Implementing long-lasting insecticide netting as a tool for diversifying integrated pest management programs of stored product insects

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Abstract

Stored products represent an enormous economic output, but insects regularly immigrate into these stored products from the surrounding landscape throughout the post-harvest supply chain, feed on these products, and cause extensive economic losses. Integrated pest management (IPM) holistically combines multiple management techniques to control an insect within a system. A key component of an IPM program is prevention. Deploying effective prevention strategies is a proactive approach to managing insects prior to them contacting and infesting food facilities and products. Long-lasting insecticide netting (LLIN), which usually contains an incorporated pyrethroid, has been used as part of a strategy to reduce the spread of malaria in tropical regions since the 1990's, and has only recently been considered for its application in pre- and post-harvest agricultural contexts as a preventative IPM tool. The goals of this thesis were to evaluate the behavioral effects of LLIN (0.4% deltamethrin) on the movement and dispersal ability of three stored product insect species: *Tribolium castaneum, Rhyzopertha dominica,* and *Trogoderma variabile* in laboratory tests. Additionally, the efficacy of LLIN in semi-field, release-recapture assays was evaluated by deploying the netting in pilot-scale warehouses alone or within attract-and-kill (AK) traps to intercept insects immigrating into food facilities and attempting to enter commodities. Within the parameters of this thesis, LLIN was found to significantly reduce distance traveled and velocity of all three focal species. The ability of the insects to disperse to a novel food resource after exposure to LLIN was significantly reduced as well. Immature stages of *T. castaneum* and *T. variabile* were also significantly reduced in their movement and dispersal ability after exposure to the netting. Both life stages showed reductions in mobility after short exposure times to the netting, and these effects were long-lasting. In the semi-field experiments, pilot-scale warehouses that deployed LLIN had significantly fewer

infestations and subsequent progeny production inside their commodities compared to warehouses without LLIN. Among the three tested methods of netting deployment, the efficacy of each deployment type was similarly effective. Finally, the efficacy of LLIN was evaluated in combination with another IPM tool, AK traps, which contained a small amount of grain and a commercial lure, all confined within two pieces of LLIN as the kill mechanism. Deploying these tools, alone or together, resulted in significant numbers of affected individuals recaptured inside the warehouse, yet outside of the commodity. Overall, LLIN is a promising tool for diversifying prevention tactics of stored product IPM. Future work should evaluate the performance of the netting in commercial food facilities and with other IPM tactics to create novel management strategies and continue evolving alongside these post-harvest pests.

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Chapter 1 - Background and Objectives

The span of the agricultural postharvest supply chain ranges from harvest at the farm to the end consumer's home. More specifically, postharvest products are moved through farm storage, elevators, processing facilities, warehouses and distribution centers, retail stores, and into consumer pantries. Collectively, these stored products include cereals, grains, legumes, and processed or durable commodities made from these products. This supply chain represents an enormous economic investment, adding up to a value of over $86 billion in the U.S. just for corn, soybean, and wheat (USDA-NASS 2020). In 2019, the U.S. stored 2 and 12 billion bushels of wheat and corn, respectively (USDA-NASS 2019). At every point in the supply chain, however, these commodities are vulnerable to insect infestation and feeding damage, leading to extensive economic losses.

An estimated 20,000 species of insects were found to infest field and stored products, with 60% of those species belonging to the order Coleoptera (Shankar and Abrol 2012). Of these, over 100 species are economically important in the post-harvest supply chain (Hagstrum and Subramanyam 2006). The type of pest infesting a commodity can be partially determined by its feeding patterns. Primary pests feed internally on whole, unprocessed seeds such as wheat kernels. On the other hand, secondary pests typically can feed only externally on broken or damaged seeds and on seeds that have been milled or processed. Knowledge of these life histories is crucial to consider when developing an integrated pest management program.

One extreme of this life history spectrum is represented by the red flour beetle (*Tribolium castaneum* (Herbst)) (Coleoptera: Tenebrionidae), a cosmopolitan, secondary stored product pest. These beetles and their larvae feed externally on both fragmented and processed products and are long-lived, causing long-term damage if left unmanaged (Phillips and Throne 2010;

Hagstrum and Subramanyam 2006). Adults can live for a few months or several years, depending on ambient temperatures, and adults can reproduce throughout their entire lifespan (Hagstrum et al. 2012).

The warehouse beetle (*Trogoderma variabile* Ballion) (Coleoptera: Dermestidae) is another secondary pest, and feeds on a variety of products, including durable commodities at mills and processing plants (Ghimire et al. 2016), and non-food items such as fabrics, furs, and preserved museum or insect specimens (Hagstrum et al. 2012). Like other dermestid species, *T. variabile* causes the most damage during its larval stage of development (Hagstrum et al. 2012). The adult stage of this beetle is shorter than red flour beetle, with the insect spending the bulk of its life stage in its larval form (Partida and Strong 1975). The larval stage is particularly problematic; larvae diapause if conditions are unfavorable so they can persist for a long time without food, and larvae also have irritating setae left in castings after molting (Ghimire et al. 2016), which can cause serious respiratory and gastric irritation if left in food and accidentally ingested (Wegner 2008). Currently available chemical control methods often work less well for dermestids than they do for other groups of stored product insects, so infestations are often difficult to control (Scheff et al. 2016; Campbell and Arbogast 2004).

