resistance (Nayak et al., 2020; Machuca-Mesa et al. 2023), corrosion of metals in structures, and efficacy from incomplete fumigation (Navarro, 2006, Brabec et al. 2019). A common alternative to phosphine is sulfuryl fluoride (Opit et al. 2016) but it is more expensive to apply. It's much more common to fumigate in bulk storage compared to large structures (Arthur 2018a). Another common management tactics in processing and storage are contact insecticides (Arthur, 1996; Morrison & Arthur, 2020; Quellhorst et al., 2022), crack-and-crevice treatments (Toews et al. 2006), spot treatments, and aerosols (Arthur, 2012). Together, these

IPM tactics, along with others, can be utilized as a multi-hurdle approach to pest management at food facilities (Scheff et al. 2019).

Pyrethroids are one of the most widely used insecticide classes after harvest (Arthur, 1996; Arthur & Subramanyam, 2012; Ghimire et al., 2016). These are synthetic insecticides that are similar to the natural pyrethrins produced in *Pyrethrum* spp. plants. Various pyrethroids including bioresmethrin (Arthur, 1992), cyfluthrin (Arthur et al. 2015), cypermethrin (Athanasios et al., 2015; Gourgouta et al. , 2019), and deltamethrin (Arthur et al. 2020) are effective against stored product pests (Stejskal et al., 2021). These active ingredients (a.i.s) can be formulated in various insecticide products such as aerosols, sprays, dust, granules, emulsifiable concentrates, and wettable powders. The effectiveness of these a.i. such as deltamethrin has been evaluated against the various stored product pests including *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Sitophilus oryzae* (Coleoptera: Curculionidae), Psocid spp. (Psocoptera: Liposcelididae) with weevils and flour beetles generally more tolerant. (Arthur, 1994; Nayak et al., 2002; Athanasios et al., 2004; Sehgal & Subramanyam, 2014; Morrison & Arthur, 2020; Ziaee & Babamir-Satehi, 2020; Trostanetsky et al., 2023). However, there has been an increasing demand for less chemical inputs after harvest, and consumers are willing to pay a price premium for this (Batte et al., 2007). Thus, to achieve this need, there has been a push to creatively diversify integrated pest management (IPM) programs for on-farm storage and other food facilities in agricultural networks.

One way to diversify IPM programs is to consider the additive, multiplicative or synergistic effects of using multiple strategies together. Long-lasting insecticide-incorporated netting (LLIN) has emerged as a promising tactic to intercept and inhibit immigrating insects

from the landscape from entering a facility by creating a physical barrier that prevents eight species of adult stored product insect from passing through the LLIN (Ranabhat et al., 2024a) can be further combined with other chemical control tactics. For example, a two-year field trial in grain bins demonstrated that alpha-cypermethrin incorporated LLIN can be leveraged in bulk storage to reduce phosphine fumigation by 68–91%, while also decreasing insect damage and dispersal by 80–95% compared to positive controls containing netting without insecticide (Ranabhat et al., 2024a). Further, Ming et al. (2024) demonstrated that adding the synergist piperonyl butoxide could increase the efficacy of the alpha-cypermethrin-based LLIN against *R. dominica* by six-fold. However, to date, no study has been conducted to assess the compatibility of LLIN with common residual contact insecticide applications.

Beyond the enhanced effects, LLIN has been shown to be effective against multiple stored product pests, with both direct mortality (Paloukas et al., 2020; Scheff et al., 2021; Altunç et al. 2024) and sublethal effects (Morrison et al., 2018; Wilkins et al., 2020, 2021; Gerken et al., 2021; Ranabhat et al., 2022; Ranabhat et al., 2024b; Ranabhat et al., 2024c). A meta-analysis of 44 peer-reviewed publications with 285 data points on the efficacy of LLIN in agriculture showed widespread efficacy of LLIN in both postharvest and to a lesser extent, preharvest agriculture (Ranabhat et al., 2024d). LLIN incorporated with various a.i. including deltamethrin, α -cypermethrin, and silica have shown direct efficacy against 13 stored product insect species (Morrison et al., 2018; Rumbos et al., 2018; Agrafioti et al., 2020; Agrafioti et al., 2023; Paloukas et al., 2020). Exposure to LLIN can reduce stored product insect progeny production (Gerken et al., 2021; Wilkins et al., 2021; Ranabhat et al., 2024a), and stored product insect dispersal (Wilkins et al., 2021; Ranabhat et al., 2024a). Moreover, LLIN was found equally effective against phosphine-resistant and -susceptible strains of *R. dominica* and, *T. castaneum*

(Ranabhat et al., 2022). Further, research also demonstrated that LLIN, when combined with an attractant lure, supports the attract-and-kill strategy's promising approach (Wilkins et al., 2021). Recently, an empirically-informed population modeling study has shown that LLIN reduces fecundity and survivorship of *T. castaneum* effectively and that populations end up extirpating in 15 generations when no immigration is present (Ranabhat et al., 2024e). Thus, LLIN presents an ideal solution for sealing diverse openings such as doors, windows, vents, cracks, crevices, intake or outtake manifolds in processing facilities, pallets in warehouses, or any other type of openings to intercept the insects as they disperse in and around food facilities.

Thus, in this study, we aimed to evaluate the potential of combining LLIN with commonly used residual contact insecticides on concrete to determine whether their joint application could enhance the effectiveness of either tactic over each alone on insect dispersal and progeny production by *T. castaneum* and *R. dominica*. We evaluated the individual or combined use of Centanyl™ EC (Central Life Sciences, Schaumburg, IL; a.i.: 4.75% deltamethrin) and Evergreen® Pyrethrum concentrate (MGK®, Aspen Lane North, MN: a.i.: 5% pyrethrins). In the US, Centynal™ EC is a conventional insecticide labeled as an emulsifiable concentrate for the treatment of empty bins and warehouses and stored grain, while Evergreen® is an organic, botanical insecticide labeled for use in food handling, processing, and storage areas. We used either LLIN (BASF Corp, Ludwigshafen, Germany; incorporated with 0.34% w/w alpha-cypermethrin) alone or in combination with either deltamethrin or pyrethrins insecticides.

Materials and Methods

Experimental insects

A field strain of the *R. dominica* and *T. castaneum* obtained from Pottawatomie Co, KS, and eastern KS, respectively, were used in this study. We used four to eight-week-old adults of both species. Cultures of *R. dominica* and *T. castaneum* have been maintained in the laboratory since 2019 and 2012, respectively at the USDA Center for Grain Animal Health Research in Manhattan, KS. *Rhyzopertha dominica* were reared on tempered organic whole wheat (Heartland Mills, Marienthal, KS, USA), while *T. castaneum* were reared on a mixture of 95% unbleached, organic flour and 5% brewer's yeast (Heartland Mills, Marienthal, KS, USA). The colonies were subcultured on a monthly basis. The colonies were maintained at 27.5°C, 65% RH, and 14:10 (L:D) h photoperiod in environmental chambers (Percival Scientific, Perry, IA, USA).

Experimental arena and its production

The experimental arena consisted of a 63 cm × 15.5 cm × 9.5 cm L: W: H metal frame. This arena also contained four square blocks of concrete (e.g., Rockite, Hartline Products Co., Cleveland, OH, USA) measuring 15.24 cm × 15.24 cm × 1.5 cm L: W: H to create a testing platform that mimics the surface of a food facility (Figure 6.1). The concrete was prepared by first mixing tap water and Rockite cement mix in a large water pitcher. The tap water was added to the dry Rockite mixture and combined until a thick paste consistency was achieved. The slurry was poured into a 1.1 L volume silicone square mold (15.24 cm × 15.24 cm × 4.57 cm L:W: H). The slurry was poured 1.5 cm thick. The cement concrete squares were left to dry and solidify at room temperature for 2–3 d. Cement squares were modular and new ones were used with each replicate performed for the assay.

In each experimental arena, there were four cement squares (Figure 6.1). We applied tape (VWR International, LLC Radnor, PA, USA) on the wall of the metal frame before placing the concrete to make it easier to remove the concrete after each replicate was conducted, and so the metal forms could be re-used. Adhesive caulk (DAP Kwik Seal, DAP Products Inc., Baltimore, MD, USA) was applied to fill any gaps between the concrete squares or between the concrete and the metal frame. The concrete at the distal end of the experimental arena was filled with 20 g of whole, organic, unbleached flour (Heartland Mills, Marienthal, KS, USA) for *T. castaneum* or organic whole wheat (Heartland Mills, Marienthal, KS, USA) for *R. dominica*. Insects were released on the concrete square at the opposite end. The two middle concrete slabs were reserved for one or two treatments as below. The inner wall of the metal frame was coated with Fluon® (polytetrafluoroethylene, Sigma-Aldrich Co., St. Louis, MO, USA) to prevent insect escape.

Insecticide formulation for concrete

We used Centanyl™ EC (formulation consisting of 49.13 mg deltamethrin/mL), and Evergreen® (formulation consisting of 8.39 mg pyrethrins/mL) which were obtained from Central Garden & Pet Company (Schaumburg, IL, USA) and MGK® Company (Aspen Lane North, MN, USA), respectively. In this study, we used a high labeled rate of Centanyl™ EC (45 ml in 3.8 L of water to treat 92.9 m²) and a high labeled rate of Evergreen® (650.6 ml of product in 3.8 L water to treat 69.7 m²) (Table 6.1). A water-only treated control was used for each replication.

For Centanyl™ EC (hereafter deltamethrin), each lot was formulated by adding 0.23 ml of product in 18.9 ml of water in a 25-ml volumetric flask. For Evergreen® (hereafter pyrethrins) each lot was formulated by adding 4.3 ml of product and 25.1 ml of water in a 50-ml volumetric flask. We evenly sprayed 1 ml of the final formulation of deltamethrin per concrete square (area:

232.3 cm²) and 1.5 ml of pyrethrins formulation per concrete square by using an artist's airbrush (Badger 100 series, Badger Corporation, Franklin Park, IL, USA). For the positive control, we sprayed 1.5 ml of tap water with the artist's airbrush. For each treatment, we used a separate airbrush to avoid the cross-contamination. We allowed the sprayed concrete squares to dry at room temperature for 24 h prior to using them for the experiment.

Treatment

We used the following six treatments: 1) untreated controls with netting identical to LLIN but lacking insecticide (Casa Collection, Mesh White, 1721-9668; Jo-Ann's, Hudson, OH, USA), and/or concrete sprayed only with distilled water, 2) LLIN only (Carifend[®] net, 0.34% alpha-cypermethrin, BASF Corp., Ludwigshafen, Germany) applied near the insect release point (15.24 cm × 15.24 cm; same size strip of the net were used for whole experiment), 3) deltamethrin only applied near the insect release position, 4) pyrethrins only applied near the insect release position, 5) a strip of the LLIN and deltamethrin (LLIN laid sequentially directly before the insecticide to intercept immigrating insects: Morrison et al., 2018, Wilkins et al., 2021), and 6) a strip of the LLIN material and pyrethrins (LLIN laid sequentially directly before the insecticide to intercept immigrating insects).

We assembled the experimental arenas for each treatment replicated on separate days. In each experimental arena, we released 30 mixed-sex adults of either *R. dominica* or *T. castaneum* (just a single species per arena) at the far end of the testing platform opposite the food source. Each dispersal apparatus was placed into a sterilite bin (86.3 × 30.5 × 39.4 cm L: H: W) to prevent insect escape and effects from neighboring apparatuses and placed on the shelf of a walk-in environmental chamber at the same conditions as the rearing colonies. After a 48-hour dispersal period, we counted the number of insects on each concrete square and also checked

their conditions in clean Petri dishes lined with filter paper. The condition of the adults was checked under the dissecting microscope and rated as alive, affected, or dead by following the definitions in Morrison et al. (2018). Briefly, the alive were moving around normally without impairment, while those that were affected showed uncertain movements, twitching of extremities, and/or were not able to right themselves after being gently prodded with a paintbrush. Those that were classified as dead were completely immobile. We collected the food source by removing the flour or wheat. Subsequently, the commodity was gently sieved using two sieves (#10 sieve, 2.0×2.0 mm mesh, W.S. Tyler, Mentor, OH; and #20 sieve, 0.841×0.841 mm mesh; W.S. Tyler, Cleveland, OH), making sure no eggs were harmed during the process and all food dust and detritus was transferred to progeny production containers. All the adults were removed from the commodity and the commodity was retained for 6 weeks at 27.5°C, 65% RH, and 14:10 (L:D) h photoperiod in environmental chambers to evaluate progeny production. There were 5–6 replications for each treatment and species combination.

Data analysis

The dispersal of *T. castaneum* and *R. dominica* was analyzed with a general linear model with separate models for each species. Treatment, zone of recapture (release zone – RZ, treatment zone 1 – T1, treatment zone 2 – T2, and food zone – FZ), and their interaction were used as fixed, explanatory variables. Residuals were inspected for assumptions of normality and homogeneity of variances, and where there were deviances, a log-transformation was adopted, which corrected issues. Upon a significant result from the model, Tukey HSD was used for multiple comparisons. Except where otherwise noted, $\alpha = 0.05$. R Software was used for all analyzes (R Core Team 2023), and *ggplot2* was used to construct figures (Wickham 2016).

The insect's condition and progeny production were analyzed with a generalized linear model based on a Quasibinomial or Quasipoisson distribution to account for overdispersion. Separate models were run for alive, affected, and dead adults. The same explanatory variables were used as above. A log-likelihood ratio was calculated based on a Chi-squared distribution to determine significance of explanatory variables. Multiple comparisons employed a Chi-squared test with a Bonferroni correction to the α -threshold.

Results

Insect dispersal in the experimental arena

Overall, the treatment did not significantly affect dispersal by *R. dominica* (Table 6.2; Figure 6.2). However, there were significantly different numbers of *R. dominica* that dispersed into each recapture zone (Table 6.2). The majority of adult *R. dominica* were found in the release zone, followed by treatment zone 1 (T1) (Figure 6.2). The fewest *R. dominica* were generally found in the food zone with the commodity. The interaction between recapture zone and treatment was also significant (Table 6.2). There was 13, 8.7, and 5.2-fold greater dispersal to the food zone in the positive control than the LLIN, deltamethrin, or pyrethrins treatment by *R. dominica*, respectively (Figure 6.2). There was no dispersal to the food zone in the LLIN + deltamethrin or LLIN + pyrethrins treatments by *R. dominica*.

For *T. castaneum*, the treatment significantly affected dispersal of adult conspecifics (Table 6.2; Figure 6.3). The recapture zone affected dispersal of *T. castaneum* (Table 6.2), with most adults transversing to the food zone in the apparatus in each treatment (Figure 6.3). There was also a significant zone by treatment interaction on the dispersal of *T. castaneum* (Table 6.2). Compared to the positive control, there was a 33%, 46%, 55%, and 60% reduction in the number

of *T. castaneum* dispersing to the food zone for the deltamethrin, LLIN, LLIN + pyrethrins, and LLIN + deltamethrin treatments, respectively (Figure 6.3). By contrast, only a 5% reduction in dispersal of *T. castaneum* adults was observed in the pyrethrins treatment compared to the positive control.

Insect condition in the experimental arena

Overall, for *R. dominica*, the treatment significantly affected the mean percentage remaining alive (Table 6.3). For example, there was a reduction in the mean percentage of live *R. dominica* by 51–95% in the pyrethrins and LLIN + deltamethrin treatment compared to the positive control (Figure 6.4). Among the treatments, pyrethrins only treatment had the highest mean percentage of alive *R. dominica* ($46 \% \pm 3.1\%$) across zones, after 48 h. The recapture zone significantly affected the mean alive adults, as well as its interaction with treatment zones (Table 6.3). There was a 96–100% reduction in the mean percentage of alive *R. dominica* remaining in each insecticide-based treatment compared to the positive control in the food zone. There were no alive adult *R. dominica* in the LLIN + pyrethrins and LLIN + deltamethrin treatment in the food zone, because there was no dispersal to the food zone in this treatment (Figure 6.4).

Treatment did not influence the percentage of affected *R. dominica* (Table 6.3). The mean affected *R. dominica* was 1.8% ($\pm 4.2\%$) in the positive control, while it varied from 13% ($\pm 2.8\%$) (pyrethrins only) to 20% ($\pm 3.9\%$) (LLIN + pyrethrins) in the insecticide-based treatments. The release zone, however, significantly changed the mean percentage of affected *R. dominica* (Table 6.3). For example, there were 150-, 40-, and 30-fold more affected *R. dominica* in the release zone, treatment zone 1 (with LLIN), and treatment zone 2 (with residual spray) than in the food zone. There was also a significant interaction between treatment and release zone (Table 6.3; Figure 6.4). There were a quarter of *R. dominica* affected in the treatment zone 2 patch of

the residual sprays, whereas this shifted to a quarter of adults affected in the treatment zone 1 patch of the LLIN, LLIN + pyrethrins, and LLIN + deltamethrin treatments (Figure 6.4).

Finally, the percentage of dead *R. dominica* after 48 h was not affected by treatment (Table 6.3). However, the release zone significantly affected the mean percentage of dead adults (Figure 6.4), as well as its interaction with treatment, as did the interaction between the release zone and treatment (Figure 6.4). The greatest percentage of dead *R. dominica* were found after 48 h in the LLIN + deltamethrin treatment, particularly in treatment zone 2 (Figure 6.4). There was 7–13-fold greater mortality of adult *R. dominica* in the insecticide-based treatments compared to the positive control (Figure 6.6).

By contrast, the mean percentage of *T. castaneum* adults remaining alive was significantly affected by treatment (Table 6.4). The recapture zone significantly affected the percentage of alive *T. castaneum* adults (Table 6.4). The mean of 55% ($\pm 8.2\%$) of *T. castaneum* adults were alive in the food zone, while only 0.35–0.44% and 2.7% ($\pm 6.1\%$) of the adults were alive in the treatment zones and release zone, respectively. There was not a significant interaction between treatment and recapture zone (Table 6.4). In every case, most *T. castaneum* adults remained alive in the food zone (Figure 6.5).

The effects on *T. castaneum* adults varied significantly among different treatments (Table 6.4). There were no affected individuals in the positive control, whereas the mean affected adults ranged from 11.3($\pm 1.5\%$)–17.1% ($\pm 2.6\%$) in the LLIN only and LLIN + deltamethrin treatments, respectively. The exception was the pyrethrins only treatment, where there were only 1.7% ($\pm 0.5\%$) adults affected. The release zone did not significantly influence the percentage of affected adults, but its interaction with treatment did (Table 6.4). There were many more affected adults in the release zone and treatment zones compared to the food zone (Figure 6.5).

Moreover, different treatments caused significantly different levels of adult mortality in *T. castaneum* (Table 6.4). There were 4–11-fold higher percent mortality in the insecticide-based treatments than that in the positive control (Figure 6.5). The recapture zone also significantly affected the percentage of dead *T. castaneum* with the highest mortality occurring in the food zone. Finally, the interaction between treatment and recapture zone significantly affected the percent mortality, with the highest mortality occurring in the deltamethrin only in the release zone (Figure 6.5). There was 14–111-fold greater mortality of adult *T. castaneum* in the insecticide-based treatments compared to the positive control (Figure 6.6).

Progeny production

We did not observe any progeny from the different treatments after six weeks for *R. dominica*, thus this has been excluded from the data analysis. For *T. castaneum*, treatment significantly affected the total progeny (larvae + pupae) observed (GLM: $\chi^2 = 12.77$, df = 5, $P = 0.02$; Figure 6.7). Total progeny observed was reduced by 45–64% in the insecticide-based treatments (with the exclusion of pyrethrins only) compared to the positive control. The mean progeny observed in the pyrethrin only treatment was $113 (\pm 37)$, which was more than the progeny (84 ± 15) observed in the positive control. Adding a residual spray to LLIN represented 8–19% fewer progeny over and beyond LLIN alone or a residual spray alone. Treatment significantly affected the number of *T. castaneum* larvae observed (GLM: $\chi^2 = 13.5$, df = 5, $P = 0.01$; Figure 6.7), with a 43–62% reduction in larvae in the insecticide-based treatments (with the exception of pyrethrins only) compared to the positive control. In the pyrethrins only treatment, the amount of larvae produced increased 154% over the positive control. Finally, the number of pupae observed did not significantly vary with treatment (GLM: $\chi^2 = 6.12$, df = 5, $P = 0.29$; Figure 6.7).

Discussion

This is the first study to evaluate whether the use of LLIN combined with a residual contact insecticide resulted in an enhanced efficacy compared to their independent usage alone on insect dispersal to a food source. This assay was meant to test the scenario of insects immigrating into a food facility by encountering LLIN as a first defense, then a treated residual insecticide surface as a second hurdle to the infestation of the commodity. Overall, we observed that the combined use of LLIN and deltamethrin or pyrethrins reduced adult insect dispersal to the food sources compared to positive control, but the level of reduction was only marginally greater than deltamethrin or pyrethrins alone. Thus, we did not observe a significantly increased efficacy in using both tactics together. In addition, the combined use of these tactics also supported reductions in the progeny production of *T. castaneum*, a common stored product insect in food processing and storage facilities. This bolsters the utility of LLIN further, as it was also shown to synergize with another chemical control tactic commonly used at food facilities, fumigations in bulk storage (Ranabhat et al., 2024a) Finally, this could help to reduce the reliance on individual tactics, which would help preserve efficacy and reduce insecticide resistance at food facilities.

In this experiment, we employed the active ingredients deltamethrin and pyrethrins, as well as alpha-cypermethrin in LLIN. Pyrethroids and pyrethrins are some of the most widely used insecticides after harvest (Arthur & Subramanyam, 2012; Ghimire et al., 2016). Deltamethrin, in particular, has been found to be highly effective when used as a grain protectant, and crack-and-crevice treatment against stored product insects, including *Sitophilus* spp., *T. castaneum*, *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae), *R. dominica*, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and others (Athanasassiou et al., 2004; Arthur & Morrison, 2020; Morrison, et al., 2021; Quellhorst et al., 2022). For its part,

alpha-cypermethrin-based LLIN is highly effective against *T. castaneum* and *R. dominica* (Paloukas et al., 2020; Galán et al., 2024). For example, complete control was achieved with *R. dominica* after 14 d exposure to LLIN (Paloukas et al., 2020). As a result, when two highly effective tactics were combined, there was not much further gain to be achieved in the present study. In addition, deltamethrin, pyrethrin, and alpha-cypermethrin all share the same mode of action, so in the future, there may be additional benefits from combining other insecticides (e.g., Spinosad) with different modes of action. Together, this may be why there was only a further 10–20% reduction in dispersal and progeny production when combining tactics.

Insecticide efficacy can be influenced by a variety of factors. These include biological, environmental, and physical factors, such as the target insect species (Arthur & Morrison, 2020, Arthur 2018b), the specific insecticide and its formulation, modes of action, the actual application rate (Arthur et al., 2020), the duration of insect exposure to the pesticide (Morrison et al., 2021), and the surface substrate (Zettler & Arthur, 2000, Lampiri et al. 2023). In our assay, we used concrete flooring to mimic the surface most commonly found in food facilities, which has been found to reduce residual spray efficacy (Zettler & Arthur, 2000, Lampiri et al. 2023). There was a 46% reduction in the LT_{95} when deltamethrin was applied to concrete compared to an impervious surface such as tile in prior work (Arthur 1997). Further, a deltamethrin emulsifiable formulation (0.1 mg/m^2) was found to be effective on a nonporous (plastic) surface but was not very effective as a residual insecticide on porous (concrete) surfaces (Trostanetsky et al. 2023). The reduced effectiveness of insecticides on porous surfaces like concrete may be due to the material's tendency to retain a portion of the active substance, rendering it unavailable to insects that come into contact with the surface (Chadwick 1985). Additionally, concrete's alkaline nature may significantly contribute to the breakdown and degradation of insecticides

(Arthur, 2012; Agrafioti et al., 2015, Lampiri et al. 2023). Various types of surfaces such as metal, wood, tile, and sealed or epoxied concrete can be found in food processing, milling, and storage facilities in the US and worldwide. Thus, it is important to note that material properties have a significant effect on the residual capacity of insecticides. In addition, we have mimicked the environmental conditions present in food processing facilities in the summer (Campbell et al., 2010). To produce a fair comparison and assess a higher concentration, the same substrate (concrete blocks) and higher concentrations of Centanyl (49 mg/ml) and Evergreen (8.4 mg/ml) were used according to the label rate.

The dispersal was influenced by insect species. Overall, *R. dominica* (an internal-infesting insect) made it to the commodity much less frequently than *T. castaneum* (an external-infesting insect). The baseline mobility of these two insect species is crucial to consider when assessing the dispersal capacity. For example, *R. dominica* is a much weaker walker than *T. castaneum* all other things equal (Morrison et al., 2018). This has been confirmed in other studies (Hagstrum & Subramanyam, 2006; Edde, 2012). While *R. dominica* is a strong flyer compared to *T. castaneum* (Edde and Phillips, 2006; Perez-Mendoza et al., 2011), it could be that the temperature was not sufficient to induce flight regularly. The low ambient threshold for *R. dominica* flight initiation is between 16–20 °C and optimum flight conditions are between 31–36 °C. The conditions in this study are just at the optimum but not well above temperatures to induce flight (Dowdy and McGughey 1994). Further, it is also important to consider how attractive the food sources are as inducement for dispersal (e.g., Van Winkle et al., 2022; Ponce et al., 2024). In our assay, we used whole wheat as a food source for *R. dominica* whereas we used flour for *T. castaneum*. The food source seems to be sufficiently attractive to both studied

species. However, their differential mobility patterns resulted in different outcomes in terms of which species was able to successfully reach the food source over the assay distance.

Interestingly, no progeny for *R. dominica* in any treatments after six weeks was observed even though some individuals were observed in food sources after 48h. However, for *T. castaneum*, the use of LLIN reduced progeny production by 40%, whereas combining LLIN with a residual insecticide (deltamethrin or pyrethrins) significantly reduced progeny an additional 10–20%. Studies have shown that the combined application of two insecticides, 0.1 mg/kg Spinosad and 0.05 mg/kg alpha-cypermethrin applied on wheat resulted in a significant reduction in progeny production against major storage pests including *T. castaneum*, *R. dominica*, *Sitophilus oryzae* (Coleoptera: Curculionidae), *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), and *Trogoderma granarium* Everts (Coleoptera: Dermestidae) when compared to the application of each alone (Wakil et al., 2023). Further, the combined use of spinosad applied at 0.1 and 0.5 ppm in addition to 5 ppm of Methoprene resulted in no progeny of *R. dominica* but progeny production was not reduced for the rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae) (Athanassiou et al., 2011). An application of Diacon® IGR Plus (e.g., active deltamethrin + methoprene IGR) and Gravista® (e.g., deltamethrin + synergist + methoprene IGR) to wheat, corn and brown rice helped to reduce the progeny production of *R. dominica*, *S. oryzae* and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) significantly (Arthur & Morrison, 2020). However, unlike these studies, we did not use grain protectants or apply insecticides directly to the grain in our study. When Diacon® IGR Plus and Gravista® were used as a residual insecticide on concrete against *S. zeamais* and *P. truncates*, their biology was significantly affected (Quellhorst

et al. 2022). Overall, we observed a significant reduction in progeny production with the combination of LLIN and a residual spray in 48 h dispersal to the food sources after six weeks.

In addition to assessing sublethal effects, our study evaluated the direct lethality of LLIN, deltamethrin, and pyrethrins, either individually or in combination after 48 h dispersal of adults. We used the maximum labeled rate for the residual insecticides, so the results demonstrated a best-case estimate for the efficacy of deltamethrin and pyrethrins against stored product insects. There was an increase in the number of affected insects in the LLIN or deltamethrin treatments compared with the pyrethrins, suggesting the former provided better control for *T. castaneum* as well as for *R. dominica*. At the maximum label rate, pyrethrins did not cause significant direct lethal effects or prevent progeny production when used alone. However, simultaneous use of pyrethrins with LLIN resulted in an increased the number of affected adults compared to their use alone

Different management strategies combination may result in varying levels of pest control, highlighting the importance of thoroughly exploring and optimizing these combinations. For instance, the combination of diatomaceous earth with plant extracts (Hanif et al., 2022), entomopathogenic fungi (Michalaki et al., 2006; Rizwan et al., 2019), or chemical pesticides (Wakil et al., 2021) has been demonstrated to enhance the efficacy against various stored product pests compared to individual insecticidal components. Additionally, the combination of fenpropathrin and spinosad can significantly enhance control efficacy against larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), maybe allowing for a reduction in the doses of one or both constituents of the combination (Ismail, 2020). Importantly, and most relevantly, Ranabhat et al. (2024a) demonstrated that LLIN enhanced the efficacy of fumigation for the protection of bulk storage of commodities by reducing the need for fumigation by 68–

91% over two years. Moreover, that research showed that using LLIN together with fumigation reduced insect dispersal and progeny production by 83–99% and 89–99%, respectively. In the current research, combined treatment resulted in a marginally increased efficacy in reducing the dispersal and progeny production of the insect.

In conclusion, the combination of LLIN and residual contact insecticide evaluated in our study may have benefits over the residual contact insecticide used alone to manage stored product insects, though this may depend on the active ingredient and rate used in the residual spray. Although our study is limited to only the adult (e.g., dispersing) stages of field strains of *T. castaneum* and *R. dominica*, these insects represent two opposite ends of life history among stored product insects. In addition, the study is also limited in that it only tested one kind of insecticide netting and two active ingredients in the residual sprays, but it gives us a proof of concept for using two common pest management tactics in concert. This knowledge will be beneficial to food facility managers moving forward so that they can better coordinate their IPM programs.

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Tables:

Table 6.1 Label, mixing, and application rates for insecticides used in residual assay with *R. dominica* and *T. castaneum*.

PRODUCT ^{1,2}	ACTIVE INGREDIENTS (A.I.)	APPLICATION RATE (MG/ML) ^{3,4,5}	MIXING RATE(ML) ⁶	AMOUNT APPLIED TO 1 CONCRETE (ML) ⁷
Centanyl EC	4.75% Deltamethrin	49	0.23	1.0
Evergreen	5% Pyrethrins	8.4	4.3	1.5

¹ Mixed 0.0112 mL of Centanyl in 0.9454 mL of H₂O to cover a concrete of 232.258 cm² area.

² Mixed 0.216 mL of Evergreen in 1.256 mL of H₂O to cover a concrete of 232.258 cm² area.

³ Corresponding to the max label rates in mL per product (mL⁻¹) H₂O.

⁴ Assumes 45 mL of product (Centanyl™ EC) is mixed in 1 gal (=3785.412 mL) of H₂O to cover area 930,000 cm².

⁵ Assumes 650.618mL of product (Evergreen®) is mixed in 1 gal (=3785.412 mL) of H₂O to cover area 700,000 cm².

⁶ In one lot, we prepared the working solution (product + H₂O) for 4645.16 cm² (Centanyl™ EC in 18.907 mL of H₂O and Evergreen® in 25.119 mL of H₂O).

⁷ Concrete of area 232.258 cm².

Table 6.2 Summary of analysis of variance (ANOVA) for *R. dominica* and *T. castaneum* after contact with long-lasting insecticide-incorporated netting and/or residual insecticide sprays on concrete in the laboratory.

VARIABLES ¹	<i>R. dominica</i>			<i>T. castaneum</i>		
	DF	F	P	DF	F	P
treatment	5	0.60	7.0×10^{-1}	5	21	4.0×10^{-14}
zone	3	20	9.4×10^{-11}	3	27	6.7×10^{-13}
treatment:zone	15	1.9	2.5×10^{-2}	15	10	3.3×10^{-14}

¹ Residual degrees of freedom = 120.

Table 6.3 Summary of generalized linear models for alive, affected, and dead *R. dominica* after dispersal over 48 h on concrete arenas with long-lasting insecticide-incorporated netting and/or residual insecticide sprays in the laboratory.

VARIABLES	DF	Alive		Affected		Dead	
		χ^2	P	χ^2	P	χ^2	P
treatment	5	33	4.1×10^{-6}	3.1	6.8×10^{-1}	7.6	1.8×10^{-1}
zone	3	46	5.1×10^{-10}	63	1.7×10^{-13}	16	1.4×10^{-3}
treatment:zone	15	46	6.2×10^{-5}	98	3.5×10^{-14}	41	3.5×10^{-4}

Table 6.4 Summary of generalized linear models for alive, affected, and dead *T.castaneum* after dispersal over 48 h on concrete arenas with long-lasting insecticide-incorporated netting and/or residual insecticide sprays in the laboratory.

VARIABLES	DF	Alive		Affected		Dead	
		χ^2	P	χ^2	P	χ^2	P
treatment	5	61	8.3×10^{-12}	33	4.0×10^{-6}	18	2.5×10^{-3}
zone	3	94	2.2×10^{-16}	7.1	7.0×10^{-2}	10	1.5×10^{-2}
treatment:zone	15	21	1.3×10^{-1}	25	4.5×10^{-2}	44	1.1×10^{-4}

Figures:

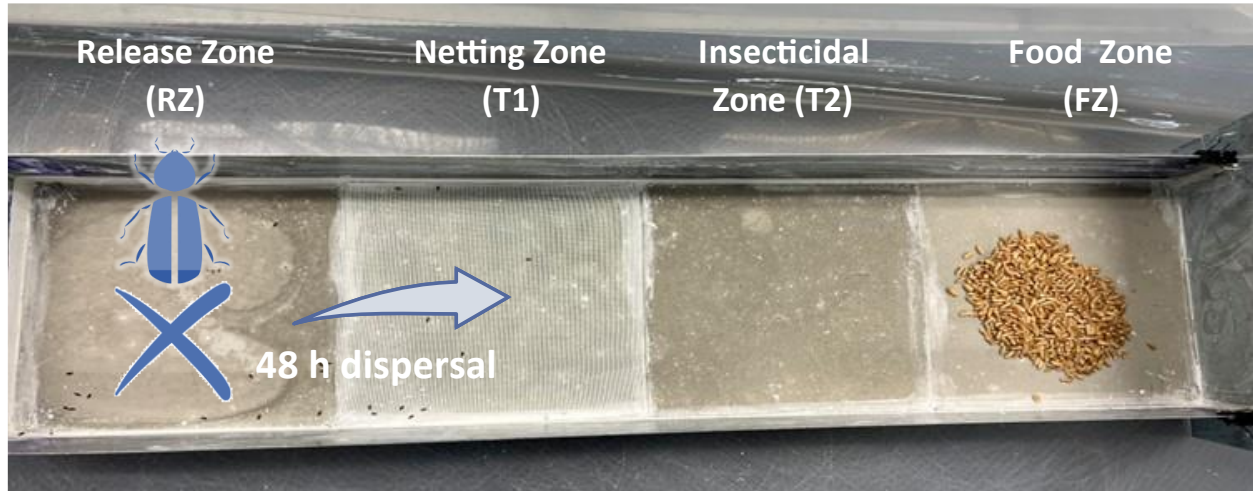


Figure 6.1 Experimental test area of residual insecticide assay

Experimental test area with 63 cm × 15.5 cm × 9.5 cm L: W: H metal frame. Each unit had a four-second form of 15.24 cm × 15.24 cm × 1.5 cm L: W: H concrete where the concrete at one end was filled with 20 g of wheat for *R. dominica* or flour for *T. castaneum* (FZ) and insects were released at the other end (RZ). Netting zone (T1) contained a net or was left empty based on treatment and the insecticidal zone (T2) consisted of sprayed concrete either with deltamethrin or pyrethrins based on treatment.

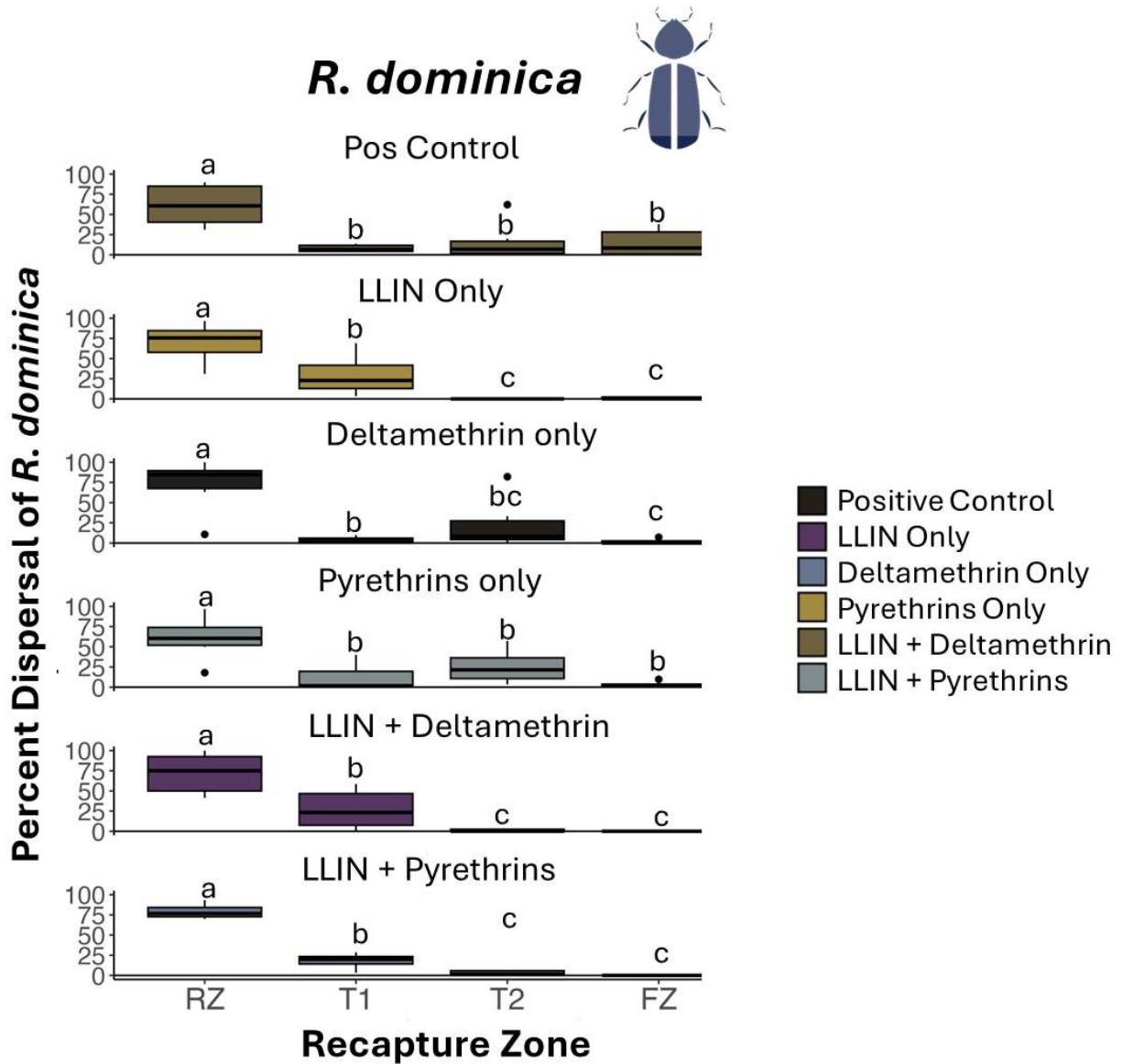


Figure 6.2 Dispersal of *R. dominica* in a residual insecticide assay when exposed to the different treatments after 48 h

Dispersal of *R. dominica* (out of 30 adults/replicate) in a residual insecticide assay when exposed to a positive control (black), long-lasting insecticide netting only (purple), deltamethrin spray only (blue), pyrethrins spray only (yellow), both LLIN and deltamethrin (brown), or both LLIN and pyrethrins (grey) after 48 h in the laboratory under constant conditions (25°C, 16:8 L:D). Box plot visualizes the median as a dark black line on each bar. Bars with shared letters are not significantly different from each other within each treatment (Kruskal Wallis, $\alpha = 0.05$). Abbreviations = RZ – release zone, T1 – treatment zone 1 (with LLIN when present), T2 – treatment zone 2 (with residual insecticide when present), FZ – food zone.

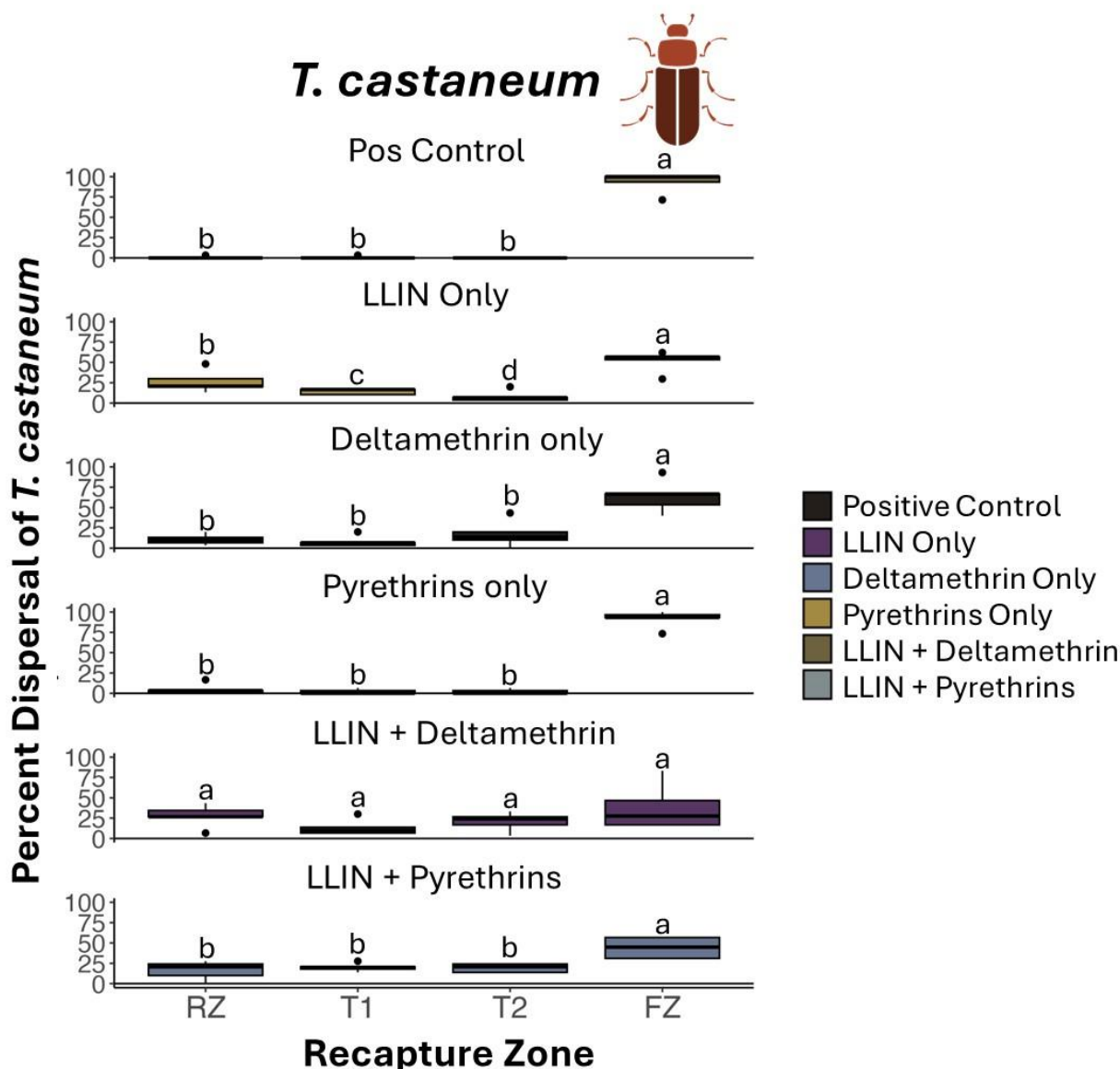


Figure 6.3 Dispersal of *T. castaneum* in a residual insecticide assay when exposed to the different treatments after 48 h

Dispersal of *T. castaneum* (out of 30 adults/replicate) in a residual insecticide assay when exposed to a positive control (black), long-lasting insecticide netting only (purple), deltamethrin spray only (blue), pyrethrins spray only (yellow), both LLIN and deltamethrin (brown), or both LLIN and pyrethrins (grey) after 48 h in the laboratory under constant conditions (25°C, 16:8 L:D). Box plot visualizes median as dark black line on each bar. Bars with shared letters are not significantly different from each other within each treatment (Kruskal Wallis, $\alpha = 0.05$). Abbreviations = RZ – release zone, T1 – treatment zone 1 (with LLIN when present), T2 – treatment zone 2 (with residual insecticide when present), FZ – food zone.