On the other end of the life history spectrum is the lesser grain borer (*Rhyzopertha dominica* Fabricius) (Coleoptera: Bostrichidae), a primary pest feeding directly on whole, stable gains rather than damaged or processed products that secondary pests thrive on. The adults and larvae cause extensive damage with larvae internally feeding on products such as wheat, corn, or rice (Phillips and Throne 2010). These beetles disperse well by flight and can potentially colonize commodities separated by great distances (Edde 2012). Females can lay up to 500 eggs in their lifetime with the eggs ranging in development time from 32 days (Edde 2012) at 18.1°C to as little as 5 days at 36°C (Hagstrum et al. 2012).

Globally, insect feeding and damage accounts for approximately $100 billion USD in postharvest losses, thus, developing an effective management strategy to reduce these economic losses is crucial (Wacker 2018). Stored product integrated pest management (IPM) ideally attempts to holistically integrate different management tactics to control insects throughout the post-harvest supply chain. However, fumigation is the most common chemical control tactic once insects have entered and infested commodities, with food facilities and bulk storage routinely fumigated every year. Methyl bromide, historically one of the most common fumigants used for structural treatments, was banned in 2005 under the Montreal Protocol since it is an ozone-depleting substance (Fields and White 2002). Phosphine remains widely used as a fumigant for commodity treatments, but insects are becoming increasingly resistant to this chemical (Huang et al. 2018; Opit et al. 2012; Schlipalius et al. 2018; Zhao et al. 2015). Fumigants don’t have residual activity and are therefore only effective in treating established infestations. Because of the issues with using fumigants and increasing demand for organic or low- to no-insecticide products by the consumer (Batte et al. 2007), there is a drive in stored product IPM to increase the efficiency of preventative management tactics to avoid insect infestations and reduce the need for remedial chemical control tactics.

One tool that can be used to bolster preventative IPM programs is long-lasting insecticide-incorporated netting (LLIN, hereafter). LLIN is a mesh netting made of polythene material, with the incorporation of the pyrethroid, deltamethrin at 0.4% (Vestergaard-Frandsen, Inc.). The LLIN usually has multi-year efficacy against insects in harsh environments (Martin et al. 2007). While LLIN is a relatively novel tool in agriculture, it has been used in tropical regions to control mosquitoes and the spread of malaria since the 1990's (Alonso et al. 1991). In the past few years, this technology has been applied in a monitoring trap in tree fruit and evaluated for reducing nuisance issues associated with home invasion by insects in the fall (Bergh and Quinn 2018; Kuhar et al. 2017). This technology has only recently been studied for management of insect pests in crops after harvest (Athanassiou et al. 2019; Morrison et al. 2018; Paloukas et al. 2020; Rumbos et al. 2018; Wilkins et al. 2020). While the netting does not create a physical barrier for stored product insects, deltamethrin is continuously brought to the netting's surface, exposing any insect that contacts the netting to the insecticide (Martin et al. 2007). One focus of this thesis is to evaluate the effectiveness of LLIN to prevent stored product insects from dispersing to and colonizing commodities. Studying the behavioral compatibility of the LLIN for the three stored product species above with their varying life histories will reveal how effective LLIN is against stored product pests.

In addition to functioning as a chemical barrier to the immigration of stored product insects, LLIN can directly kill the insects in an attract-and-kill device. Attract-and-kill (AK) is an IPM tactic that attempts to control pest populations by using an attractant to modify an insect's behavior and subsequently lure the insect with an attractant to a killing mechanism (El-sayed et al. 2009; reviewed in Gregg et al. 2018). For example, LLINs were used in AK traps or baited trees to control *Halyomorpha halys* (Stål) in pear orchards (Giuseppino et al. 2018) and apple orchards (Morrison et al. 2016b, 2019c), respectively. Coupling an attractant with the LLIN could result in greater exposure times to the netting due to repeated dosing by insects orienting to the attractive stimuli, and thus higher rates of mortality. Multiple exposures have been shown to have a similar effect on stored product insects as single, longer exposures (Gerken et al. 2020).

For an AK device to be successful, the chosen attractant must produce the greatest 1) attraction to, and 2) capture by a trap from a large suite of stored product insects in an area. Types of attractants for these devices vary depending on the species of insect that requires management. Stimuli in AK programs have included pheromones (Morrison et al. 2016a; Giuseppino et al. 2018), host or plant volatiles (reviewed in Gregg et al. 2018; Morrison et al. 2016b; Wallingford et al. 2018), sound (Laumann et al. 2017), and visual stimuli (Rice et al. 2017; Giuseppino et al. 2018). Probably one of the most important categories in AK programs are pheromones, which are chemical signals produced by individuals of a species and serve to communicate information to other conspecifics. Aggregation pheromones usually bring individuals of each sex and sometimes immature stages together in large numbers for reproduction, host-finding, feeding, and other purposes (reviewed in Wertheim et al. 2005), and are characterized by attraction to an area and not a point source emission. Aggregation pheromones are likely to be the most useful for an AK device.

Prior work has demonstrated that the male-produced aggregation pheromone is 4,8-dimethyldecanal in *T. castaneum* (Boake and Wade 1984) which attracts both sexes. A synthetic form of 4,8-dimethyldecanal is used in current commercial lures. The degree of attraction to this synthetic pheromone can be both concentration and species-dependent among closely related taxa (Boake and Wade 1984). Two different stereoisomers make up the aggregation pheromone in *R. dominica*. In particular, these are (*S*)-(+)-1-methylbutyl (*E*)-2-methyl-2-pentenoate and (*S*)-(+)-1-methylbutyl (*E*)-2,4-dimethyl-2-pentenoate, but are commonly referred to by the names dominicalure 1 and dominicalure 2 in the primary literature (Williams et al. 1981). These commercially available lures contain male-produced pheromones and are used to attract both male and female *R. dominica* to an area (Williams et al. 1981).

Kairomones are chemical signals that are produced by one species but used by another species to the benefit of the species responding to the cue. Many commercial traps for stored product insects use oils typically derived from grains as kairomone attractants (e.g. Morrison et al. 2020). These can be used as both an attractant and a mechanism for killing insects in the traps (Campbell et al. 2002; Phillips and Throne 2010). Both kairomones and aggregation pheromones are good candidates to pair with LLIN and to create an AK trap.

The following chapters evaluate LLIN and AK-based interception traps as novel tools for stored product IPM programs at food facilities. First, the movement and dispersal ability of three key stored product insects—*Tribolium castaneum*, *Rhyzopertha dominica*, and *Trogoderma variabile*—were studied after exposure to LLIN. Second, the movement and dispersal ability of immature *T. castaneum* and *T. variabile* were evaluated after exposure to LLIN to understand the effects of LLIN among life stages. Third, optimal stimuli at different doses were evaluated for inclusion in an interception trap using wind tunnel and release-recapture assays. Fourth, an interception trap with optimal stimuli was assessed over two years as a proof-of-concept in capturing a variety of stored product insects at commercial food facilities. Finally, semi-field studies were conducted in pilot scale warehouses to evaluate the efficacy of LLIN deployment methods, and to understand the efficacy of LLIN when used alone or in combination with AK-based interception traps. Specimens used in this research are deposited as voucher number 261 in the Kansas State University Museum of Entomological and Prairie Arthropod Research.

Chapter 2 - Mobility of adult *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) after exposure to long-lasting insecticide-incorporated netting

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Introduction

The major three stored grains (corn, soybean, and wheat) in the US alone represent a value of $85.9 billion (USDA-NASS 2018), and as they are processed into different food products, the economic value increases. The key to integrated pest management (IPM) programs for food facilities that handle grain and grain-based products is prevention (Phillips and Throne 2010). Prevention of insect infestation is important for food facilities because of the cost associated with disinfestation once insects enter a facility and the risks associated with infestation of the finished product (Hagstrum and Subramanyam 2006). Common preventative tactics include sealing cracks and other routes of entry into a food facility, keeping doors and windows closed, and screening all openings. While screening can be effective, the small size openings needed for preventing insects from passing through often does not allow adequate air flow and can easily be clogged with food dust (Phillips and Throne 2010). Prevention is often difficult because of the abundance of insects outside food facilities, food accumulations present in and around anthropogenic structures, and natural refugia (Dowdy and McGaughey 1998). The difficulty can be further intensified by the flight activity of stored product insects around food facilities and their success in immigrating to new facilities (Campbell and Arbogast 2004, McKay et al. 2017), with documented dispersal distance of at least 1.6 km (Mahroof et al. 2010), though this is probably much greater. Additionally, transportation of insect-infested products into a facility (Campbell and Arthur 2007) can further complicate matters. Concurrently, there has been an increasing demand for organic or low insecticide-input products by consumers, even for multi-ingredient processed products (Batte et al. 2007). As a consequence, new management tools that help prevent infestation of commodities and structures with insects while reducing insecticide inputs are highly desirable.