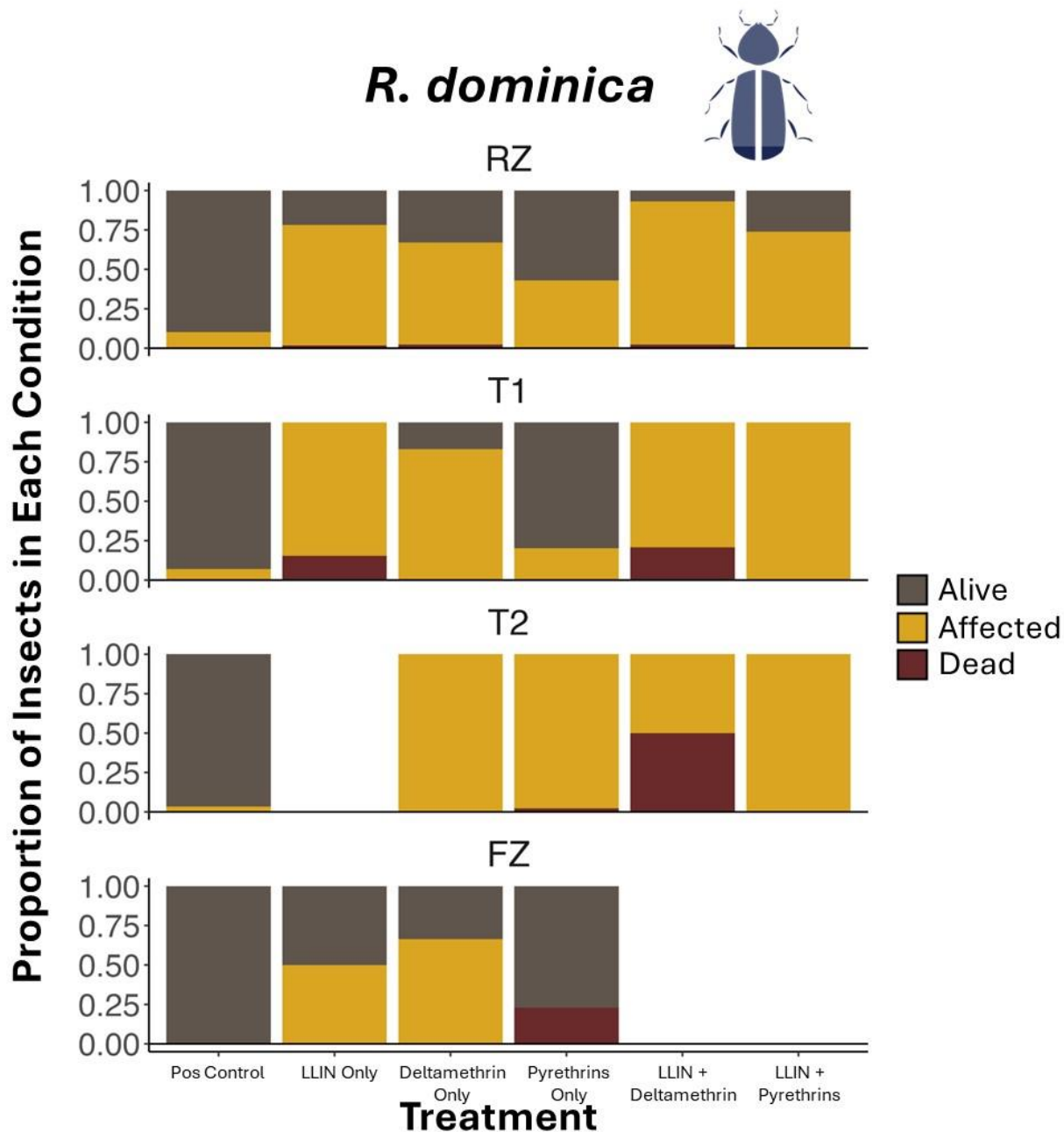


Figure 6.4 Proportion of *R. dominica* rated as alive, affected, or dead by recapture zone when exposed to different treatments after 48 h

Proportion of *R. dominica* (out of 30 adults/replicate) rated as alive (brown), affected (yellow), or dead (red) by recapture zone when exposed to a positive control, long-lasting insecticide netting only, deltamethrin spray only, pyrethrins spray only, both LLIN and deltamethrin, or both LLIN and pyrethrins after 48 h in the laboratory under constant conditions (25°C, 16:8 L:D). Abbreviations = RZ – release zone, T1 – treatment zone 1 (with LLIN when present), T2 – treatment zone 2 (with residual insecticide when present), FZ – food zone. Missing bars indicate no insects recovered in that treatment and zone.

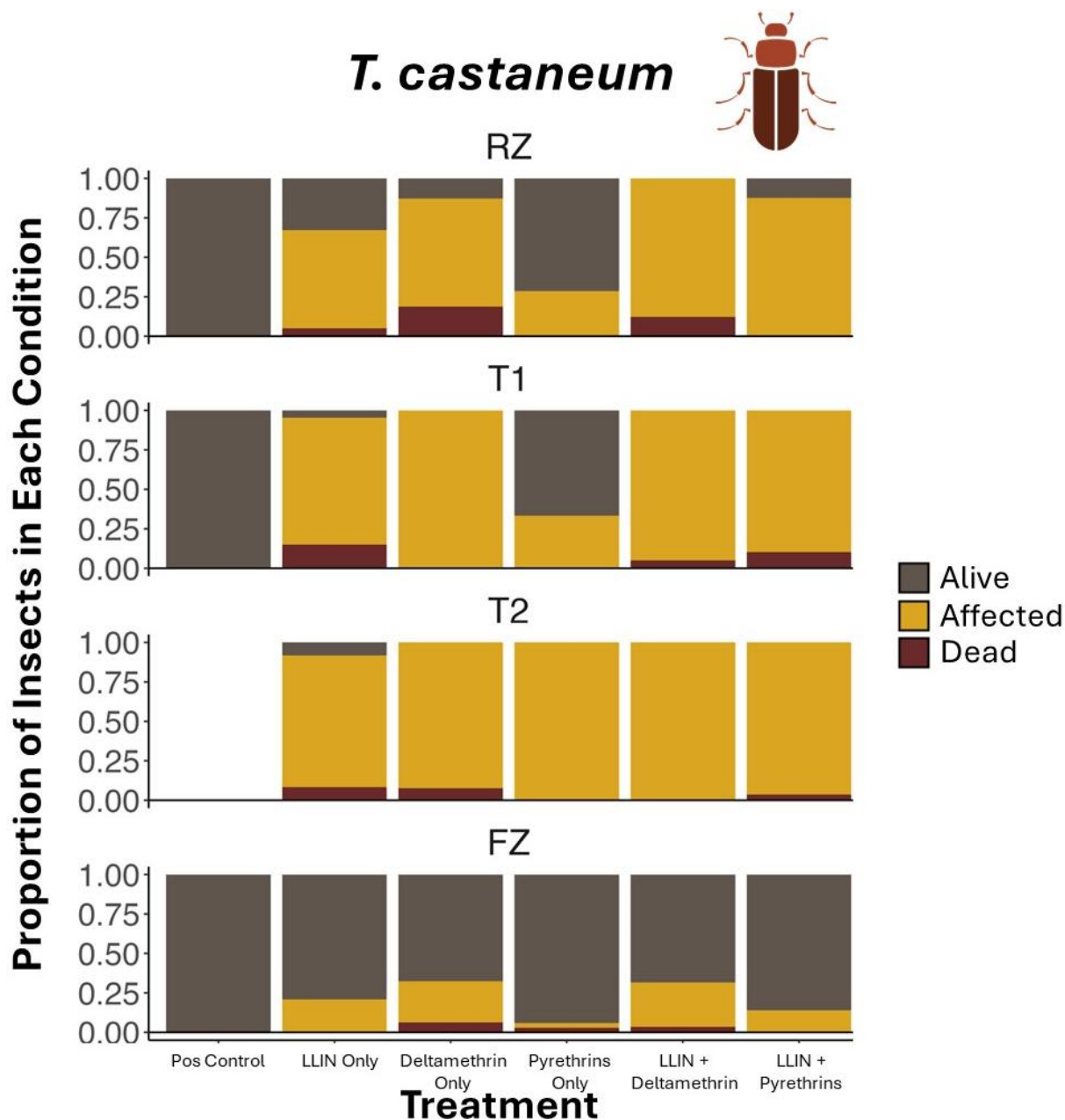


Figure 6.5 Proportion of *T. castaneum* rated as alive, affected, or dead by recapture zone when exposed to different treatments after 48 h

Proportion of *T. castaneum* (out of 30 adults/replicate) rated as alive (brown), affected (yellow), or dead (red) by recapture zone when exposed to a positive control, long-lasting insecticide netting only, deltamethrin spray only, pyrethrins spray only, both LLIN and deltamethrin, or both LLIN and pyrethrins after 48 h in the laboratory under constant conditions (25°C, 16:8 L:D). Abbreviations = RZ – release zone, T1 – treatment zone 1 (with LLIN when present), T2 – treatment zone 2 (with residual insecticide when present), FZ – food zone. Missing bars indicate no insects recovered in that treatment and zone.

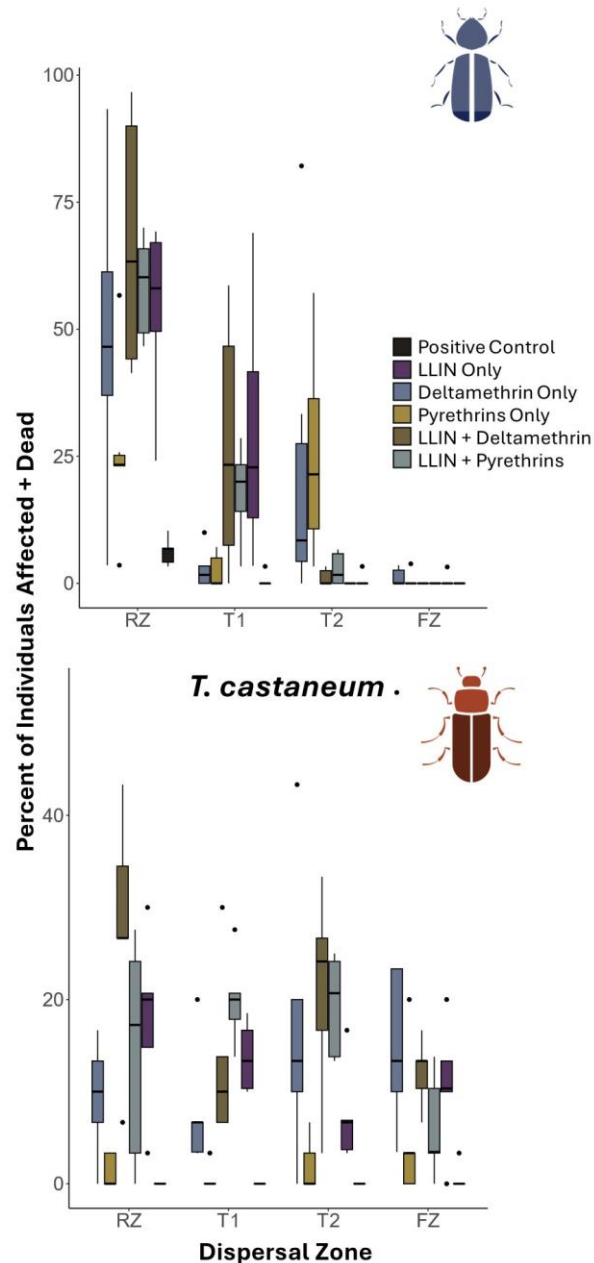


Figure 6.6 Percent mortality (affected + dead) of *R. dominica* and *T. castaneum* by recapture zone when exposed to a different treatments after 48 h

Percent mortality (affected + dead) of *R. dominica* (top panel) and *T. castaneum* (bottom panel) (out of 30 adults/replicate) by recapture zone when exposed to a positive control (black), long-lasting insecticide netting only (purple), deltamethrin spray only (blue), pyrethrins spray only (yellow), both LLIN and deltamethrin (brown), or both LLIN and pyrethrins (grey) after 48 h in the laboratory under constant conditions (25°C, 16:8 L:D). Box plot visualizes the median as a dark black line on each bar. Bars with shared letters are not significantly different from each other (Kruskal Wallis, $\alpha = 0.05$). Abbreviations = RZ – release zone, T1 – treatment zone 1 (with LLIN when present), T2 - treatment zone 2 (with residual insecticide when present), FZ – food zone.

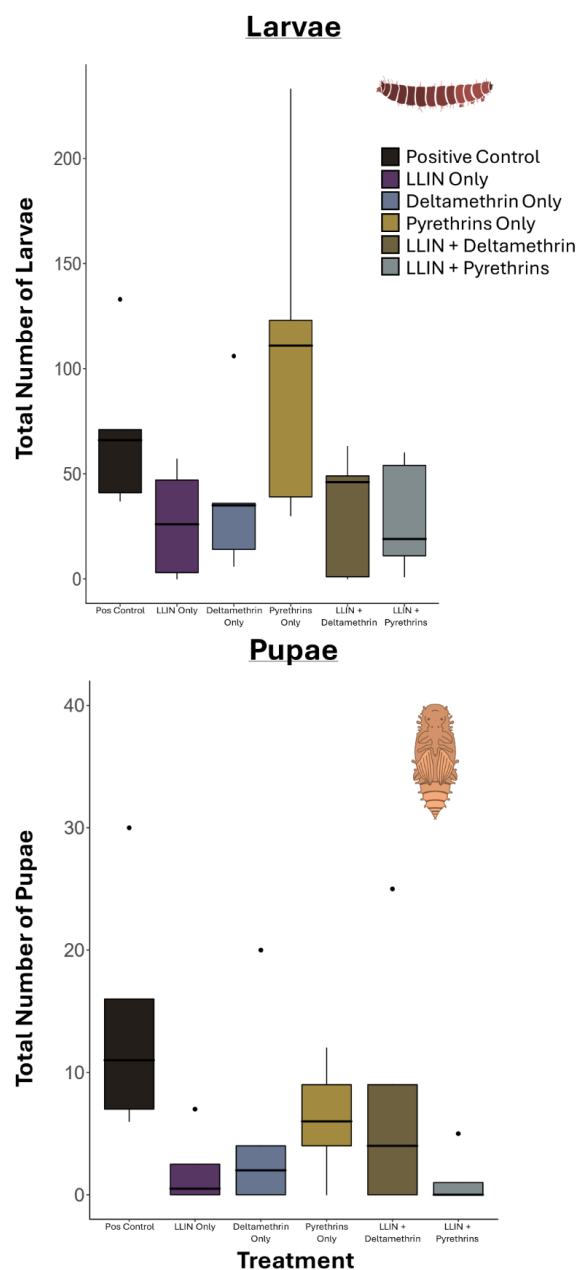


Figure 6.7 Progeny production by *T. castaneum* after 6 weeks in flour placed at the opposite end in the food zone after exposure to different treatment after 48 h

Progeny production (larvae – top panel, pupae – bottom panel) by *T. castaneum* after 6 weeks in flour placed at the opposite end in the food zone after exposure to a positive control (black), long-lasting insecticide netting only (purple), deltamethrin spray only (blue), pyrethrins spray only (yellow), both LLIN and deltamethrin (brown), or both LLIN and pyrethrins (grey) after 48 h in the laboratory under constant conditions (25°C, 16:8 L:D). Box plot visualizes median as dark black line on each bar. Bars with shared letters are not significantly different from each other (Kruskal Wallis, $\alpha = 0.05$). Abbreviations = RZ – release zone, T1 – treatment zone 1 (with LLIN when present), T2 - treatment zone 2 (with residual insecticide when present), FZ – food zone. There was no progeny production by *R. dominica*.

**Chapter 7 - Modeling long-term, stage-structured dynamics of
Tribolium castaneum at food facilities with and without two types of
long-lasting insecticide-incorporated netting**

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Abstract

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a cosmopolitan and destructive external-infesting pest at many food facilities. The use of deltamethrin- and α -cypermethrin-incorporated long-lasting insecticide netting (LLIN) has shown incredible promise for the management of stored product insects. However, it is unknown how LLIN deployed within food facilities may affect the long-term population dynamics of *T. castaneum* compared to populations where no LLIN is present. Exposure to LLIN has been shown to affect mortality in the current generation and decrease progeny production in the subsequent generation. Thus, we modeled the long-term population dynamics of *T. castaneum* at food facilities over 15 generations by incorporating realistic estimates for mortality and progeny reduction after contact with LLIN compared to baseline growth by the species. We parameterized the model with estimates from the literature and used a four-stage structured population (eggs, larvae, pupae, and adults). The model was implemented using the package popbio in R. Our models suggest that deploying LLIN led to significant population reduction based on the estimates of mortality and progeny reduction from prior work, whereas the baseline model exhibited exponential population growth. In addition, there were differences in the frequencies of each life stage under each scenario modeled. As a result, it appears deploying LLIN may contribute to the local extirpation of *T. castaneum* within as few as 15 generations. Our work contributes to a growing literature about the effectiveness of incorporating LLIN into existing pest management programs for managing stored product insects in food facilities.

Key Words: Post-harvest, stored product pest, stage-structured model, insecticide netting, integrated pest management

Introduction

The movement and dispersal of insects have a significant impact on the integrated pest management (IPM) programs at food facilities. There are multiple ways that insects move around food facilities: one is through anthropogenic-mediated or insect-mediated means to and within a facility, and the other is natural immigration from the landscape. Prior work has shown that common stored product insects can be found and subsist on hosts found in the natural landscape (Jia et al., 2008), and in mark-release-recapture studies, stored product insects dispersed as far as 267–375 m (Mahroof et al., 2010). Finally, prior work has found that these insects can be found far from food facilities (Scheff et al., 2022). Many food facilities have a variety of entry points that allow insects to gain access from the surrounding natural landscape. As a result, employing an innovative tactic to intercept immigrating insects before they can reach commodities could be a first line of defense against infestation.

One such tactic that has proven effective at intercepting immigrating insects after harvest is the use of long-lasting insecticide-incorporated netting (LLIN). The duration of efficacy is 1–1.5 years in harsh conditions of a south-facing wall outdoors (Scheff et al. 2024). LLIN has shown both direct lethal (Paloukas et al., 2020; Scheff et al., 2021) and sublethal effects on multiple species of stored product pests (Morrison et al., 2018; Wilkins et al., 2020). In fact, two types of LLIN (a 0.4% deltamethrin, Vestergaard Frandsen Inc., and a 0.34% α -cypermethrin, BASF, -based netting) have shown direct efficacy against several life stages of multiple important stored product insect species (Morrison et al., 2018; Rumbos et al., 2018; Paloukas et al., 2020; Agrafioti et al., 2021; Scheff et al., 2021). Further, LLIN exposure can reduce stored product insect reproduction in subsequent generations by half in laboratory trials (Gerken et al., 2021). In a pilot-scale warehouse, LLIN exposure reduced stored product insect dispersal by 93–

95% and progeny production in commodities by 99% (Wilkins et al., 2021). Prior research has also found that LLIN is equally effective against phosphine-resistant and -susceptible strains of the lesser grain borer *Ryzopertha dominica* (F.) (Coleoptera: Bostichidae) and the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Ranabhat et al., 2022). Thus, LLIN may be ideal for covering openings such as doors, windows, vents, cracks, crevices, intake or outtake manifolds in processing facilities (McKay et al., 2024), pallets in warehouses (Wilkins et al., 2021), headspace on grain silos (Ranabhat et al., 2024) or any other types of opening to intercept insects as they disperse in and around the food facilities and prevent entry into facility.

A pest that has been the focus of prior LLIN research is the red flour beetle, *T. castaneum*, an important, cosmopolitan pest of stored products, which feeds externally on damaged or broken grains (Hagstrum and Subramanyam 2006). This species is a strong walker (Morrison et al., 2019), but a weak flier that is largely confined to food facilities and the nearby areas where grain is processed (Drury et al., 2009; Ridley et al., 2011). *Tribolium castaneum* has been found to be susceptible to sublethal reductions in movement and dispersal after exposure to LLIN (Morrison et al., 2018; Wilkins et al., 2020). In addition, there was high direct lethality to *T. castaneum* when no food was present, and there was no repellency observed either at close- or long-range (Scheff et al., 2021). With netting containing α -cypermethrin, researchers found 85% mortality of *T. castaneum* after 14 d (Paloukas et al., 2020). Overall, research on LLIN against *T. castaneum* appears promising, but it is unknown how it affects *T. castaneum* populations in the long-term.

For effective integration into an IPM program, it is critical to understand how exposure to LLIN affects the long-term population dynamics of economically important insect pests such as *T. castaneum*. As Gerken and Morrison (2023) noted, it is not always possible to conduct

experiments at a commercial scale at food facilities (e.g., release insects to determine the efficacy of LLIN), but data-informed modeling can be a potential solution for this. For example, given realistic parametrization, modeling helps us to determine what a baseline population may look like in terms of population dynamics and how LLIN implemented as a management tactic may affect populations over time at the food facility. Furthermore, modeling can be used to predict patterns or increases in insect infestation based on data from stakeholders which in turn can be used to direct insect pest management tactic employed. While ecologists have sought to mathematically describe insect populations for over a century for various insect species (Wennergren and Stark, 2000; Caprio et al., 2009; Wiman et al. 2016; Caprio et al., 2016; Nakanishi et al., 2021), there have been great strides in understanding and modeling stored product insect populations in the last several decades (Lefkovitch and Currie, 1967; Cuff and Hardman, 1980; Hagstrum and Flinn, 1990; Throne 1995; Meikle et al., 1998; Meikle et al., 1999; Briggs et al., 2000; Pedersen et al., 2020).

Studying animal population growth in a tractable way was harnessed by estimating population growth change from age-specific birth and survival rates through projection matrices (Leslie, 1945). This gave rise to the fundamental importance of the Leslie matrix to divide an animal's life cycle into distinct stages, with each stage representing an age class. Typically, there are N age classes, and the population size is recorded for each class. Over time, individuals either age (progressing to the next age class) or die. The survival rate for each age class indicates the proportion of the population transitioning to the next age class. Additionally, new individuals can be born, and the birth rate or fecundity denotes the rate per capita of births arising from each age class. Later, Lefkovitch (1965) proposed a similar model, but with improvements for animals that have distinct life stages instead of age classes where individuals could both remain

temporarily in the same life stage or move on to the next one. This model incorporates a survival probability within a life stage, allowing for the possibility that individuals could persist in the same stage for a certain duration (e.g., a day) before progressing to the next stage, which makes the generational timing of stage-structured projection matrices more realistic.

Our aim in this study was to use *T. castaneum* as a model species to simulate the long-term effects of LLIN on the population dynamics of conspecifics, taking into account mortality, fecundity, or both compared to base population projections and parameterizing the model using data from the literature, including Sokoloff (1974), Wilkins et al. (2021), Gerken et al. (2021), Scheff et al. (2021) and Scheff et al. (2024). In particular, we developed a stage-structured Lefkovitch matrix to project the growth of a *T. castaneum* population under different mortality and fecundity scenarios using two types of LLIN containing different insecticide active ingredients.

Materials and Methods

Study Organism

The red flour beetle, *T. castaneum* was used as a model organism for this study. The life cycle of *T. castaneum* has been extensively described in the literature and in this study, we mainly included the information reviewed by Sokoloff (1974). In brief, the life cycle of *T. castaneum* consists of four main life stages, including egg, pupa, larva, and adult. Their life cycle is influenced by environmental factors (temperature, humidity, and photoperiod), rearing diet, and population density (White, 1987; Sokoloff, 1974), but for the sake of the modeling we have narrowed abiotic conditions to near ideal temperature (25–30°C) and RH (60–70%), maximizing both longevity and reproductive output, and held other conditions constant. These abiotic

conditions also reflect those within a typical food facility over the course of a year (Campbell et al. 2010).