One potential new tool to reduce insect infestation is long-lasting insecticide incorporated netting (LLIN). These fine mesh nets are usually formulated with a pyrethroid insecticide such as permethrin or deltamethrin, and can be effective for up to a year or even longer (Rozendaal 1997; Martin et al. 2007). Since the mid-1990s, LLINs have been employed in tropical and subtropical regions of the world to control mosquitoes and reduce the spread of malaria (Alonso et al. 1991, Lengeler 2004, Kitchen et al. 2009) and to kill vectors of other arthropod-borne diseases (Dutta et al. 2011). This strategy has also been used to protect livestock against arthropod-borne diseases, such as suppressing the abundance of the bluetongue virus vector *Culicoides imicola* Kieffer (Diptera: Ceratopogonidae) among cattle in sheds (Calvete et al. 2010). Unlike the early versions of the nets, which required repeated treatment with insecticides over time, LLINs are constructed such that insecticide moves to the surface of the netting material over time, producing multi-year residual efficacy (Martin et al. 2007). In the past few years, LLINs have been evaluated for their utility in protecting crops before harvest in agriculture. For example, in squash ecosystems, LLINs were used successfully to exclude aphids and decrease the spread of Cucumber mosaic virus and Cucurbit aphid-borne yellows virus (Dáder et al. 2015). LLINs are also being evaluated as a potential tool in the management of invasive *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) to directly kill the insect and for use as a killing agent in a monitoring trap (Kuhar et al. 2017, Morrison III et al. 2017). Most recently, LLINs are being considered for their ability to control post-harvest insects (Scheff et al. 2018). However, Scheff et al. (2018) found that very long exposure times to the LLIN were required to induce mortality and knockdown, thus it remains uncertain whether brief bouts of exposure to LLINs will be sufficient to prevent dispersal of insects to commodities. Additionally, another challenge with currently available LLINs is that they do not provide a substantial physical barrier because most stored product insects are small enough to pass through the netting material. As a result, the question remains as to whether exposure to the netting will have sufficiently pronounced effects on the behavior of stored product insects to reduce their dispersal after contact and thus serve as an effective barrier to insect immigration.

There are over 100 species of insects that attack products in food facilities, with a diverse set of life histories (Hagstrum and Subramanyam 2006). However, two vastly different life histories are represented by *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Both species are widely distributed and economically important post-harvest insects: *T. castaneum* in mills and *R. dominica* in bulk stored grain. *Tribolium castaneum* is a secondary feeder (Hagstrum and Subramanyam 2006), feeding on already broken grain, a relatively weaker flier, and mostly confined to facilities and local areas around which grain is processed (Drury et al. 2009, Ridley et al. 2011). By contrast, *R. dominica* is a primary feeder, with larvae feeding and developing on kernels internally (Hagstrum and Subramanyam 2006), while also being a strong flier (Edde and Phillips 2006) and long-distance disperser (Mahroof et al. 2010). If LLIN can be shown to be behaviorally compatible for two species with such divergent life histories, it is probable that LLIN will be a promising technology against a variety of other stored product insects.

Exposure to insecticides, whether from LLIN or in other contexts, not only have direct lethal effects, but may have sublethal effects on the behavior of insects. Sublethal effects may consist of alterations in host-finding, mating behavior, feeding behavior, dispersal ability, and locomotion (Haynes 1988, Desneux et al. 2007). Especially important to consider for potentially immigrating stored product insects is locomotory behavior and dispersal ability. Prior work has documented both increased locomotion after contact with insecticides, for example with exposure of *Triatoma infestans* (Hemiptera: Reduviidae) to films of deltamethrin (Alzogaray et al. 1997), and decreased horizontal movement, vertical climbing, and flight capacity in *H. halys* after brief exposure to insecticides (Morrison III et al. 2017). In an insecticide-susceptible strain of the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), movement decreased when adults were in deltamethrin-treated filter paper-lined plastic arenas (Guedes et al. 2009). However, the behavioral effects of insecticides on stored product insects have been neglected in the past (Guedes et al. 2011), despite their importance to evaluating overall insecticide efficacy (Boyer et al. 2012).

For LLINs to be an effective control measure, they must be compatible with the biology and behavior of stored product insects. Pyrethroids, which are the active ingredient in many LLINs, may have deleterious behavioral side effects in some arthropods, such as repellency (Katz et al. 2008). This would prevent the use of LLIN from effectively intercepting pests as they immigrate to stored product facilities. However, Scheff et al. (2018) importantly found no evidence of long-distance or contact repellency from LLIN against *T. castaneum* and *R. dominica*. Nonetheless, there are several other considerations that must be met for LLIN to be behaviorally compatible with stored product pests and be potentially effective as a control tactic. Most importantly, LLIN must ultimately reduce dispersal by stored product insects to new food patches after brief contact with the material. Therefore, the objectives of this study were to 1) evaluate changes in locomotor behavior for *T. castaneum* and *R. dominica* after varying exposure times to LLIN and post-exposure holding durations, and 2) elucidate whether brief contact with LLIN prevents dispersal of both species.