Insecticide Netting

In this study, we focused on two types of LLIN that have been found to be effective for managing various stored product insect pests. One is an LLIN consisting of a polyethylene netting (2 × 2 mm mesh; 36 knots/cm²; D-Terrence, Vestergaard, Inc., Lausanne, Switzerland) with 0.4% deltamethrin active ingredient, while the second one is Carifend[®] net (40 deniers with mesh size 97 knots/cm²; BASF AG, Ludwigshafen, Germany) containing 0.34% α-cypermethrin active ingredient. Both types of nets were included in the study because of slower knockdown by the latter netting for stored product insects and to verify long-term management potential.

Foundational Model

We used a standard Lefkovitch matrix model to project population growth for *T. castaneum*, with four life stages (e.g., egg, larva, pupa, and adult; Lefkovitch, 1965). In equation (1), the Lefkovitch matrix *L* matrix (4 × 4) represents the life-stage structure of *T. castaneum* which has an egg, larvae, pupae, and an adult, where only the adults contribute to the fecundity, *F*. By multiplying *L* with the population vector *n_{i(t)}*, where *t* is the time step (e.g., generation) and *i* is a life stage, we obtain the resultant vector *n_{i(t+1)}*, which reveals the distribution of individuals across different life stages in the subsequent time period.

$$\begin{bmatrix} n_{1(t)} \\ n_{2(t)} \\ n_{3(t)} \\ n_{4(t)} \end{bmatrix} \times \begin{bmatrix} P_1 & 0 & 0 & F \\ G_1 & P_2 & 0 & 0 \\ 0 & G_2 & P_3 & 0 \\ 0 & 0 & G_3 & P_4 \end{bmatrix} = \begin{bmatrix} n_{1(t+1)} \\ n_{2(t+1)} \\ n_{3(t+1)} \\ n_{4(t+1)} \end{bmatrix} \quad (1)$$

In equation (1), *P*₁ represents the probability of staying in the egg stage, *G*₁ is the probability of moving from the egg to the larval stage, *P*₂ is the probability of staying in the larval stage, *G*₂ is the probability of moving from the larval stage to pupal stage, *P*₃ is the

probability of staying in the pupal stage, G_3 is probability of moving from the pupal stage to adult, while P_4 is the probability of staying in the adult stage (Figure 7.1).

Model Parameterization and Scenarios

We simulated population outcomes for up to 15 generations by using the life table data for *T. castaneum* using the R package *popbio*. Fifteen generations equaled an equivalent duration of 1.4 years, which was bracketed by the period of effectiveness for LLIN (1–1.5 years). Survivorship, fecundity, and transition information for each stage were derived from the literature (summarized in Table 7.1). The developmental duration of eggs, larvae, and pupae were 3.82 ± 0.005 , 22.81 ± 0.67 , and 6.24 ± 0.071 days (Kollros, 1944). The average life duration of the adult used in this study was 221.16 days (Park et al., 1961). We used 94 offspring for fertility based on a previous study (Park et al., 1965) and a 99% rate of eclosion from pupae to adult.

To explore the sensitivity of the base model to changes in mortality and fecundity, both of these parameters were systematically varied from near zero to their maximum value given in the base model (e.g., $F = 94$, $P_4 = 0.871$). The parameters were varied alone or in combination and the resulting population growth was plotted. All plots were created using ggplot2 (Wickham, 2016) in R software (R Core Team, 2022).

Four empirical scenarios from the literature were modeled containing estimates of fecundity reduction only, survivorship reduction only, or both fecundity and survivorship reduction when using LLIN (Wilkins et al., 2021; Gerken et al., 2021; Scheff et al., 2021, Scheff et al., 2024; Table 7.2). The Wilkins et al. 2021, Gerken et al. 2021, and Scheff et al. 2021 were collectively the Vestergaard models or deltamethrin LLIN, while the Scheff et al. 2024 model was also called the BASF model. An individual projection matrix was constructed for each of the

four scenarios and combinations of the reductions in fecundity, survivorship, or both. Population growth and proportion in each life stage were projected for 15 generations for each case, including the base model. Overall variation and oscillation were calculated to compare trends among the proportion of life stages in each case. A sensitivity analysis was performed to determine which stage should be targeted to most greatly affect the population growth after exposure to the netting. Moreover, a mortality function based on empirical data with LLIN exposure collected in the laboratory on *T. castaneum* was implemented. Mortality was implemented for adults (cell 4 x 4 in the matrix) as $[P_4 - m_{LLIN}]$, where m_{LLIN} is the mortality resulting from the insecticide netting above and beyond the control.

Results

Baseline *T. castaneum* population

The population of *T. castaneum* increased exponentially through the 15th generation without any use of LLIN (Figure 7.2A). There were frequent oscillations in life stages, especially in eggs and larvae, with what appears to be three peaks over 15 generations. Eggs of *T. castaneum* were in the highest abundance followed by larvae compared to the pupae and adults, which were both substantially lower (Figure 7.2B).

Sensitivity by *T. castaneum* in model to modifications of mortality and fecundity

Regardless of reductions in survivorship, population size starkly increases near generation 15 after exposure to deltamethrin LLIN (Figure 7.3). For example, even large changes in survivorship did not greatly affect the population size, because when mortality was 97% (and survivorship was correspondingly low), there was still an increase in population abundance by the 12th generation onwards (Figure 7.3, top-left panel). Thus, the total population size was relatively insensitive to the change in mortality and resulted in only delayed effects on

population growth. By contrast, the population size was more greatly affected by a reduction in the fecundity of *T. castaneum* after exposure to deltamethrin LLIN with a tipping point of greater than 23.6 eggs laid by conspecifics (Figure 7.4). Prior to that point, the total population size of *T. castaneum* flatlined over 15 generations. When mortality and fecundity are changed together, fecundity appeared to matter more for the total population size (Figure 7.5).

Efficacy of LLIN among empirical scenarios over 15 generations

From the Wilkins et al. (2021) study, when we took fecundity alone into account, we observed that deltamethrin LLIN causes a greater than 99% reduction in the total population size by the 15th generation compared to the baseline model, with the total population size after 15th generation rising just above 400 (Figure 7.6A, left panel). However, when we took only survivorship into account, LLIN reduced the total population size by 60% by the 15th generation compared to the baseline model (Figure 7.6A, -middle panel). When we consider the effect of both fecundity and survivorship reductions from LLIN on *T. castaneum* populations, the total population size actually decreased from 200 in the 1st generation to near zero by the 15th generation, showing local extirpation of conspecifics (Figure 7.6A, right panel). In Gerken et al. (2021), similarly, with a significant fecundity reduction from deltamethrin LLIN, we observed an 80% reduction in the total population size by generation 15 with insecticide netting compared to the baseline model (Figure 7.6B, -left panel). Finally, in Scheff et al. (2021), with just a survivorship reduction from deltamethrin LLIN, we observed a total population size that was reduced by 53% in the presence of insecticide netting compared to the baseline model by generation 15 (Figure 7.6C, middle panel).

Proportion in each life stage among empirical scenarios over 15 generations

When only fecundity reductions from deltamethrin LLIN were considered, the number of oscillations in populations of life stages was reduced relative to the baseline population of *T. castaneum* (Figure 7.7). In the case of Wilkins et al. (2021), reducing fecundity resulted in monotonic dampening of oscillations in life stages and roughly equal proportions of eggs, larvae, and adults compared to pupae after exposure to deltamethrin LLIN (Figure 7.7A, left panel). When just considering survivorship reductions from deltamethrin LLIN, there were actually a greater number of oscillations in all life stages, with larvae much more mesokurtic while adults and pupae were much more platykurtic compared to leptokurtic egg populations. This was true for both the cases of Wilkins et al. (2021), (Figure 7.7A, middle-panel) and Scheff et al. (2021), (Figure 7.7C, middlepanel) after exposure to deltamethrin LLIN. When fecundity and survivorship reductions were taken together, all populations showed much more leptokurtic peaks and were more similar in proportion to each other.

Life Stage Sensitivity Analysis

In the baseline model, the most susceptible *T. castaneum* life stage to interventions was adult to egg (e.g., ovipositing females), adult to larvae, and pupae to egg (Figure 7.8A). To a lesser extent, larvae to pupae transitions were also susceptible to perturbations. By and large, these were the same four transitions that were most sensitive in all the models, including in the Vestergaard-based models, Wilkins et al. 2021 (survivorship reduction: Figure 7.8B), Gerken et al. 2021 (Figure 7.8C), Scheff et al. 2021 (Figure 7.8D), as well as the Scheff et al. 2024 (BASF model) (Figure 7.8E). However, when fecundity was reduced or both fecundity and survivorship were reduced in the Wilkins et al. (2021) model, none of the life stages showed notable susceptibility to perturbations (Figure 7.8B).

Performance of BASF LLIN

Furthermore, α -cypermethrin incorporated LLIN from the Scheff et al. 2024 (BASF model) significantly reduced the population of *T. castaneum* by 98.5% over 15 generations compared to the baseline population without LLIN (Figure 7.9A). After the 8th generation, there was a significant and exponential increase in the total population size without LLIN, but this was not observed for the population of *T. castaneum* with LLIN. In terms of life stages, BASF LLIN exposure resulted in a greater number of oscillations in all life stages compared to the baseline population (Figure 7.9B). Larval peaks were much more mesokurtic while adults and pupae were much more platykurtic compared to leptokurtic egg populations. Peaks for eggs emerged first, followed by larvae, then pupae and adults, with each subsequent life stage in lower proportion than the one preceding it. The maxima were relatively stable among all life stages. The most sensitive life stage transitions to intervention by the BASF netting were the same as the Vestergaard netting: adult to egg, adult to larvae, pupae to egg, and larvae to pupae (Figure 7.8E).

Discussion

For the first time, we have modeled the long-term population dynamics of *T. castaneum*, a highly destructive pest, after being exposed to LLIN as an IPM tactic. Encouragingly, we find that regardless of the active ingredient, the use of this tactic can result in the long-term suppression of *T. castaneum* when we appropriately take the sublethal effects of insecticide exposure into account. Thus, the sublethal effects are critically important for the long-term suppression of insect populations, including this species. LLIN has been demonstrated to cause both lethal and sublethal effects to a variety of stored product insects (Morrison et al., 2018; Rumbos et al., 2018; Paloukas et al., 2020; Wilkins et al., 2020; Wilkins et al., 2021; Scheff et

al., 2021; Gerken et al., 2021), including reducing mobility, dispersal, progeny production, and mating. This is important to consider for immigrating and dispersing stored product insects that may try to gain access to commodities.

We found fecundity reduction was a more important determinant of LLIN efficacy than merely survivorship reduction. To some extent this makes sense, because by obviating the production of a single egg, one negates both the survivorship and all the future offspring that an individual could produce, whereas negating an adult may or may not do anything for future offspring. In studies where fecundity reductions were considered, there were marked reductions in the total population of *T. castaneum* compared to the baseline population by the end of the simulation in generation 15. Not many studies have considered the combined effects of LLIN on survivorship and progeny production, but Wilkins et al. (2021) did so, and this resulted in a decreasing population model compared to exponential growth in the baseline population model. Practically, this likely means it is better to deploy the LLIN at the perimeter of the facility or structure, so there is a greater chance adults succumb to LLIN exposure prior to reproducing in commodities.

In addition to the practical results of comparing lethal and sublethal effects of LLIN, this study also demonstrates the benefit of employing ecological modeling to interrogate the long-term success of IPM tactics, which may not always be possible in the field given time, costs, or personnel constraints. Our model demonstrates how LLIN may be successful in the long-term background suppression of *T. castaneum* based on realistic, empirically parametrized data from the literature. This included well-established population parameters for *T. castaneum* (Sokaloff, 1974), but also LLIN-relevant measures of efficacy and success from empirical studies (Gerken et al., 2021; Scheff et al., 2021; Wilkins et al., 2021; Scheff et al., 2024). This approach is

especially useful when it's not possible to conduct field-level experiments at commercial food facilities, as is the situation here. It is all the more appropriate given recent calls to adopt ecological tools in stored product entomology, such as modeling (Gerken and Morrison, 2023). Though there has been ecological modeling in stored products to evaluate questions of biology related to host commodities or dispersal, few have used it as a tool to assess the efficacy of IPM tactics (e.g., Kawamoto et al., 1989; Throne, 1995; Meikle et al., 1998). In our study, Wilkins et al. (2021) was the most realistic scenario for LLIN exposure of *T. castaneum*. This scenario accounted for a comprehensive range of sublethal effects in pilot-scale warehouses, distinguishing it from other laboratory-based studies. Consequently, when we consider the case of Wilkins et al. (2021), we observed that the utilization of LLIN resulted in the most substantial population suppression in each generation. Our work highlights the importance of ecological modeling for assessing long-term outcomes of IPM.

There are some caveats to this work. These include the fact that we did not use spatially explicit models (e.g., Deangelis and Yurek 2017), take into account climate variables (e.g., Harman et al. 2024), account for sanitation at food facilities (e.g., Morrison et al. 2019), or repeated exposures to insecticide netting (Gerken et al. 2020). Importantly, we also did not include immigration, which is an opportunity for further model improvements. There is a tradeoff between a model being tractable and too complex to be useful or generalizable. For our purposes, a model is a mathematical simplification that is intended to be generalizable, and with these limitations in mind, it serves as a suitable starting point for exploring the IPM of using LLIN at food facilities.

In this study, we used the data from studies that employed LLIN with either 0.4% deltamethrin or 0.34 % α -cypermethrin (Gerken et al., 2021, Wilkins et al., 2021, Scheff et al.

2021, Scheff et al., 2024). Both deltamethrin and α -cypermethrin are type II pyrethroids and function through contact with the organism. These pyrethroids are relatively “newer” and less studied chemistry in stored product insects (Arthur and Morrison, 2020; Stejskal et al., 2021). However, there are documented cases of pyrethroid resistance in stored product insects already outside the US, including in *Sitophilus* spp. in Brazil, Argentina, and Uruguay (Haddi et al. 2018). Resistance to active ingredients has already been registered as a concern by stakeholders in the use of LLIN (Ranabhat et al. 2022; Ranabhat et al. 2024a). Incorporating any empirical measures of resistance into the modeling process to determine how it affects long-term performance of LLIN at food facilities may be a critical next step. To ameliorate this, there are also multiple other active ingredients, such as permethrin, indoxacarb, and dinotefuran, which have been shown effective against the major stored product insects after being incorporated into surrogate LLIN material (Ranabhat et al., 2022). Nonetheless, current use of LLIN in a bulk storage environment showed that it can reduce fumigation by 68–100% compared to controls (Ranabhat et al. 2024b), demonstrating that LLIN has a role to play in resistance management as part of a holistic pest management program. Overall, our work shows that LLIN with both deltamethrin and α -cypermethrin are effective at reducing the population of *T. castaneum* in the long-term.

When deploying LLIN at food facilities, it is likely to succeed as a low-maintenance, background suppression tactic. LLIN may be deployed in a variety of ways, including on vents, windows, doors, partitions, intake manifolds, or other areas where insects may gain access to a facility. It is likely that the total effect of incorporating LLIN is an underestimate in this study, because LLIN is meant to be used as part of a comprehensive IPM program with multiple hurdles to pest infestation. Thus, one must imagine the few individuals not being removed from

the foraging population by this tactic are likely being removed by other existing IPM tactics at facilities. These other tactics may include residual insecticide treatments, grain protectants, aerosols, and fumigants, among other semiochemical-mediated, behaviorally-based tactics (Morrison et al., 2021). Use of LLIN as part of a comprehensive IPM program is more likely to obviate resistance development because different tactics could employ multiple modes of action and active ingredients. It is likely that greater sublethal effects would speed up the development of resistance compared to direct lethal effects. Overall, however, the total population size would likely be reduced even further in the context of a comprehensive IPM program, leaving commodities more frequently insect-free. This would ineluctably reduce consumer complaints due to infested goods.

Overall, our study showed that population growth was suppressed when LLIN was used compared to a baseline population of *T. castaneum* without LLIN. Future work should model LLIN exposure with internal-infesting insects and evaluate the efficacy of LLIN when combined with other IPM tactics such as fumigation or residual insecticide usage. In addition, the development of models incorporating other active ingredients for phosphine-susceptible and -resistant species will offer valuable insights for addressing the growing issue of insecticide resistance among different stored product insects. The benefits of ecological modeling are clear and should be applied more often in applied management work on stored product insects. In addition to long-term suppression, we found the two netting products tested were suitable for hampering *T. castaneum* populations, with changes in fecundity playing a greater role than survivorship. This study underscores the potential of LLIN as an additional strategy for the long-term suppression of *T. castaneum* populations, offering a valuable tool for diversifying management practices within food facilities.

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Tables:

Table 7.1 Life history values for *T. castaneum* used in the model.

Stage	Parameter	Value	Sources
Egg	Development duration	3.82 ± 0.005 d	Kollros (1944)
Larvae	Development duration	22.81 ± 0.67 d	Kollros (1944)
Pupae	Development duration	6.24 ± 0.071 d	Kollros (1944)
		217.25, 288.75, 206.5,	
Male adult	Average life duration	119.25 d	Park et al. (1961)
		236.25, 247.75, 241.75,	
Female adult	Average life duration	211.75 d	Park et al. (1961)
	Fertility	94 ± 0.86 eggs	Park, Mertz, Grodzinski, and Prus (1965)
Pupae	Pupae to adult	>99%	White (1988)

Table 7.2 Different exposure scenarios for LLIN were used in modeling growth of *T. castaneum*.

Stages	Parameter	Reduction		Source	Scenario
		in Value	Net		
Female adult	Fecundity	63%	0.4 % delatmethrin	Vg ¹	Gerken et.al (2021)
Mixed sex adult	Survivorship	96%	0.4 % delatmethrin	Vg	Scheff et al. (2021)
Mixed sex adult	Fecundity	99%	0.4 % delatmethrin	Vg	Wilkins et al. (2021)
Mixed sex adult	Dispersal/Survivorship	95%	0.4 % delatmethrin	Vg	Wilkins et al. (2021)
Mixed sex adult	Fecundity	45%	0.34 % α -cypermethrin	BASF ²	Scheff et al. (2024)
Mixed sex adult	Survivorship	68%	0.34 % α -cypermethrin	BASF	Scheff et al. (2024)

¹ Vestergaard Inc. (Lusanne, Switzerland)

² BASF Corps. (Ludwigshafen, Germany)

Figures:

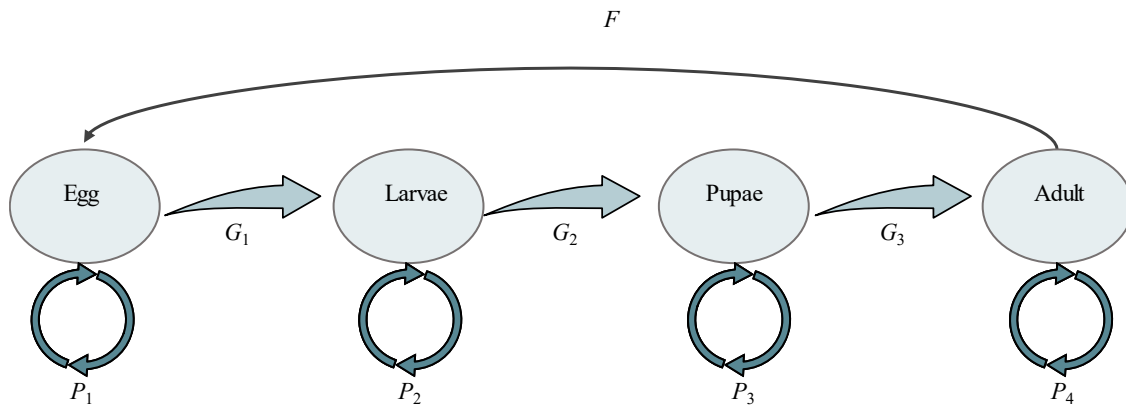


Figure 7.1 Schematic diagram representing the stage structure of the base model for *T. castaneum* in the study

The letters in each box represent the model parameters of the appropriate state variables; P_i is the individual's survival probability and staying within the same stage and G_i is the individual's growth probability and moving to the next stage, while F is the fecundity for the adult female *T. castaneum*.

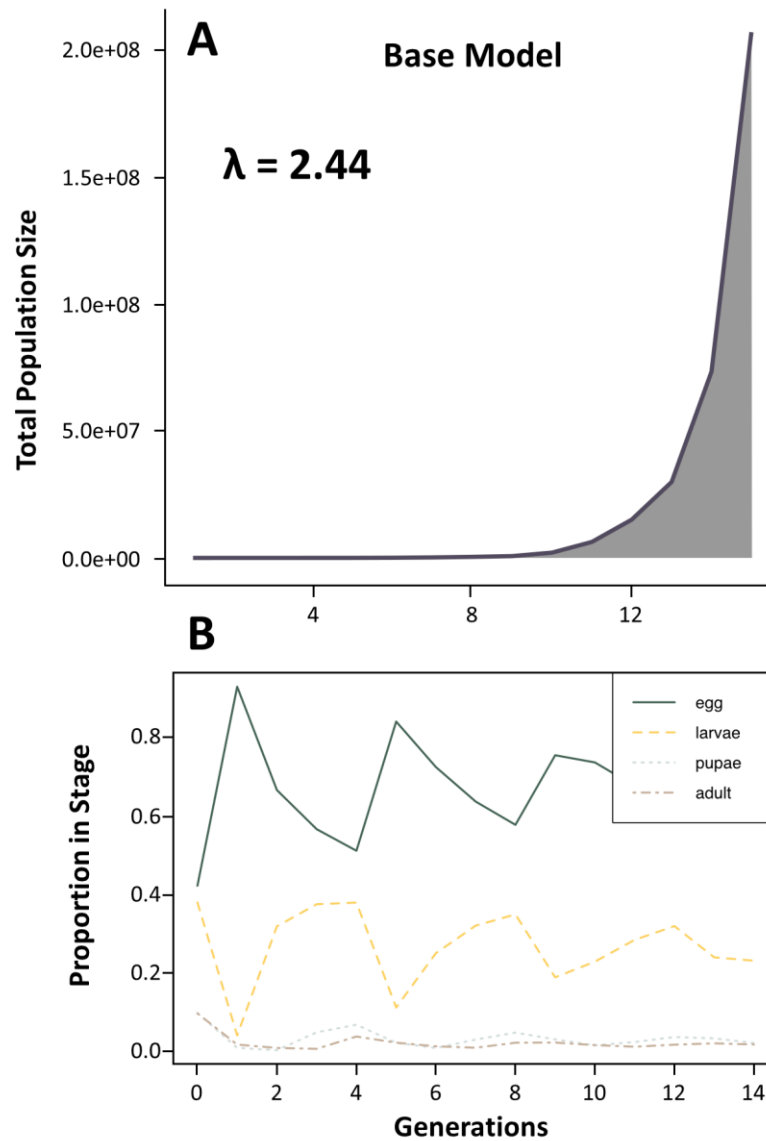


Figure 7.2 Baseline model of *T. castaneum* without exposure to LLIN showing total population size and proportion in each life stage over 15 generations

Baseline model of *T. castaneum* without exposure to long-lasting insecticide-incorporated netting (LLIN) showing total population size (A) and proportion in each life stage (B) over 15 generations. Population estimates were parameterized from empirical data from the primary literature, mainly Sokaloff (1974), see Table 1. Lambda (λ) is given in the graph and represents the population growth rate.