Materials and Methods

Source Insects

For each assay, 4- to 8-week-old adult *T. castaneum* (field-derived colony from Hudson, KS in 2012) and *R. dominica* (field-derived colony from outside a mill in Russell, KS in 2012) were used. *Tribolium castaneum* was continuously reared on 95% unbleached, organic flour with 5% brewer's yeast added, while *R. dominica* was reared on organic rice, and both were held in an environmental chamber set at 27.5˚C, 60% RH, and 14:10 L:D photoperiod. All individuals were starved for 24 h prior to use.

Movement Assay

To understand the sublethal effects of netting exposure on the locomotion of *T. castaneum* and *R. dominica*, the following 3-way full factorial assay was used. Mixed-sex adult beetles were exposed to long-lasting insecticide polyethylene screen netting (D-Terrence, Vestergaard Inc., Lausanne, Switzerland) incorporated with 0.4% deltamethrin or control netting that had identical physical properties but lacked insecticide. Adults were exposed in sets of three for 1, 5, or 10 min intervals in 24 × 24 cm square Petri dishes with the netting secured on the bottom of the dish with tape. After exposure, adults were tested immediately, or had a post-exposure holding duration of 24, 72, or 168 h individually in plastic containers (4 × 4 cm H:D) under the same environmental chamber conditions as the colonies (see section above), but held without supplemental food. Movement of adults was tracked in six individual Petri dishes (90 × 15 mm D:H) with a piece of filter paper (85 mm D, Grade 1, GE Healthcare, Buckinghamshire, UK) lining the bottom for 2 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 × 30 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. 10.0.828, Noldus Inc., Leesburg, VA, USA). The program was used to calculate the total distance moved (cm) and the mean angular velocity (deg/s) over the 2-h period for each adult. Angular velocity was tracked because it is a measure of how erratic movements are for individuals, with higher angular velocity indicating less directed, more erratic movements. Each adult was considered a replicate and was never used more than once. The condition of each adult was recorded as either alive (normal movement speed and activity), affected (sluggish movements, or on back with legs twitching), or dead (motionless even after prodding) according to the definitions in (Morrison III et al. 2017b). A total of 18 replicates were performed per treatment combination, and a total of 432 adults were tested per species.

For all analyses of movement after netting exposure, only those adults that were recorded as alive or affected at the end of the trials were used in the analysis. To analyze the data from the movement assay, two 4-way ANOVAs with the same form were used per species, one for the total distance moved and another for the mean angular velocity over the 2-h period. There were four fixed explanatory variables: presence of insecticide in netting (LLIN or control netting), exposure time (1, 5, or 10 min), and post-exposure holding duration (0, 24, 72, or 168 h), and sex (male or female, for *T. castaneum* only). The second and third order interactions between the first explanatory three variables were also represented in the model. The model for *R. dominica* did not include sex as a variable, because the sex of adult *R. dominica* beetles are not possible to differentiate (see Edde 2012). Because the data did not fulfill the assumptions of normality, they were log-transformed, and inspection of residuals confirmed that assumptions were subsequently fulfilled. Upon a significant result from the ANOVA, pairwise comparisons were performed with Tukey's HSD. For this and all other tests, R Software (R Core Team, 2017) was used, with $\alpha = 0.05$ except where otherwise noted.

The associated mortality data for the movement assay was analyzed with logistic regression, with condition of the beetle (alive, or affected + dead) treated as the response variable. Fixed, explanatory factors in the model included the same as those above, with the exception of sex. Because of over-dispersion in the dataset, a quasi-poisson distribution was used to model the data. Tests for significance were conducted using a log-likelihood test based on a χ^2-distribution. Upon a significant result from the test, pairwise comparisons were performed with χ^2-tests using a Bonferroni correction to the *P*-value. Finally, to assess if exposure to insecticide netting started to impair mobility during an Ethovision trial, the initial 2-h Ethovision trials were split up into four, 30-min intervals (0–30 min, 30–60 min, 60–90 min, 90–120 min), and the explanatory factors listed above for the model were collapsed into the following four categories: 1) immediate exposure (adults immediately run after exposure), and LLIN-exposed, 2) immediate exposure, and control netting-exposed, 3) non-immediate exposure (adults run 24, 72, and 168 h later) and LLIN-exposed, and 4) non-immediate exposure and control netting-exposed. These data were graphed for both species, and post-hoc contrasts using Fisher's LSD and Bonferroni correction to *P*-values was performed for treatments at 0–30 and 90–120 min for distance moved and angular velocity.