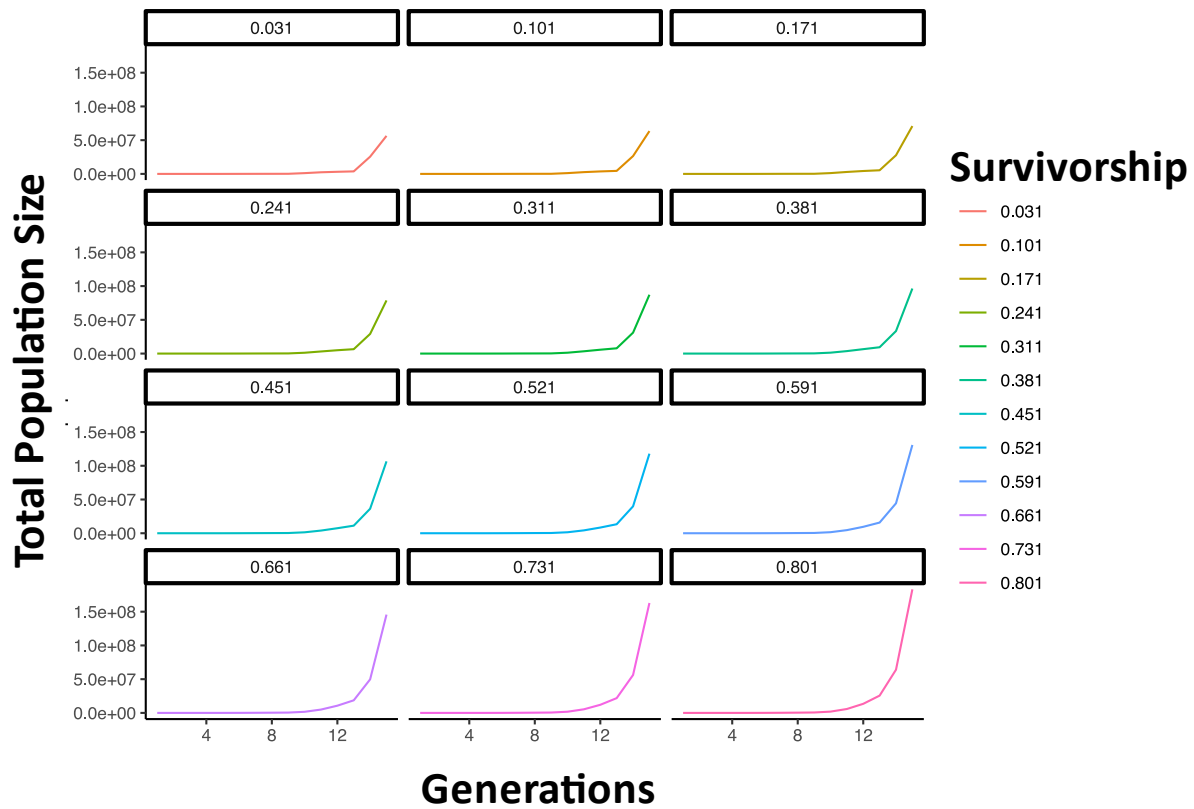


Figure 7.3 Individual effect of reducing survivorship by 12-equally spaced lower steps for *T. castaneum* adults from baseline population model maximum on total population size
The individual effect of reducing survivorship by 12-equally spaced lower steps for *T. castaneum* adults from baseline population model maximum (0.871) on total population size over 15 generations. The survivorship must be reduced very low to have a substantial effect on the population size.

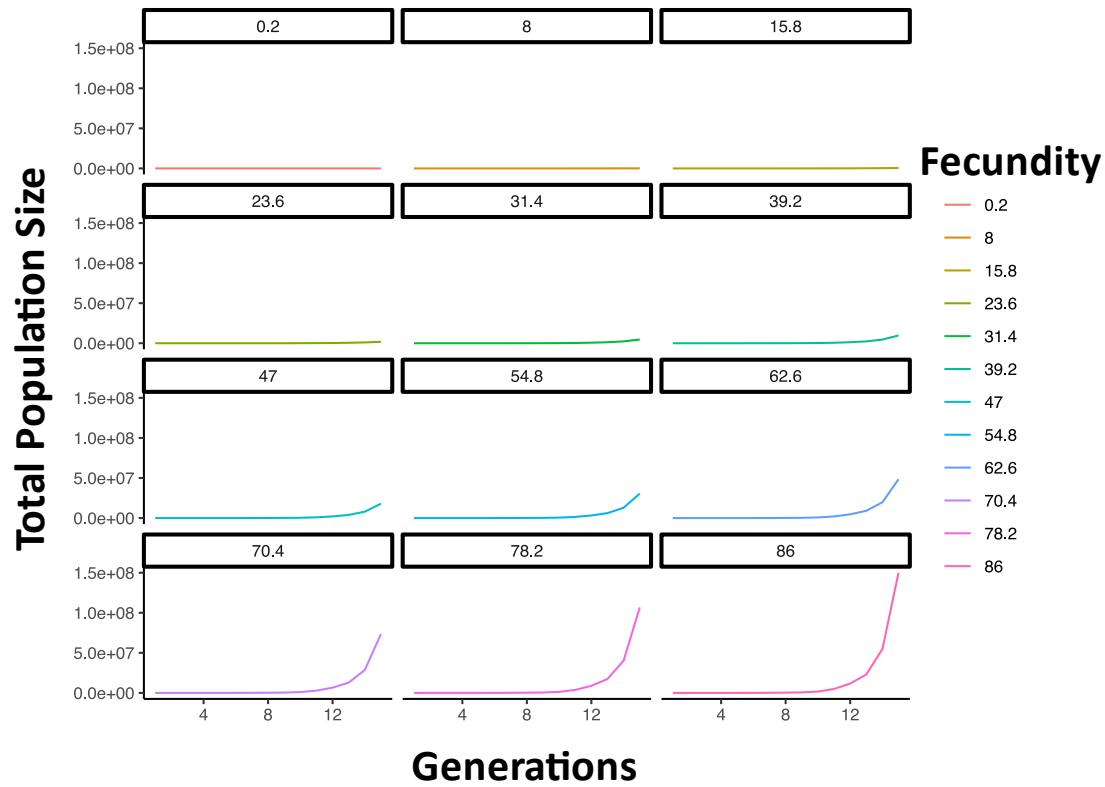


Figure 7.4 Individual effect of reducing fecundity by 12-equally spaced lower steps for *T. castaneum* adults from baseline population model maximum on total population size

The individual effect of reducing fecundity by 12-equally spaced lower steps for *T. castaneum* adults from baseline population model maximum (94 eggs) on total population size over 15 generations. Fecundity reductions have an outsized effect on the population size of *T. castaneum*.

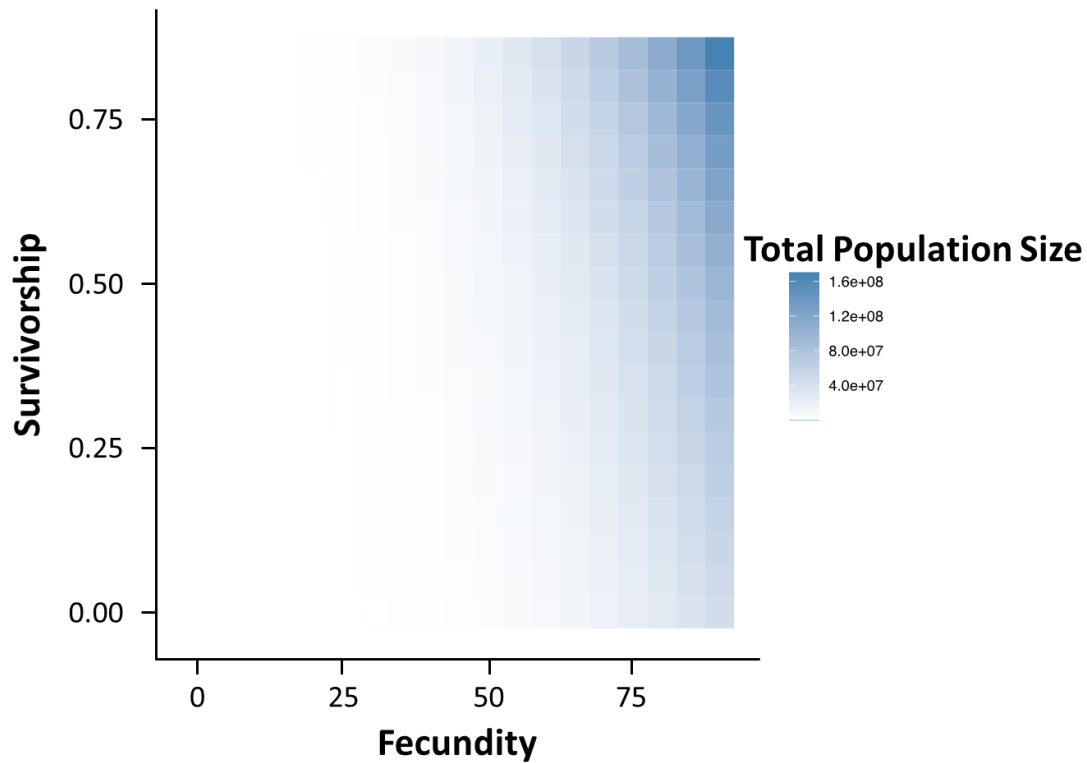


Figure 7.5 Combined effect of reducing fecundity and survivorship by 18-equally spaced lower steps for *T. castaneum* adults from the baseline population model maximums on the total population size at the end of the 15th generation

The combined effect of reducing fecundity and survivorship by 18-equally spaced lower steps for *T. castaneum* adults from the baseline population model maximums (0.871 survivorship and 94 eggs) on the total population size at the end of the 15th generation. Fecundity reduction had a greater impact on total population size of *T. castaneum* than survivorship.

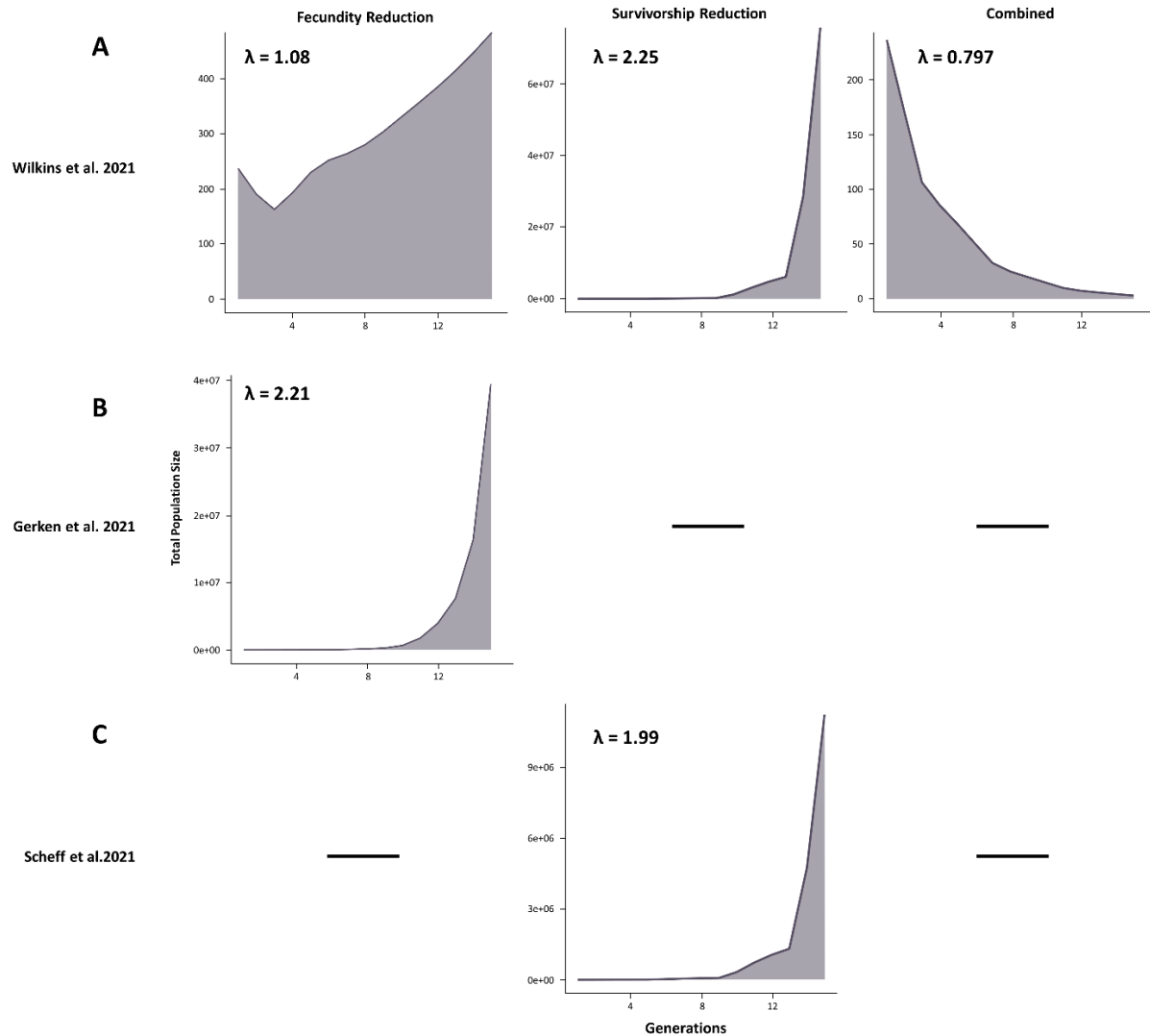


Figure 7.6 Effect of empirically-derived cases of 0.4% deltamethrin-based LLIN exposure on the total population size of *T. castaneum*

Effect of empirically-derived cases of 0.4% deltamethrin-based LLIN exposure on the total population size of *T. castaneum* considering only fecundity reductions (left column), survivorship reductions (middle column), or both (right column). Data is derived from A) Wilkins et al. (2021), B) Gerken et al. (2021), and C) Scheff et al. (2021). Solid line denotes no empirical data available for parameterization of model for the study. Lambda (λ) is given in the graphs and represents the population growth rate.

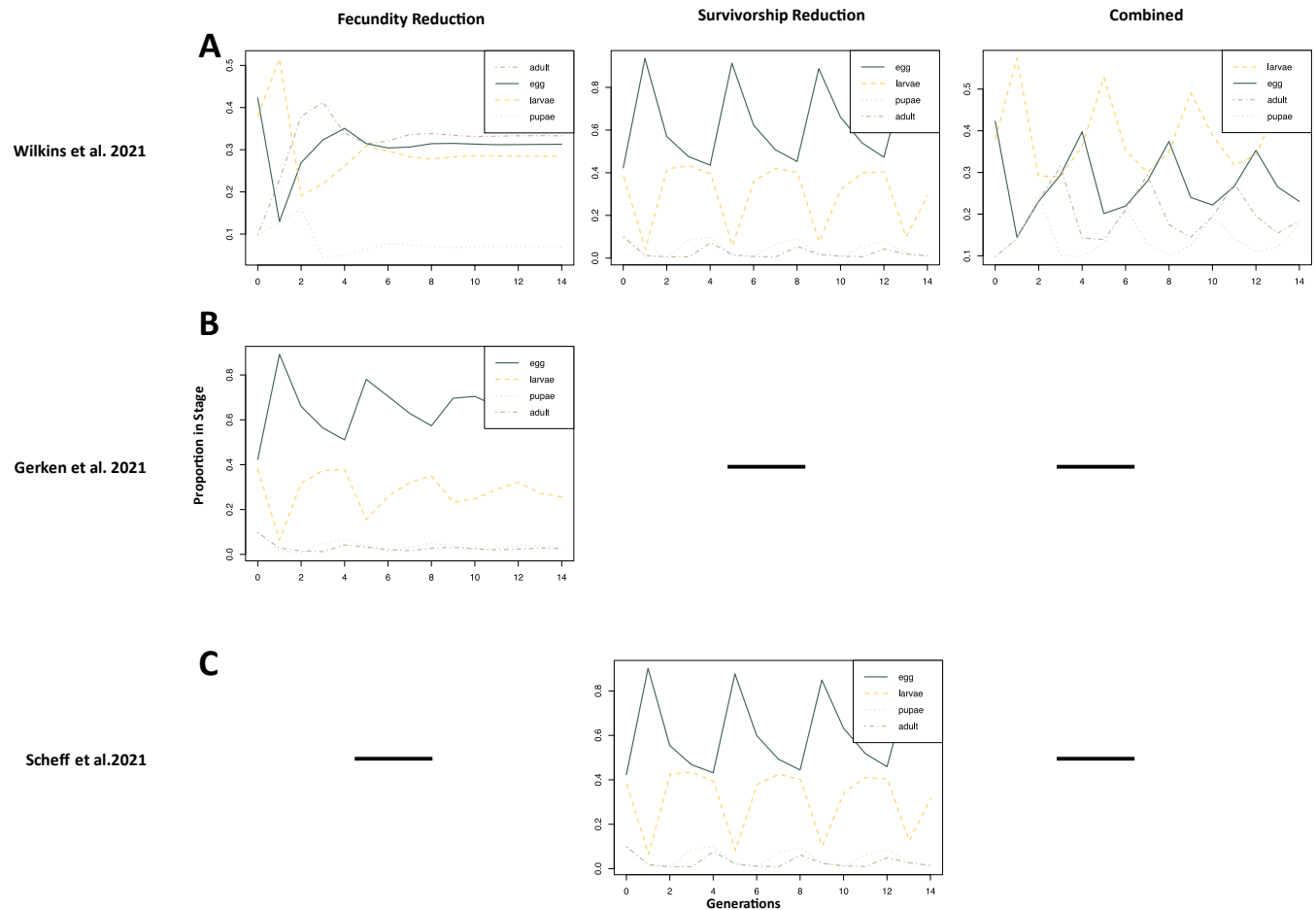


Figure 7.7 Effect of empirically-derived cases of 0.4% deltamethrin-based LLIN exposure on the proportion of individuals in each life stage for *T. castaneum*

Effect of empirically-derived cases of 0.4% deltamethrin-based LLIN exposure on the proportion of individuals in each life stage for *T. castaneum* considering only fecundity reductions (left column), survivorship reductions (middle column), or both (right column). Data is derived from A) Wilkins et al. (2021), B) Gerken et al. (2021), and C) Scheff et al. (2021). Solid line denotes no empirical data available for parameterization of model for the study.



Figure 7.8 Results of the sensitivity analysis from the baseline stage-structured model of *T. castaneum* and those expressing a fecundity reduction, survivorship reduction, or both. Results of the sensitivity analysis from the baseline stage-structured model of *T. castaneum* and those expressing a fecundity reduction (left column), survivorship reduction (middle column), or both (right column). Data is derived from A) base model B) Wilkins et al. (2021), C) Gerken et al. (2021), D) Scheff et al. (2021), and E) Scheff et al. (2024). Darker red shades correspond to higher sensitivity transitions.

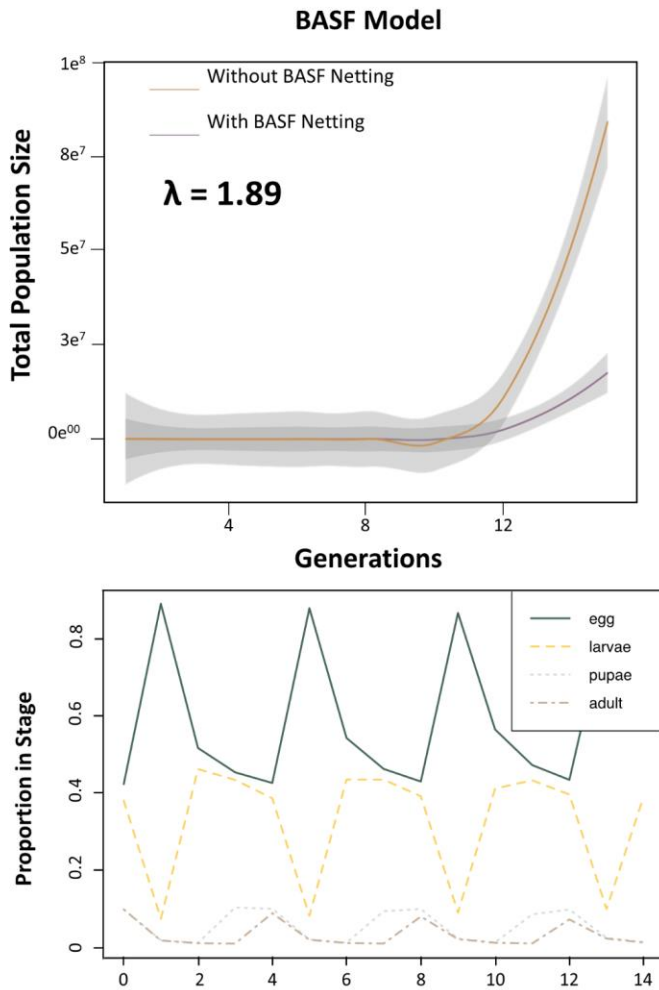


Figure 7.9 Baseline model of *T. castaneum* without exposure to LLIN and with 0.34% α -cypermethrin LLIN and showing the proportion of individuals in each life stage

Baseline model of *T. castaneum* without exposure to long-lasting insecticide-incorporated netting (LLIN) (e.g., yellow line) and with 0.34% α -cypermethrin LLIN (e.g., purple line) (A) and showing the proportion of individuals in each life stage (B) over 15 generations. Population estimates were parameterized from empirical data from the primary literature, mainly Sokaloff (1977) and Scheff et al. (2024). Lambda (λ) is given in the graph and represents the population growth rate.

Chapter 8 - Research summary and future directions

Prevention of infestation is key to post-harvest IPM programs. Prevention can focus on both insects entering structures from the outside environment or on mitigating the spread of insects within structures. Long-lasting insecticide-incorporated netting (LLIN) is one of the novel preventative tactics to prevent insect dispersal in and around food facilities. While prior work has demonstrated lethal and sublethal effects of LLIN on stored product insects, I have significantly added to the state of the art in this dissertation. In Chapter One, I provided an updated review and conducted a meta-analysis to assess the combined pattern across studies on LLIN in pre- and post-harvest agriculture to determine the direct and sub-lethal efficacy of exposure. In the end, this left us with a total of 44 peer-reviewed publications with 285 data points on the efficacy of LLIN in agriculture. LLIN exposure was 8.6-fold and 257-fold more effective in causing direct mortality and sublethal effects in the laboratory, respectively, compared to the field. However, broad generalization was limited because of the low sample size for field studies. Direct mortality was equally observed after exposure in preharvest and postharvest systems, but sublethal effects were 4.9-fold stronger after harvest. Deltamethrin and alpha-cypermethrin-incorporated netting inflict similar direct mortality across taxa. LLIN exposure showed direct efficacy against 19 out of 23 tested species and has induced sublethal effects in 10 out of 16 tested species. This provided adequate framing for the rest of the work.

In Chapter Two, I evaluated whether LLIN can support resistance management programs for phosphine. In particular, the efficacy of different CRMs (LLIN or packaging material) with each of four active ingredients (AI) (deltamethrin, permethrin, indoxacarb, and dinotefuran) was compared to control CRMs in reducing movement of phosphine-susceptible and -resistant *Rhyzopertha dominica* and *Tribolium castaneum*. Their movement was assessed using video-

tracking and Ethovision software. I also tested higher rates of each active ingredient (AI) on standardized-sized cheesecloth and varied exposure time to obtain median lethal time (LT₅₀) for each compound and susceptibility. Exposure to LLIN with deltamethrin significantly reduced the movement of both species compared to the other CRMs regardless of their susceptibility to phosphine. Deltamethrin was the most effective AI for both species, while dinotefuran and indoxacarb were the least effective for *R. dominica* and *T. castaneum*, respectively. Most AIs resulted in appreciable and approximately equivalent mortality at higher concentrations among phosphine-susceptible and resistant strains. Overall, my findings demonstrate that LLIN can be an additional approach to combat phosphine-resistant populations of stored product insects around food facilities. In the future, other active ingredients such as the synergist piperonyl butoxide is worth testing extensively against a variety of pyrethroid-resistant stored product insects.

In Chapters Three and Four, I examined whether exposure to 0.4% deltamethrin-incorporated LLIN disrupts the normal olfactory and chemotactic behavior of immature, male and female (or mixed) of *Trogoderma variabile* and *T. inclusum* movement in the presence of important semiochemicals, including food kairomones (e.g., flour) and pheromones, e.g., (Z)-14-methyl-8-hexadecenal. The total distance moved, velocity, frequency of entering each half of the petri dish and stimulus zone and latency of entering each zone over 30 min were logged immediately or 24 h after exposure to LLIN. I evaluated this with a video-tracking protocol and confirmed volatile emissions with headspace collection coupled with gas chromatography and mass spectrometry (GC/MS). Generally, the olfaction and orientation of larval and adult dermestids were affected after exposure to LLIN compared to control netting. Pheromonal stimuli were more important for males, but food and pheromones were equally important for

females. There were also species-linked differences in effects on olfaction after the insects were exposed to LLIN. These findings suggest the use of LLIN may enhance the effectiveness of other behaviorally-based management strategies when used as part of a comprehensive IPM program. Follow-up work should understand whether these findings translate to pilot-scale warehouses and commercial food facilities.

In Chapter 5, I investigated whether the use of LLIN can improve the efficacy of fumigation against stored product insects. For this study, I used three 110 MT capacity grain bins, and in each, 60 perforated buckets (e.g., miniature silos), which were filled with 500 g of uninfested wheat. Miniature silos were protected either by 0.34% α -cypermethrin incorporated LLIN, netting without insecticide (positive control), or no netting (negative control). Monthly samples of 100 g of grain from three miniature silos in one of four blocks from three-grain bins were taken between June and October both in 2022 and 2023. Half of each treatment was randomly assigned to phosphine fumigation treatment based on a conservative FGIS tolerance level, while the remainder were not fumigated. We determined whether phosphine fumigation could be reduced with the use of LLIN over the season. Overall, we found that miniature silos protected with LLIN showed insect dispersal and progeny production that was reduced by 83–99% and 89–99%, respectively, compared to insecticide-free netting and no-netting controls. Furthermore, damage in miniature silos was reduced by 37–99% compared to controls. Most importantly, the total number of fumigations could be reduced by 68–91% by using LLIN compared to controls. This study validates that LLIN is consistently effective for existing pest management tactics such as enhancing phosphine fumigation in bulk storage structures. A future direction includes understanding whether LLIN could work in other parts of the supply chain like

the shipping industry to prevent insect immigration and reduce fumigation of shipping containers.

In Chapter 6, I assessed whether the use of LLIN with residual contact insecticides may enhance the efficacy of residual insecticide to reduce dispersal and damage by stored product insects. For this study, I used 0.34% alpha-cypermethrin-based LLIN and the common residual contact insecticides such as CentanylTMEC (a.i. deltamethrin) and Evergreen®(a.i. pyrethrins) against the *T. castaneum* and *R. dominica* and recorded their dispersal and progeny production in treatments with LLIN alone, deltamethrin or pyrethrins alone, LLIN + insecticide, or no treatment (positive control). I demonstrated the use of both LLIN and residual insecticide with either deltamethrin or pyrethrins reduced adult insect dispersal to the food sources compared to the control. Further, I found LLIN alone was marginally effective in decreasing progeny production of *T. castaneum* by 10-20% compared to the positive control. Notably, pyrethrins did not cause significant direct lethal effects or prevent progeny production when used alone against *T. castaneum*. However, the simultaneous use of pyrethrins with LLIN may offer complementary effects on mortality and progeny reduction. The finding of this study suggests the combined use of LLIN, and the residual contact insecticides evaluated in our study may have some benefits over using residual contact insecticide alone to manage stored product insects, though this may depend on the active ingredient and rate used in the residual spray. Overall, this knowledge will be beneficial to food facility managers moving forward so that they can better coordinate their IPM programs. In the future, we can assess the combined use of residual sprays with more active ingredients and different kinds of insecticide netting.

Finally, Chapter Seven focuses on how the use of LLIN within food facilities may affect the long-term population dynamics of stored product insects compared to populations where no

LLIN is present. Thus, I modeled the long-term population dynamics of *T. castaneum* at food facilities over 15 generations by incorporating realistic estimates for mortality and progeny reduction after contact with LLIN compared to baseline growth by the species. I parameterized the model with estimates from the literature and used a four-stage structured population (eggs, larvae, pupae, and adults). The model was implemented using the package popbio in R. This model suggests that deploying LLIN led to significant population reduction based on the estimates of mortality and progeny reduction from prior work, whereas the baseline model exhibited exponential population growth. In addition, there were differences in the frequencies of each life stage under each scenario modeled. Overall, this study showed that population growth was suppressed when LLIN was used compared to a baseline population of *T. castaneum* without LLIN. In addition to long-term suppression, we found the two netting products tested were suitable for impeding *T. castaneum* populations, with changes in fecundity playing a greater role than survivorship. This study highlights the potential of LLIN as an additional strategy for the long-term suppression of *T. castaneum* populations, offering a valuable tool for diversifying management practices within food facilities. Future work should model LLIN exposure with internal-infesting insects and evaluate the efficacy of LLIN when combined with other IPM tactics such as fumigation or residual insecticide usage. In addition, the development of models incorporating other active ingredients for phosphine-susceptible and -resistant species will offer valuable insights for addressing the growing issue of insecticide resistance among different stored product insects. Overall, the findings of my research may help in the speed of adoption of LLIN by stakeholders at food facilities and help to increase the sustainability of IPM.

The most commonly assessed active ingredients in netting have been alpha-cypermethrin and deltamethrin due to limitations in the availability of commercial options on the international market.

One of the possible consequences of the consistent utilization of LLINs in pest management programs is the potential development of pyrethroid resistance. So, novel approaches such as employing nets coated with dual active ingredients with different modes of action and targets in the insect need to be explored and should be adapted for use in postharvest production systems. The effects of LLIN on natural enemies that exist on stored commodities, either naturally or through biological control programs, remain mostly unknown. Therefore, it's crucial to conduct trials to assess the effect of LLIN on various natural enemies. While laboratory experiments provide valuable insights into LLIN dynamics and effectiveness, field trials are crucial for evaluating practical feasibility, as well as understanding the impact of environmental factors. More comprehensive field studies reflecting real-world application scenarios are required for the widespread adoption of LLIN. Continued research efforts focused on field-level understanding of LLIN efficacy, standardizing practices, resistance management, and environmental impact assessment are necessary to unlock the full potential of LLINs and endorse their use as a standard tool in stored product pest management programs. However, it is clear from the current research that LLINs hold enormous promise for stored product protection from insects.

Appendix A - Supporting information for Chapter 3

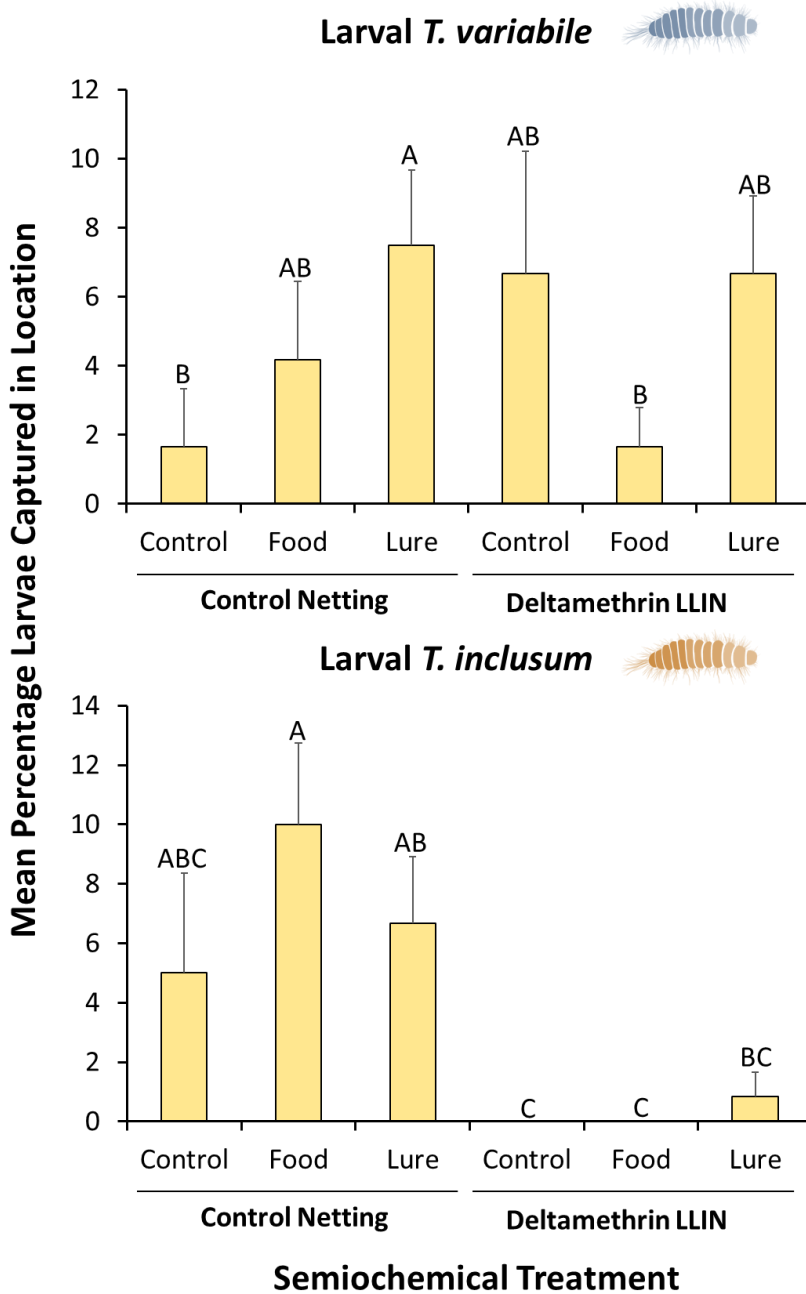


Figure S3-1 Mean percentage ($\pm$ SE) of larval *T. variable* (top panel) and larval *T. inclusum* (bottom panel) recaptured inside the trap (yellow) after 1-min exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting netting (LLIN) in a laboratory release-recapture 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. Bars with shared letters are not significantly different (Tukey HSD, $\alpha = 0.05$).

Appendix B - Supporting information for Chapter 4

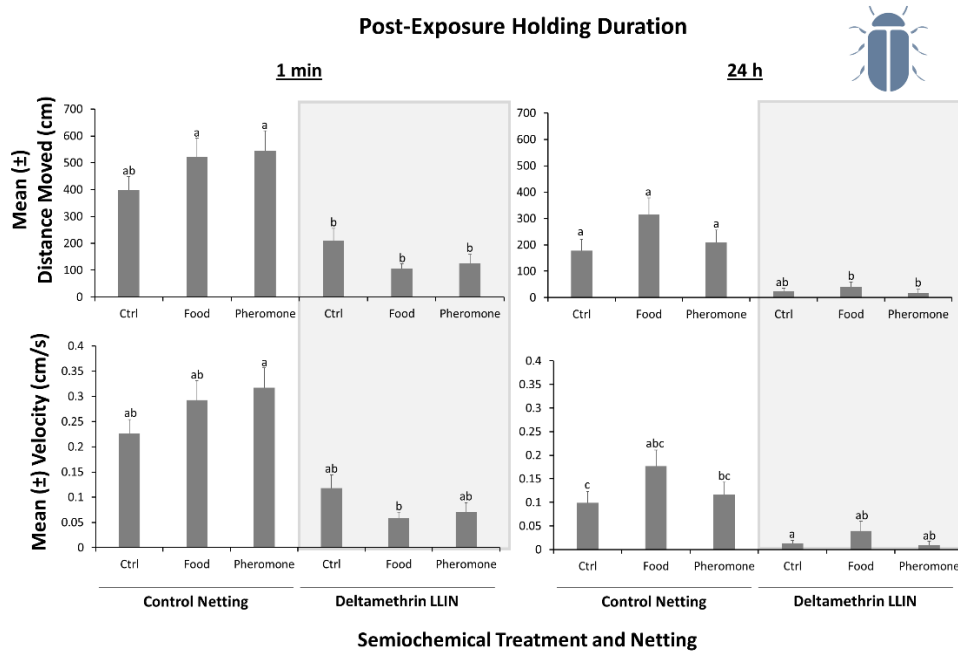


Figure S4-1 Distance moved ($\pm$ SE, cm) (top panels) or instantaneous velocity ($\pm$ SE, cm/s) (bottom panels) by mixed-sex populations of *T. variable* after 1-min exposure to control netting without insecticide or long-lasting 0.4% deltamethrin-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24 h later (right). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments.

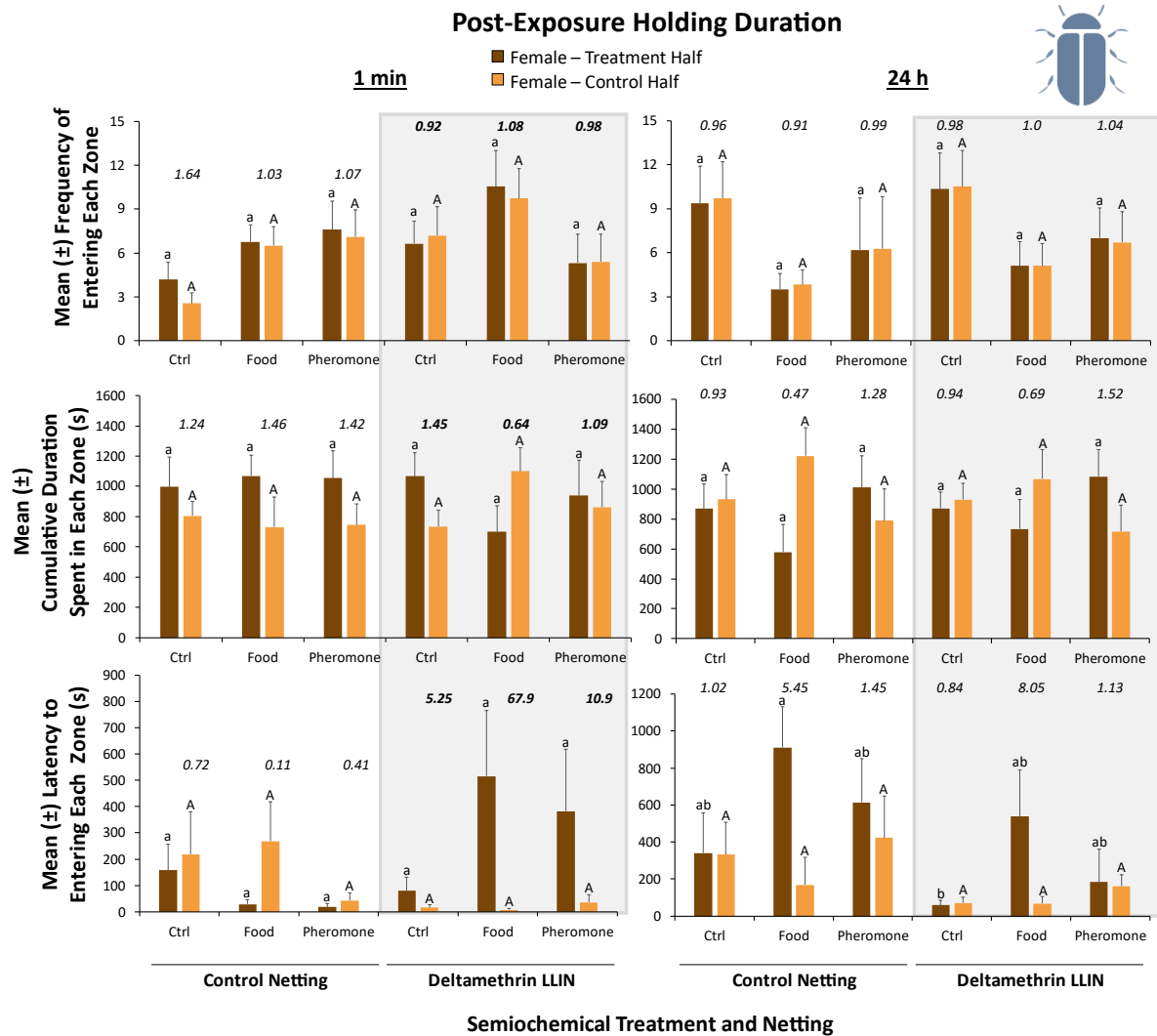


Figure S4-2 Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels), or latency to finding (bottom panels) the treatment half (dark orange) or control half (light orange) of the arena by female *T. variabile* after 1-min exposure to control netting without insecticide or long-lasting 0.4% deltamethrin-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control half of arena, while lower case letters represent multiple comparisons among treatments in the treatment half of arena. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italacized numbers over bars represent ratio of female *T. variabile* behavioral response in the treatment:control half of the arenas, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

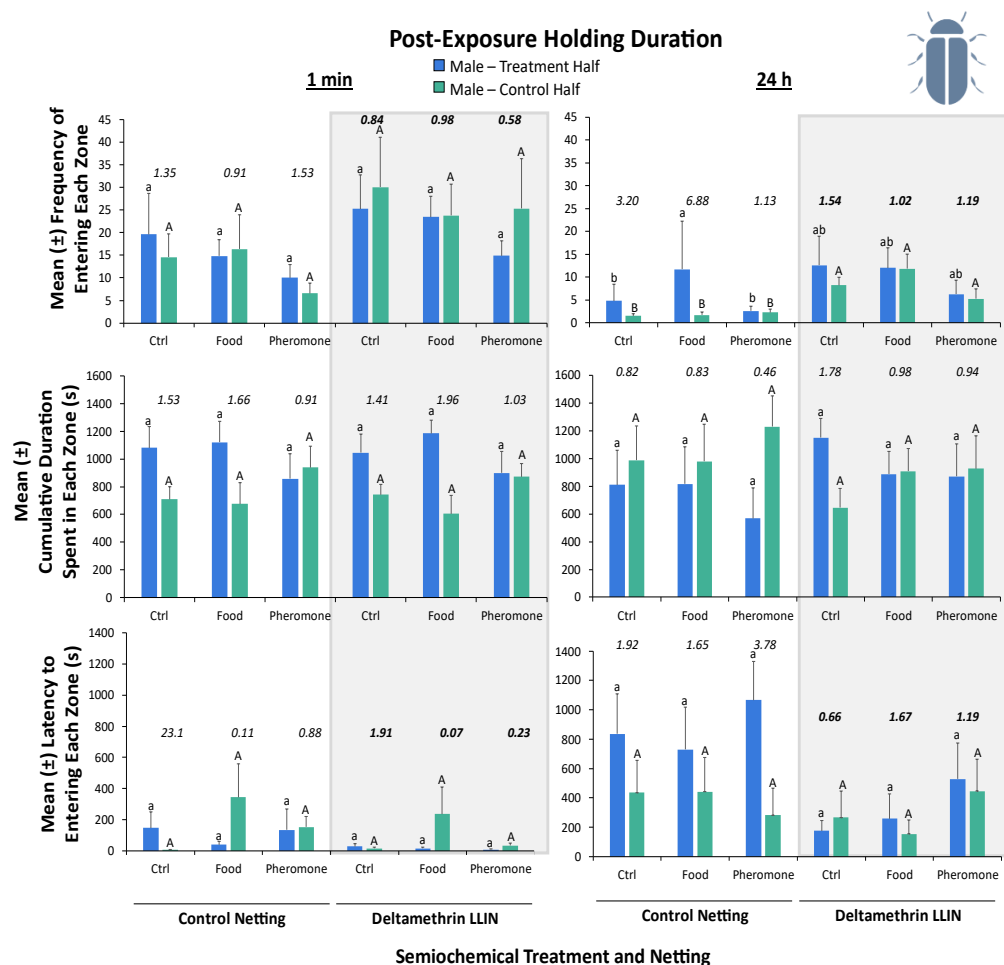


Figure S4-3 Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels), or latency to finding (bottom panels) the treatment half (light blue) or control half (light green) of the arena by male *T. variabile* after 1-min exposure to control netting without insecticide or long-lasting 0.4% deltamethrin-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control half of arena, while lower case letters represent multiple comparisons among treatments in the treatment half of arena. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italized numbers over bars represent ratio of male *T. variabile* behavioral response in the treatment:control half of the arenas, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