Dispersal Assay

In order to evaluate whether *T. castaneum* and *R. dominica* can reach new food patches after exposure to netting, we employed a dispersal assay modified from (Arnold et al. 2017). The dispersal apparatus consisted of an introduction container with no food material (5 × 6.5 cm D:H), PVC pipe (5 mm I.D.) of variable lengths, and a container with 20 g of unbleached, organic flour for *T. castaneum* or rice for *R. dominica* that represented a "new" food patch. The two containers were fixed at a spacing of 25, 75, or 175 cm apart using a piece of wood (5 x 10 cm H:W) with recessed seating for the containers. A single piece of cotton twine was threaded through the apparatus for the beetles to walk along, with one end contacting the bottom of the introduction chamber and the other end terminating halfway into the container with food so that there was no return movement once beetles had committed to dispersing to the new food patch. Adult beetles were exposed to either LLINs or control netting for 5 min as described above, and then held post-exposure for 1 min, 10 min, or 24 h before being placed in sets of 20 single-sex (*T. castaneum*) or mixed sex adults (*R. dominica*) in the introduction container of the apparatus. Adults were given 48 h to disperse to the new food patch in an environmental chamber set at 30˚C, 65% RH, and 14:10 L:D photoperiod. At the conclusion of the trial, the number of adults reaching the new food patch were counted. As above, the condition of each adult was recorded as either alive, affected, or dead. In total, there were 12–18 replicates per treatment combination, with a total of 218 separate runs.

For the dispersal assay, a 3-way ANVOA was used with the number of adults reaching the new food patch (dispersal cup) as the response variable. Each model used the dispersal distance (25, 75, and 175 cm), the post-exposure holding duration (1 min, 10 min, or 24 h), and sex (male or female) as fixed, explanatory variables. As above, the model for *R. dominica* did not use sex as an explanatory variable because of difficulty in sexing adult beetles. Residuals were inspected to confirm assumptions of normality and homogeneity of variances was fulfilled, and no transformation was deemed necessary. Upon a significant result from the ANOVA, Tukey's HSD was used for pairwise comparisons. The associated mortality data was analyzed with a logistic regression using the same form as for the ANOVA above, and with the same procedure as the mortality for the movement assay.

Results

Movement Assay: T. castaneum

In total, 864 h of movement was recorded for *T. castaneum*. Exposure to LLINs significantly decreased the distance moved by *T. castaneum* (F = 102; df = 1, 404; P < 0.0001) over 3-fold compared with exposure to control netting (Fig. 2-1). The exposure time did not significantly affect the distance moved by *T. castaneum* (F = 0.073; df = 1, 404; P < 0.93). There was no significant interaction between exposure time and presence of insecticide in the netting (F = 2.05; df = 2, 404; P = 0.13). The post-exposure holding duration, however, significantly altered the distance moved by *T. castaneum* (F = 17.0; df = 3, 404; P < 0.0001), with adults moving twice as much when immediately assessed compared with 168 h later. In addition, there was a quantitative interaction between presence of insecticide in the netting and holding duration (F = 17.6; df = 3, 404; P < 0.0001), with a greater reduction in distance moved at 24 and 72 h post-exposure than immediately or 168 h after. Importantly, exposure to LLIN significantly reduced the movement of adults by two- to nine-fold compared with exposure to control netting regardless of holding duration (Fig. 2-1). The interaction between exposure time and holding duration was not significant (*F* = 1.51; df = 6, 404; *P* = 0.18), nor was the three-way interaction that also included presence of insecticide in the netting (*F* = 1.26; df = 6, 404; *P* = 0.28). There was no significant difference in the movement of females and males after netting exposure (*F* = 2.38; df = 1, 404; *P* = 0.20), with a mean ± SE distance moved of 1817 ± 104 cm and 1963 ± 316 cm after exposure to control netting, respectively, which decreased to 514 ± 48 cm and 625 ± 80 cm after exposure to LLIN. Over the course of the 2-h trial, the distance moved by *T. castaneum* adults immediately after exposure to LLIN decreased from being equivalent to the distance moved by the controls to statistically indistinguishable from the distance moved by adults exposed to the netting after a post-exposure holding duration of 24–168 h (Fig. 2-2, post-hoc contrasts at 0–30 and 90–120 min), which is an over 5-fold reduction.

The angular velocity of adult *T. castaneum* was also affected by exposure to LLIN (*F* = 289; df = 1, 404; *P* < 0.0001; Fig. 2-3), which resulted in a 1.5-fold increased angular velocity compared with control netting. In addition, exposure time had a small but significant effect on the angular velocity of adults (*F* = 3.87; df = 2, 404; *P* < 0.05), with the angular velocity of adults about 100 deg/s greater after 10 min exposures compared with 1 min exposures. There was also a significant quantitative interaction between presence of insecticide in netting and exposure time (*F* = 6.69; df = 2, 404; *P* < 0.01), with the angular velocity over 250 deg/s greater after exposure to LLIN for 10 min compared to 1 min, while there was a uniform angular velocity of between 1089–1129 deg/s after exposure to control netting regardless of specific exposure time (Fig. 2-3). The post-exposure holding duration significantly affected the angular velocity (*F* = 17.1; df = 3, 404; *P* < 0.0001), with the angular velocity over 300 deg/s greater after examining adults 168 h later compared with immediately after applying the treatments (Fig. 2-3). While there was not a significant interaction between the exposure time and the holding duration ($F = 0.440$; df = 6, 404; $P = 0.85$), there was a significant interaction between the presence of insecticide and holding duration ($F = 3.50$; df = 3, 404; $P < 0.05$). The 3-way interaction was not significant ($F = 0.295$; df = 6, 404; $P = 0.94$). At the beginning of the 2-h trials, the angular velocity exhibited by adult *T. castaneum* immediately after exposure to LLIN was statistically indistinguishable from the controls, but significantly less than for adults in longer post-holding conditions. However, the angular velocity for the immediately-exposed cohort rapidly rose until it was statistically similar to the adults exposed to LLIN after 24, 72, and 168 h (Fig 2-4, post-hoc contrasts).

Exposure to LLIN significantly affected the mortality of adult *T. castaneum* ($\chi^2 = 755$; df = 1; $P < 0.0001$; Fig. 2-5). Overall, about 13% and 54% of the adults exposed to LLIN were alive or affected at the end of the 2-h trials, whereas 99% and 0% of the adults exposed to control netting were alive or affected. Mortality of adults was similar across the exposure times ($\chi^2 = 2.10$; df = 2; $P = 0.35$), but there was a significant interaction of exposure time and presence of insecticide on mortality ($\chi^2 = 22.9$; df = 2; $P < 0.0001$). In particular, the number of alive adults was 5–14 times greater for control netting-exposed compared with LLIN-exposed beetles (Fig. 2-4). Adult mortality was significantly affected by the post-exposure holding duration ($\chi^2 = 17.9$; df = 3; $P < 0.001$; Fig. 2-5). Specifically, there was a significant interaction with the presence of insecticide ($\chi^2 = 167$; df = 3; $P < 0.0001$), such that there was some low amount of recovery after 72 and 168 h, but not at earlier time points (Fig. 2-5). However, after a week, 66.7% of the adults exposed to LLINs were still considered affected or dead, compared to 0% of the control beetles. Finally, there was no significant interaction between holding duration and exposure time ($\chi^2 =$

0.27; df = 6; *P* = 0.99), or the 3-way interaction among holding time, exposure time, and presence of insecticide on the distance moved by *T. castaneum* (χ^2 = 0.73; df = 6; *P* = 0.99).

Movement Assay: R. dominica

In total, 864 h of movement was recorded for *R. dominica*. For adults exposed to LLIN, distance moved was decreased by over 3-fold compared with control netting (*F* = 28.2; df = 1, 407; *P* < 0.0001). Exposure to LLIN for 1, 5 or 10 min resulted in non-significant, similar reductions in distance moved by adult *R. dominica* (*F* = 1.38; df = 2, 407; *P* = 0.25; Fig. 2-1). There was no significant interaction between the presence of insecticide in the netting and exposure time (*F* = 1.19; df = 2, 407; *P* = 0.31). The post-exposure holding duration significantly affected the distance moved by adults (*F* = 3.56; df = 3, 407; *P* < 0.05), with beetles moving almost half the distance after 168 h compared with immediately after exposure to the netting (Fig. 2-1). The interactions between holding duration and presence of insecticide in netting (*F* = 0.781; df = 3, 407; *P* = 0.51) and exposure time (*F* = 1.02; df = 6, 407; *P* = 0.41) were not significant, nor was the 3-way interaction among the variables (*F* = 1.68; df = 6, 407; *P* = 0.12). Importantly, at every post-exposure holding duration, the total distance moved by *R. dominica* exposed to LLIN was 3–4 times less than beetles exposed to control netting (Fig. 2-1). Unlike *T. castaneum*, the decrease in distance moved by adult *R. dominica* after LLIN exposure was immediate, and did not change over the 24, 72, and 168 h holding periods, and was always less than control adults (Fig. 2-4, post-hoc contrasts).

As with *T. castaneum*, exposure to LLIN significantly increased the angular velocity of adult *R. dominica* by over 1.3-fold compared with control netting (*F* = 38.1; df = 1, 407; *P* < 0.0001). Exposure time did not significantly affect the angular velocity of adults (*F* = 3.56; df = 3, 407; *P* < 0.05), and regardless of the exposure time, the angular velocity increased by 335–366 deg/s for adults exposed to LLIN compared with control netting (Fig. 2-3). In addition, the interaction between exposure time and the presence of insecticide in the netting on the angular velocity of adults was not significant ($F = 0.029$; df = 2, 407; $P = 0.97$). The post-exposure holding duration significantly affected the angular velocity of adults ($F = 3.56$; df = 3, 407; $P < 0.05$), with the highest angular velocity for adults held for 168 h and the lowest for adults held for 72 h (Fig. 2-3). There was a significant interaction between the holding duration and the presence of insecticide ($F = 6.24$; df = 3, 407; $P < 0.001$), but not exposure time ($F = 0.798$; df = 6, 407; $P = 0.57$) or the 3-way interaction among all the variables ($F = 0.364$; df = 6, 407; $P = 0.90$). Importantly, the adults exposed to LLIN had significantly elevated angular velocities 0–72 h after exposure compared with control netting, although by 168 h, the angular velocity between LLIN-exposed and control netting-exposed adults had equilibrated (Fig. 2-3). The increase in angular velocity for LLIN-exposed *R. dominica* was immediate during the 2-h trials and elevated at every interval over all the other treatments, while adults exposed to LLIN but held for 24, 72, or 168 h exhibited a lower angular velocity that was closer to the controls (Fig. 2-4, post-hoc contrasts).