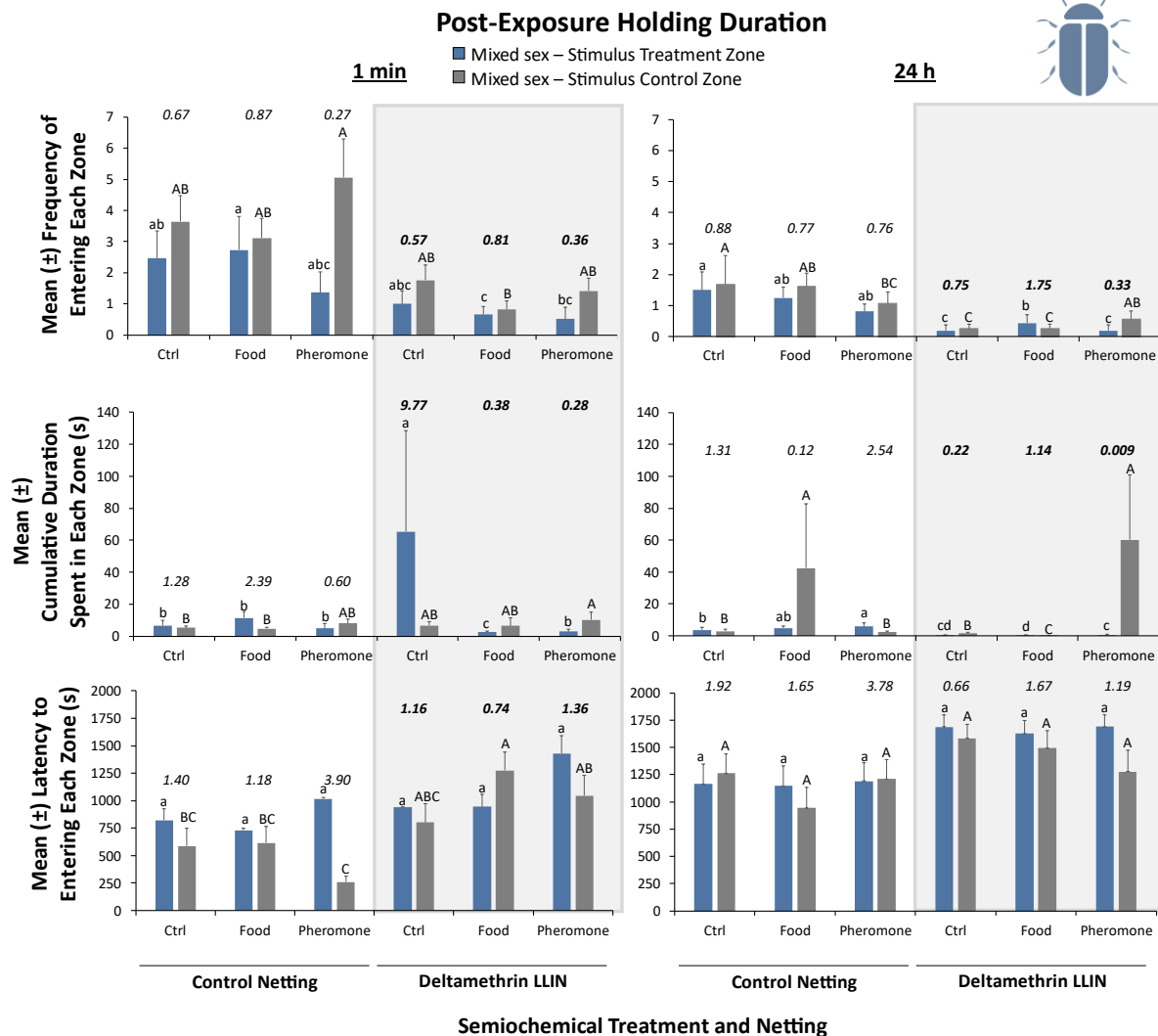


Figure S4-4 Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels) or latency to finding (bottom panels) the treatment stimulus zone (dark blue) or control stimulus zone (dark gray) by mixed-sex populations of *T. variabile* after 1-min exposure to control netting without insecticide or long-lasting 0.4% deltamethrin-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control stimulus zone, while lower case letters represent multiple comparisons among treatments in the treatment stimulus zone. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italicized numbers over bars represent ratio of mixed-sex *T. variabile* behavioral response in the treatment:control stimulus zones, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

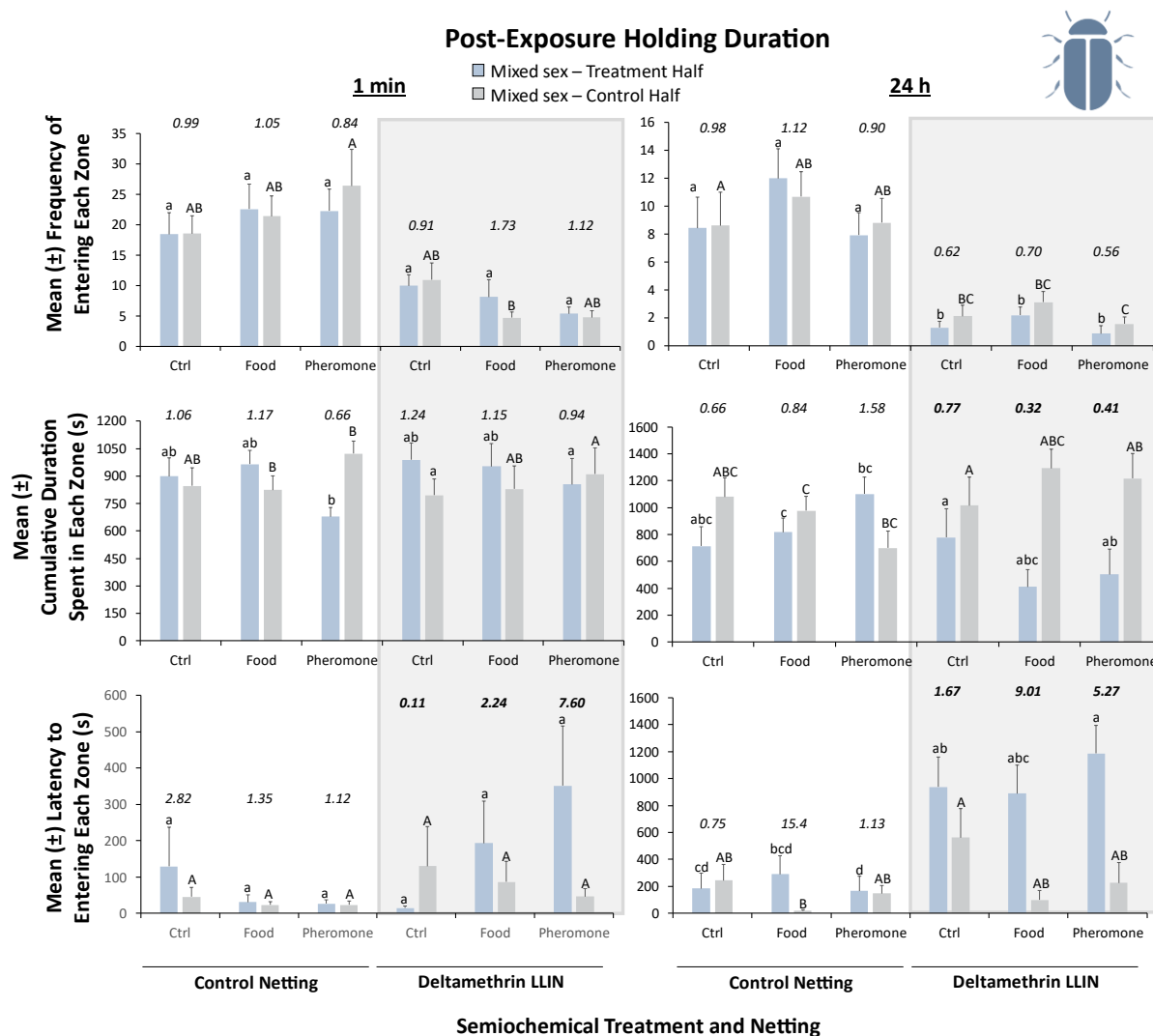


Figure S4-5 Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels), or latency to finding (bottom panels) the treatment half (light blue) or control half (light gray) of the arena by mixed-sex populations of *T. variable* after exposure to control netting without insecticide or long-lasting 0.4% deltamethrin-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control half of arena, while lower case letters represent multiple comparisons among treatments in the treatment half of arena. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italicized numbers over bars represent ratio of mixed-sex *T. variable* behavioral response in the treatment:control half of the arenas, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

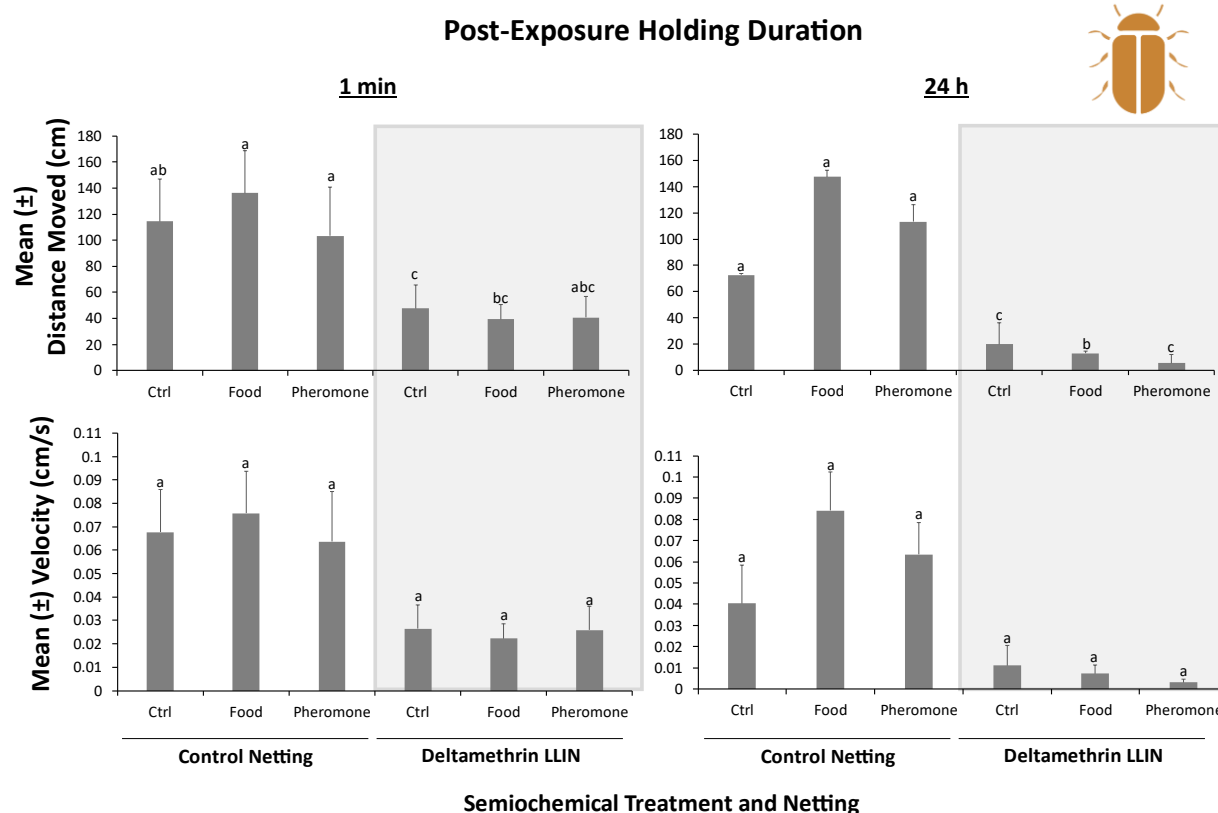


Figure S4-6 Distance moved ($\pm$ SE, cm) (top panels) or instantaneous velocity ($\pm$ SE, cm/s) (bottom panels) by mixed-sex populations of *T. inclusum* after 1-min exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24 h later (right). Bars with shared letters are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments.

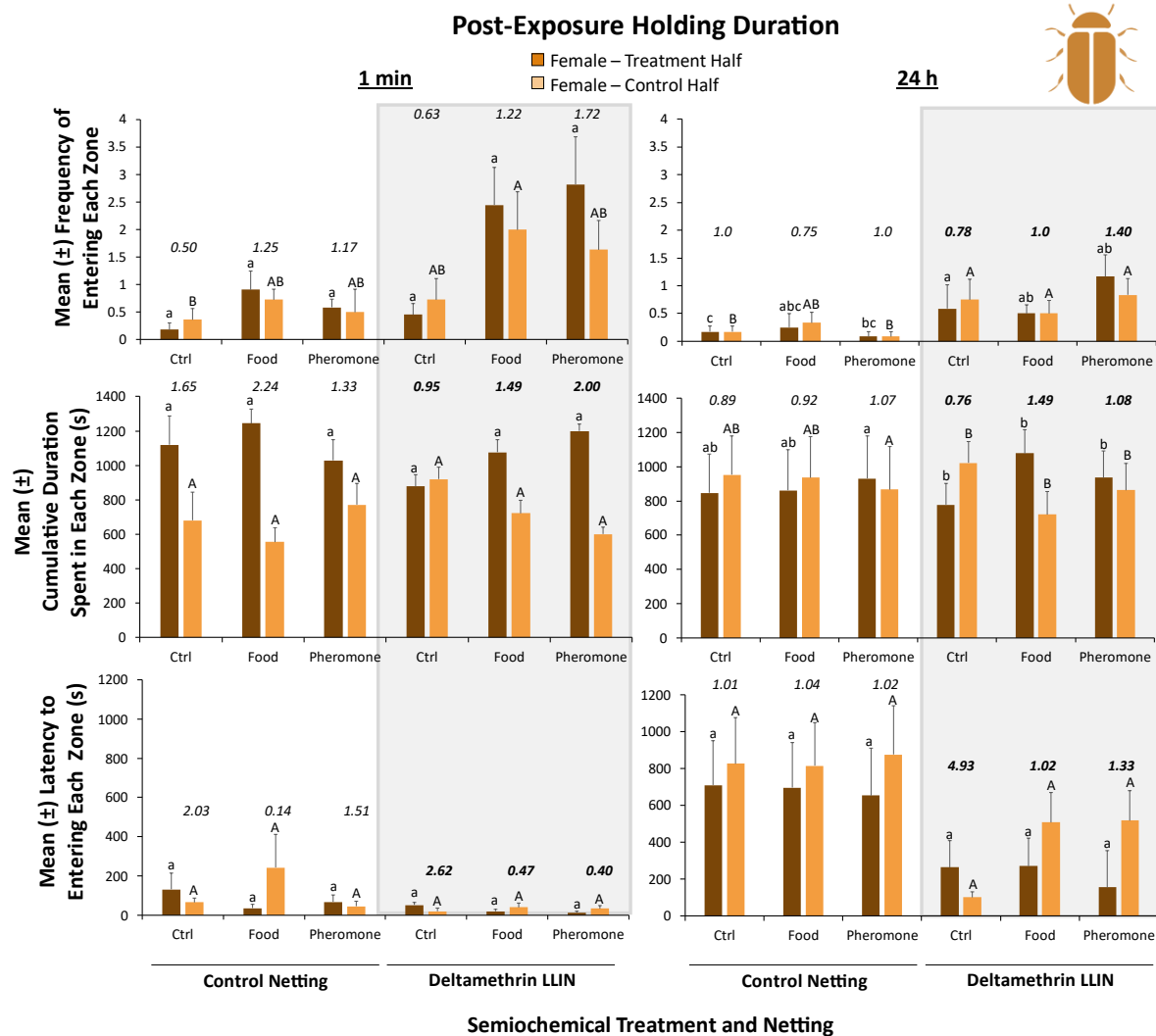


Figure S4-7 Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels), or latency to finding (bottom panels) the treatment half (dark orange) or control half (light orange) of the arena by female *T. inclusum* after 1-min exposure to control netting without insecticide or long-lasting 0.4% deltamethrin-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control half of arena, while lower case letters represent multiple comparisons among treatments in the treatment half of arena. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italacized numbers over bars represent ratio of female *T. inclusum* behavioral response in the treatment:control half of the arenas, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

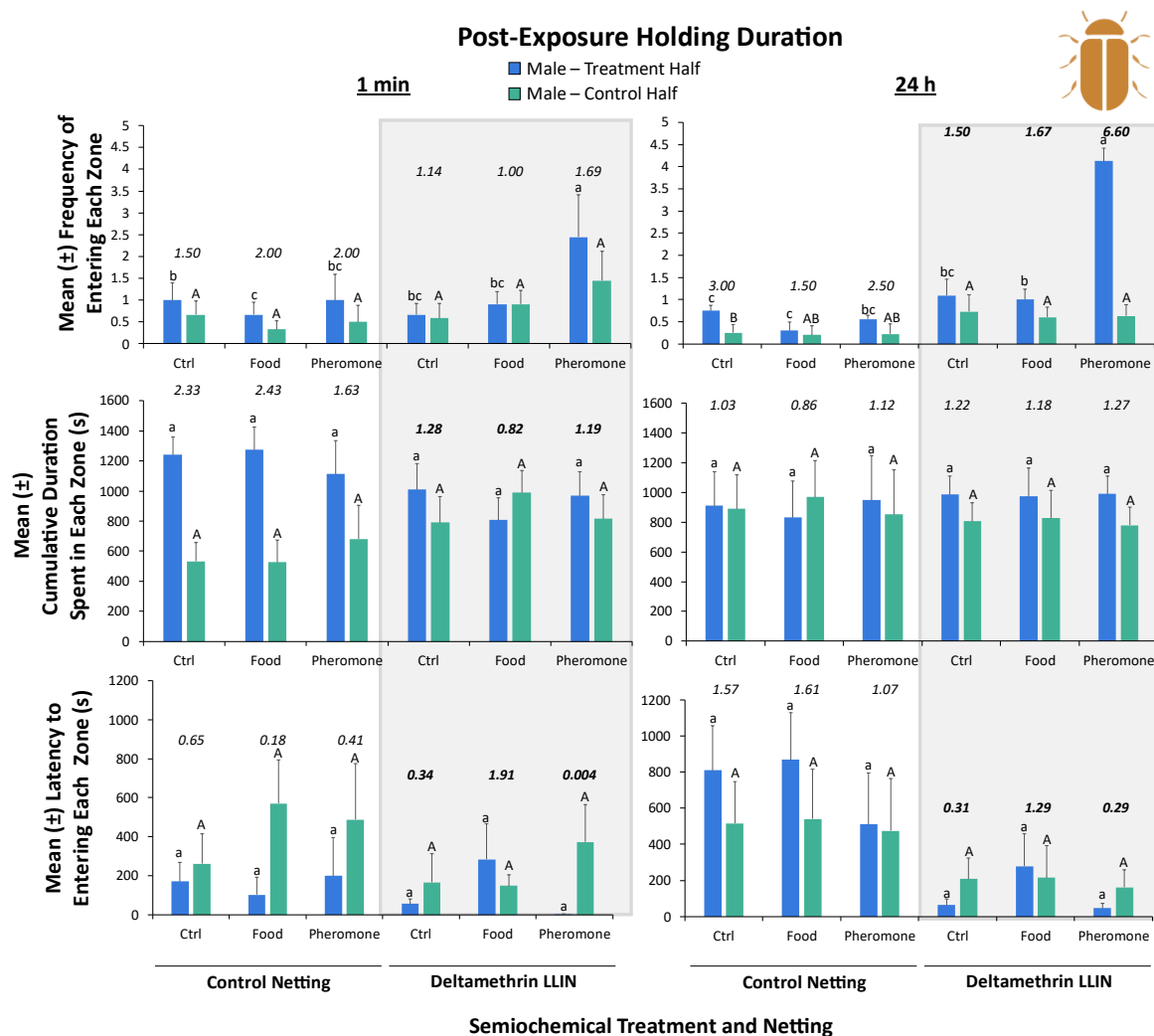


Figure S4-8. Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels), or latency to finding (bottom panels) the treatment half (light blue) or control half (light green) of the arena by male *T. inclusum* after 1-min exposure to control netting without insecticide or long-lasting 0.4% deltamethrin-incorporated netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control half of arena, while lower case letters represent multiple comparisons among treatments in the treatment half of arena. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italicized numbers over bars represent ratio of male *T. inclusum* behavioral response in the treatment:control half of the arenas, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

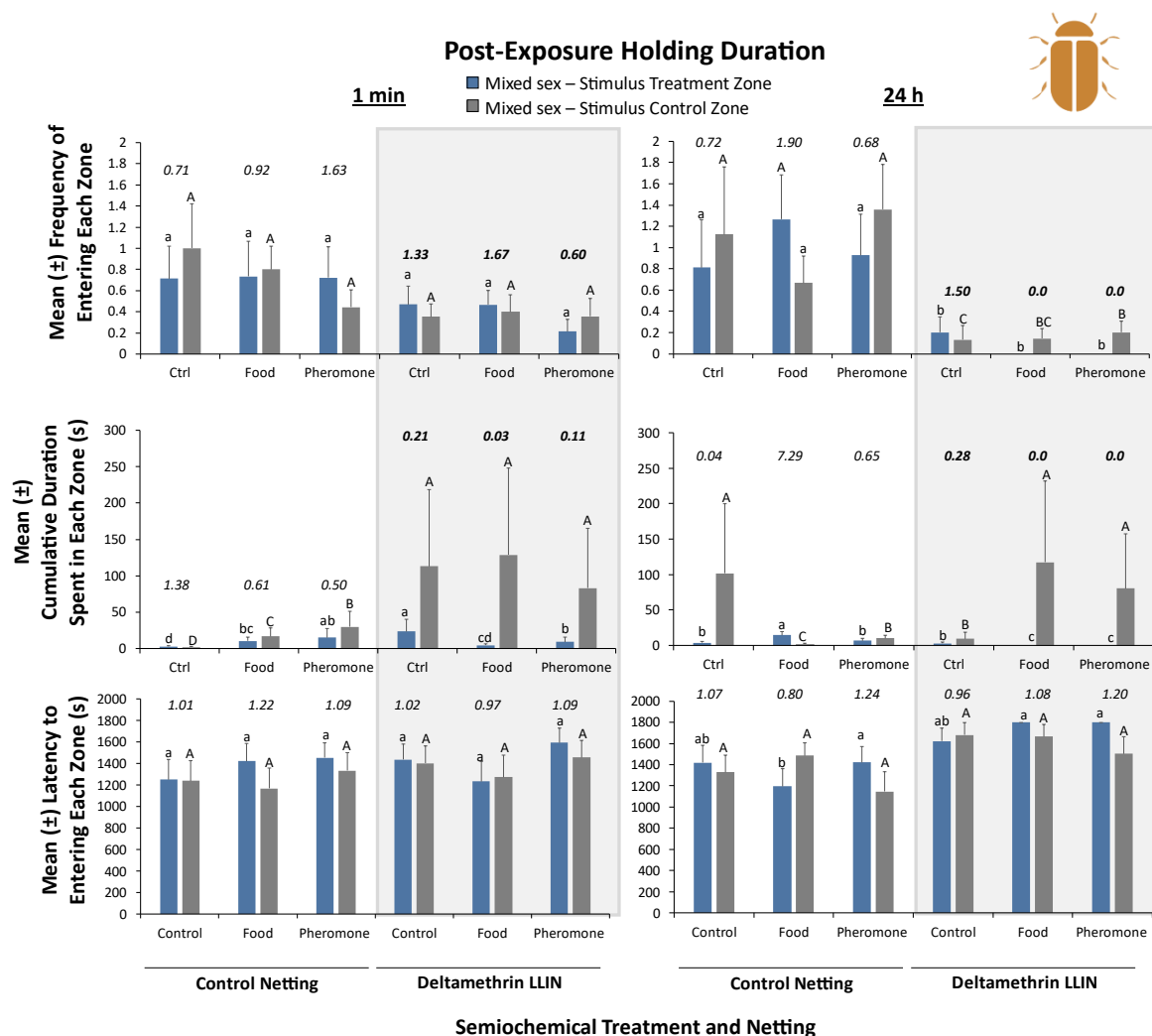


Figure S4-9. Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels) or latency to finding (bottom panels) the treatment stimulus zone (dark blue) or control stimulus zone (dark gray) by mixed-sex populations of *T. inclusum* after 1-min exposure to control netting without insecticide or 0.4% deltamethrin-incorporated long-lasting netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control stimulus zone, while lower case letters represent multiple comparisons among treatments in the treatment stimulus zone. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italicized numbers over bars represent ratio of mixed-sex *T. inclusum* behavioral response in the treatment:control stimulus zones, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

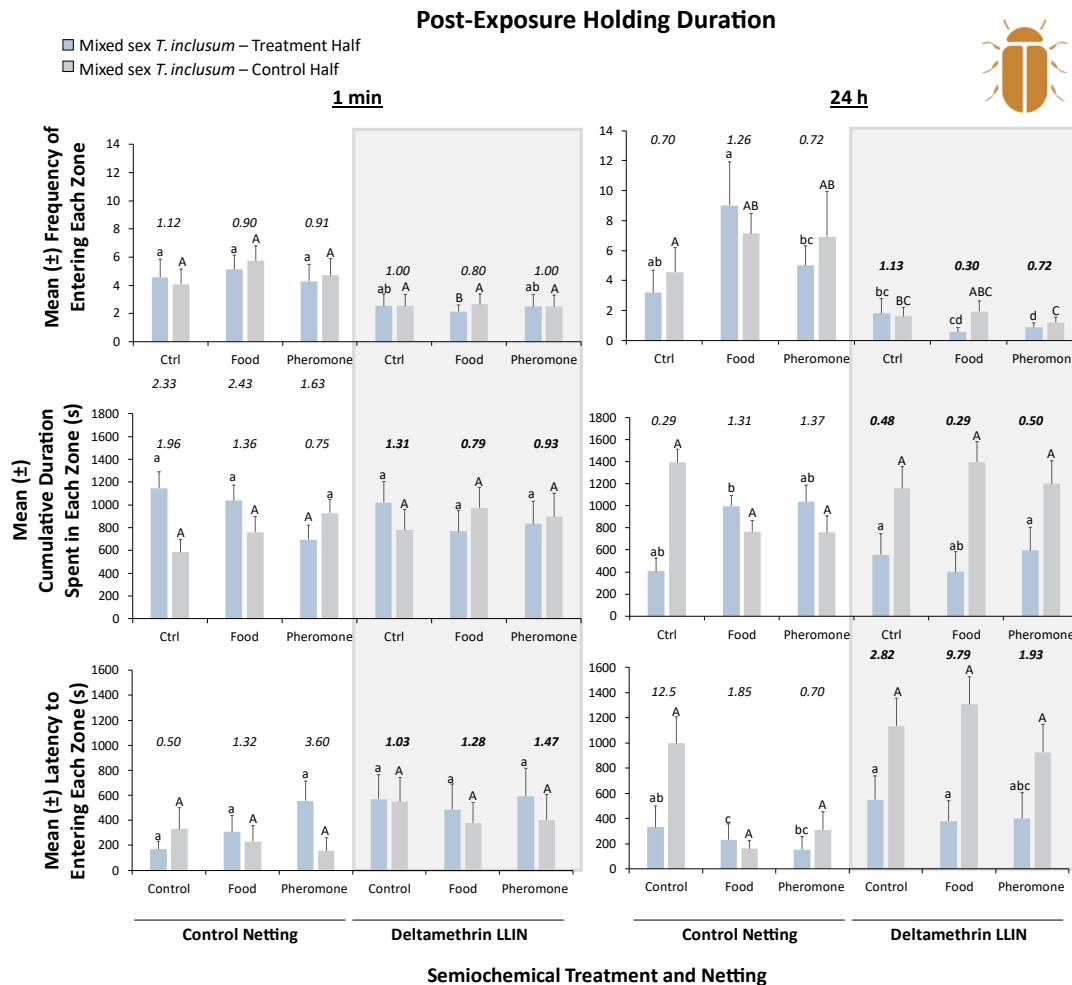


Figure S4-10. Frequency of entering ($\pm$ SE) (top panels), cumulative duration spent in ($\pm$ SE) (middle panels), or latency to finding (bottom panels) the treatment half (light blue) or control half (light gray) of the arena by mixed-sex populations of *T. inclusum* after exposure to control netting without insecticide or long-lasting deltamethrin-incorporated 0.4% netting (LLIN) in the presence of important semiochemicals including food (e.g., 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) or 24-h later (right). Capitalized letters represent multiple comparisons among treatments in control half of arena, while lower case letters represent multiple comparisons among treatments in the treatment half of arena. Bars with shared letters of the same case are not significantly different from each other (Tukey HSD, $\alpha = 0.05$). Shaded grey area represents LLIN-exposed treatments. Italicized numbers over bars represent ratio of mixed-sex *T. inclusum* behavioral response in the treatment:control half of the arenas, and bolded numbers represent significant deviations from patterns found from control-netting exposed individuals (χ^2 -test, $\alpha = 0.05$).

Table S4-1 Summary of statistical model results for the log of the distance moved by mixed-sex dermestids after exposure to 0.4% deltamethrin long-lasting insecticide netting in response to semiochemicals over a 30-min period.

Variable	Distance Moved (ANOVA)			Velocity (ANOVA)		
	Df	F	P	Df	F	P
Mixed sex <i>T.variabile</i>						
treatment	2	1.06	0.35	2	2.01	0.14
netting	1	2.04	0.15	1	0.01	0.94
postexposure	1	0.92	0.34	1	3.16	0.08
treatment:netting	2	0.58	0.56	2	2.93	0.06
treatment:postexposure	2	1.53	0.22	2	3.59	0.03
netting:postexposure	1	0.78	0.38	1	3.23	0.07
treatment:netting:postexposure	2	1.54	0.22	2	1.18	0.31
Residuals	186			186		
Mixed sex <i>T.inclusum</i>						
treatment	2	0.11	0.90	2	0.40	0.67
netting	1	13.53	0.00	1	1.74	0.19
postexposure	1	0.14	0.71	1	0.49	0.48
treatment:netting	2	0.07	0.93	2	0.26	0.77
treatment:postexposure	2	0.21	0.81	2	1.61	0.20
netting:postexposure	1	1.10	0.30	1	0.19	0.67
treatment:netting:postexposure	2	2.02	0.14	2	0.88	0.42
Residuals	170			170		

Table S4-2Summary of statistical model results for the log of frequency of entering into zone/kernel zone by mixed-sex dermestids after exposure to 0.4% deltamethrin long-lasting insecticide in response to important semiochemicals over a 30 -min period.

Variable	Sequential ANOVAs										
	MANOVA			Freq. ¹ of Stimulus Trt Zone		Freq.of Half Trt Zone		Freq. of Stimulus Ctrl Zone		Freq. of Half Ctrl Zone	
	df	F	P	F	P	F	P	F	P	F	P
Mixed sex <i>T.variabile</i>											
treatment	2	1.57	0.18	0.22	0.80	2.52	0.08	0.47	0.62	1.91	0.15
netting	1	20.8	0.0001	18.4	0.0001	34.2	0.0001	43.2	0.0001	34.7	0.0001
postexposure	1	17.3	0.0001	23.2	0.0001	14.9	0.001	43.4	0.0001	16.0	0.0001
treatment:netting	2	3.89	0.001	2.04	0.13	0.82	0.44	4.35	0.01	4.86	0.01
treatment:postexposure	2	2.31	0.06	0.66	0.52	1.21	0.30	2.62	0.08	1.77	0.17
netting:postexposure	1	5.46	0.001	7.70	0.01	8.00	0.01	7.25	0.01	10.9	0.01
treatment:netting:postexposure	2	5.62	0.001	7.03	0.01	0.19	0.83	4.35	0.01	4.65	0.01
Residuals	186										
Mixed sex <i>T.inclusum</i>											
treatment	2	9.00	0.0001	16.2	0.0001	1.91	0.15	3.21	0.04	0.93	0.40
netting	1	71.9	0.0001	194	0.0001	38.1	0.0001	89.5	0.0001	47.7	0.0001
postexposure	1	2.94	0.02	11.4	0.001	0.28	0.60	0.46	0.50	0.24	0.62
treatment:netting	2	9.37	0.0001	18.8	0.0001	0.62	0.54	2.01	0.14	1.72	0.18
treatment:postexposure	2	1.84	0.12	0.99	0.37	1.78	0.17	0.94	0.39	0.11	0.89
netting:postexposure	1	3.94	0.01	14.4	0.001	0.04	0.84	0.77	0.38	0.23	0.63
treatment:netting:postexposure	2	0.64	0.63	0.19	0.83	0.21	0.81	0.32	0.73	0.16	0.85
Residuals	170										

¹Abbreviations: Freq. – Frequency, Trt – Treatment, Ctrl – Control

Table S4-3 Summary of statistical model results for the log of cumulative duration spent in zone/kernel zone by mixed-sex dermestids after exposure to 0.4% deltamethrin long-lasting insecticide in response to important semiochemicals over a 30 -min period.

				Sequential ANOVAs							
				Cum. Duration in Stimulus Trt Zone		Cum. Duration in Half Trt Zone		Cum. Duration in Stimulus Ctrl Zone		Cum. Duration in Half Ctrl Zone	
Variable	df	F	P	F	P	F	P	F	P	F	P
Mixed sex <i>T.variabile</i>											
treatment	2	46.7	0.0001	75.6	0.0001	2.13	0.12	18.5	0.0001	3.88	0.02
netting	1	10.8	0.0001	21.6	0.0001	13.9	0.001	2.98	0.09	19.1	0.0001
postexposure	1	53.2	0.0001	123	0.0001	13.1	0.001	75.9	0.0001	13.5	0.001
treatment:netting	2	21.1	0.0001	33.0	0.0001	1.03	0.36	9.99	0.0001	0.71	0.49
treatment:postexposure	2	2.71	0.03	4.77	0.0001	0.66	0.52	3.08	0.05	1.34	0.26
netting:postexposure	1	29.9	0.0001	119	0.0001	0.13	0.72	0.02	0.88	0.07	0.79
treatment:netting:postexposure	2	25.0	0.0001	44.4	0.0001	0.75	0.47	9.10	0.001	0.37	0.69
Residuals	186										
Mixed sex <i>T.inclusum</i>											
treatment	2	1.55	0.19	1.23	0.29	0.74	0.48	1.77	0.17	0.37	0.69
netting	1	38.0	0.0001	8.13	0.01	12.1	0.00	122	0.0001	25.7	0.0001
postexposure	1	11.6	0.0001	36.8	0.0001	1.01	0.32	4.15	0.04	4.71	0.03
treatment:netting	2	2.70	0.03	3.64	0.03	0.79	0.46	1.18	0.31	0.13	0.88
treatment:postexposure	2	1.57	0.18	1.58	0.21	0.38	0.68	1.55	0.21	0.40	0.67
netting:postexposure	1	3.52	0.01	2.83	0.09	0.85	0.36	2.89	0.09	0.77	0.38
treatment:netting:postexposure	2	1.09	0.36	0.19	0.82	1.56	0.21	0.55	0.58	0.99	0.37
Residuals	170										

1Abbreviations: Cum. – Cumulative, Trt – Treatment, Ctrl – Control

Table S4-4 Summary of statistical model results for a latency to zone/kernel zone by dermestids after exposure to 0.4% deltamethrin long-lasting insecticide in response to important semiochemicals over a 30 -min period

Variable	Df	MANOVA		Sequential ANOVAs							
		F	P	Lat. to Stimulus Trt Zone		Lat. to Half Trt Zone		Lat. to Stimulus Ctrl Zone		Lat. to Half Ctrl Zone	
		F	P	F	P	F	P	F	P	F	P
Mixed sex <i>T.variabile</i>											
treatment	2	2.47	0.05	2.15	0.12	0.77	0.47	0.78	0.46	4.19	0.02
netting	1	15.1	0.0001	15.5	0.001	32.5	0.0001	21.0	0.0001	3.93	0.05
postexposure	1	20.6	0.0001	21.5	0.0001	38.1	0.0001	31.0	0.0001	8.52	0.01
treatment:netting	2	1.04	0.39	0.20	0.82	1.93	0.15	1.07	0.35	0.74	0.48
treatment:postexposure	2	2.46	0.05	1.35	0.26	0.00	1.00	2.00	0.14	2.80	0.06
netting:postexposure	1	9.13	0.0001	1.81	0.18	18.0	0.0001	1.61	0.21	0.90	0.34
treatment:netting:postexposure	2	0.93	0.45	0.23	0.79	0.64	0.53	1.69	0.19	0.39	0.68
Residuals	186										
Mixed sex <i>T.inclusum</i>											
treatment	2	1.99	0.10	1.32	0.27	0.98	0.38	0.12	0.89	1.09	0.34
netting	1	8.08	0.00	6.51	0.01	14.1	0.001	4.39	0.04	4.26	0.04
postexposure	1	3.22	0.01	3.03	0.08	11.3	0.001	2.53	0.11	0.57	0.45
treatment:netting	2	1.11	0.35	0.05	0.95	1.51	0.22	0.19	0.83	0.36	0.70
treatment:postexposure	2	2.41	0.05	0.12	0.88	3.37	0.04	1.58	0.21	1.93	0.15
netting:postexposure	1	1.59	0.18	4.17	0.04	3.97	0.05	0.89	0.35	0.12	0.73
treatment:netting:postexposure	2	2.01	0.10	1.87	0.16	3.01	0.05	0.03	0.97	0.28	0.76
Residuals	170										

1Abbreviations: Lat. – Latency, Trt – Treatment, Ctrl – Control

Appendix C - Supporting information for Chapter 5

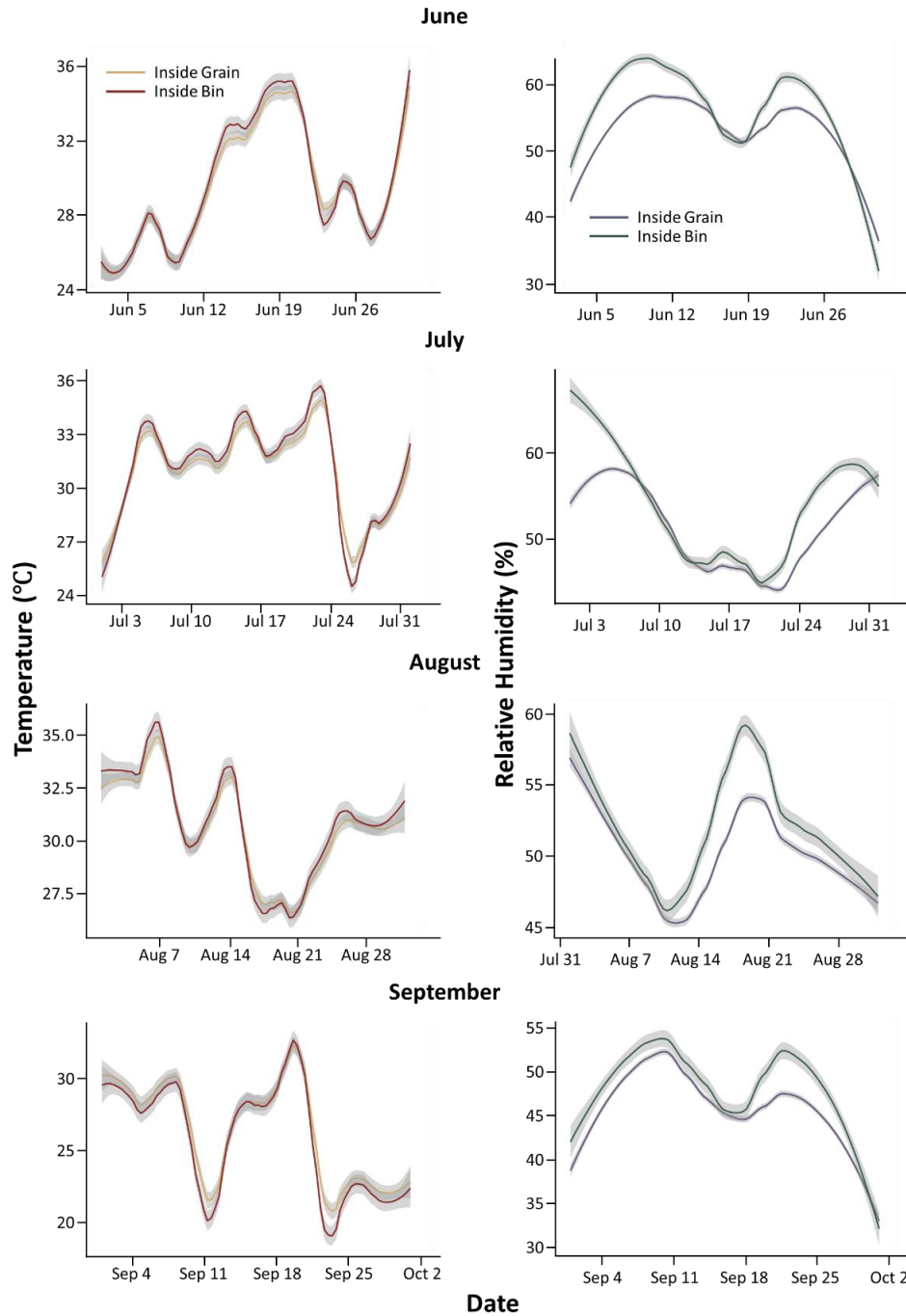


Figure S5-1 Monthly mean temperature (°C) (left column) and relative humidity (%) (right column). Variables were recorded by the data logger inside grain (yellow or purple curve) and inside grain bin (red or green curve) every 10 min from June to September 2022 from three 110-MT capacity grain bins at the USDA-ARS Center for Grain and Animal Health Research in Manhattan, KS.

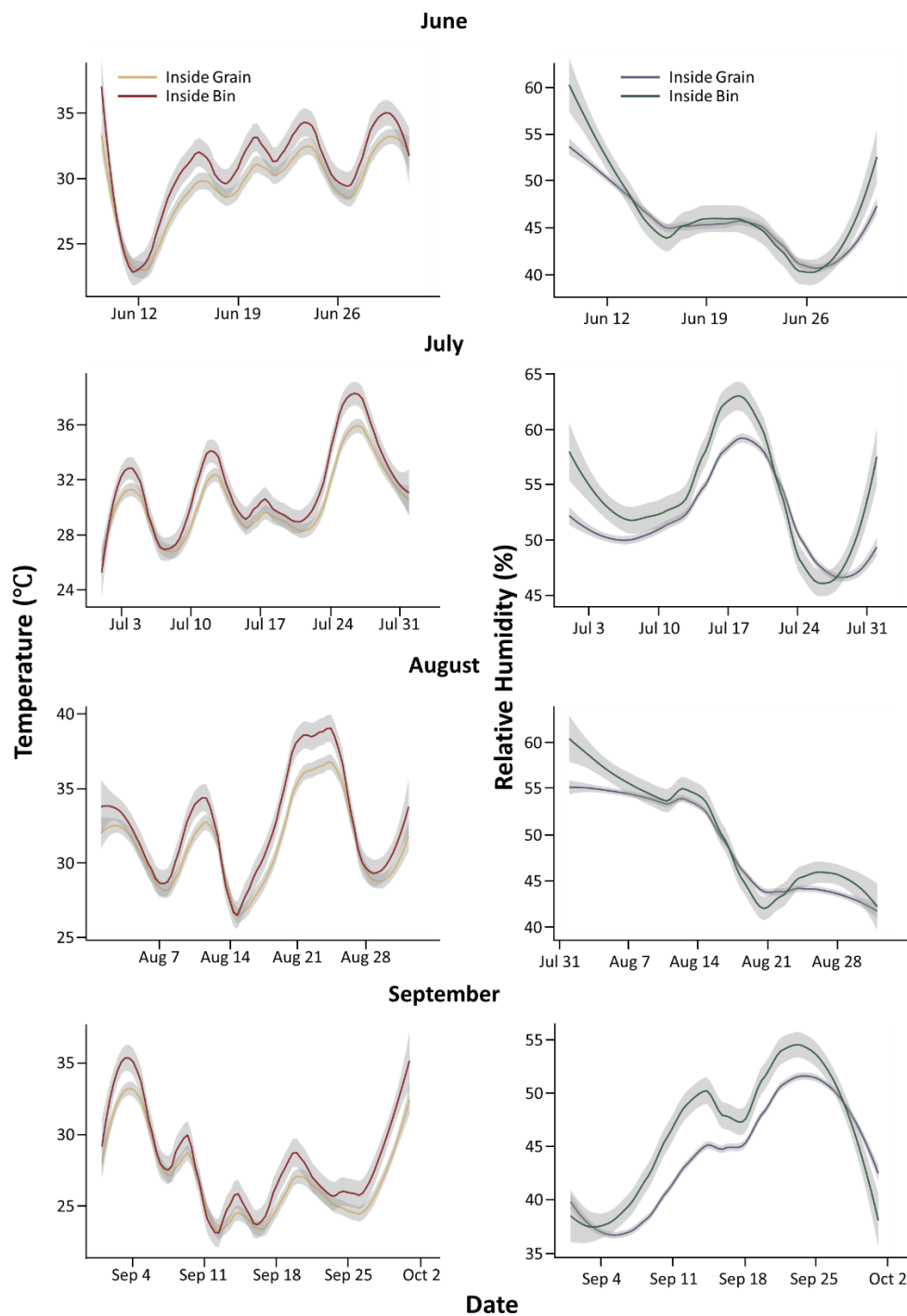


Figure S5-2 Monthly mean temperature (°C) (left column) and relative humidity (%) (right column). Variables were recorded by the data logger inside grain (yellow or purple curve) and inside grain bin (red or green curve) every 10 min from June to September 2023 from three 110-MT capacity grain bins at the USDA-ARS Center for Grain and Animal Health Research in Manhattan, KS.

Appendix D - Voucher specimens

Specimens used in my PhD research are deposited as **voucher number 270** in the KSU Museum of Entomological and Prairie Arthropod Research.