The effect of exposure of LLIN on *R. dominica* adult mortality was even more pronounced than for *T. castaneum* ($\chi^2 = 617$; df = 1; $P = 0.0001$), with only 3.7% of adults alive after exposure to insecticide netting compared with 85% of adults alive after exposure to control netting. An additional 17% of adults were considered affected after exposure to LLINs, while there were no adults affected after exposure to control netting. Mortality of adults was not affected by exposure time ($\chi^2 = 0.68$; df = 2; $P = 0.71$), with uniformly high mortality that was 5–8 times greater for adults exposed to LLIN compared with those exposed to control netting (Fig. 2-5). The interaction between exposure time and presence of insecticide was also not significant (χ^2 = 0.17; df = 2; P = 0.92). The post-exposure holding duration had a significant impact on the mortality of *R. dominica* (χ^2 = 47.5; df = 3; P = 0.0001), with mortality almost double for adults held 168 h compared with those tested immediately. This was an interactive effect with the presence of insecticide (χ^2 = 29.5; df = 3; P = 0.0001), and was driven by mortality in the control after 168 h (Fig. 2-5), likely exacerbated by the lack of food for a prolonged period. Importantly, the number of affected and dead adults after exposure to LLIN ranged from 93–100% compared with 0–14% after exposure to control netting. There was no significant interaction between holding duration and exposure time (χ^2 = 4.14; df = 6; P = 0.66), or all three variables (χ^2 = 0.19; df = 6; P = 0.85) on the mortality of *R. dominica* adults.

Dispersal Assay: T. castaneum

A total of 4,320 adult *T. castaneum* were tested for their ability to disperse to a new food resource. The presence of insecticide in the netting significantly affected their capacity to disperse (F = 2151; df = 1, 89; $P < 0.0001$), with almost 19-fold fewer adults reaching the new food patch after being exposed to LLINs compared with control netting. The distance subtly but significantly affected the dispersal of adults to the new food patch (F = 8.60; df = 2, 89; $P < 0.001$; Fig. 2-4), with slightly fewer adults reaching the new resource at 175 cm after exposure to control netting compared with the other distances. There was no significant interaction between presence of insecticide in the netting and distance (F = 1.59; df = 2, 89; P = 0.21). Importantly, regardless of distance, the number of adult *T. castaneum* reaching the new food patch after exposure to LLINs was 14- to 51-fold less compared with exposure after control netting, with the highest fold decrease at the longest distance (Fig. 2-4). By contrast, the post-exposure holding duration did not significantly affect the dispersal of adults (F = 1.06; df = 2, 89; P = 0.35; Fig. 2-4). In addition, the interaction between the holding duration and presence of insecticide (F =

2.45; df = 2, 89; *P* = 0.092) or distance (*F* = 0.284; df = 4, 89; *P* = 0.89), or the 3-way interaction between all the variables (*F* = 0.493; df = 4, 89; *P* = 0.74), did not significantly affect the number of adults reaching the new food patch. Finally, sex also did not affect the dispersal capacity of adult *T. castaneum* (*F* = 1.63; df = 1, 89; *P* = 0.21), with an average of 18.5 ± 0.4 and 18.4 ± 0.4 females and males, respectively, reaching the new food patch after exposure to control netting while only 1.4 ± 0.4 and 0.52 ± 0.2, respectively, made it to the food patch after exposure to LLIN.

Only about 2% of the control adult *T. castaneum* were considered dead or affected, which was 48-fold lower than adults exposed to LLIN (χ^2 = 2091; df = 1; *P* = 0.0001; Fig. 2-5). The post-exposure holding duration did not impact the percentage of adults that were dead or affected (χ^2 = 3.09; df = 2; *P* = 0.21). There was no significant interaction between the holding duration and presence of insecticide (χ^2 = 0.328; df = 2; *P* = 0.90) on mortality. There was no significant difference among the associated mortality of adults in the distance treatments (χ^2 = 2.45; df = 2; *P* = 0.29). Finally, neither the two-way interactions between distance and presence of insecticide (χ^2 = 2.07; df = 2; *P* = 0.26) or holding duration (χ^2 = 2.05; df = 4; *P* = 0.73), nor the 3-way interaction (χ^2 = 0.168; df = 4; *P* = 0.77) were significant.

Dispersal Assay: R. dominica

A total of 1,440 *R. dominica* adults were tested for their ability to disperse to a new food patch after exposure to control netting or LLINs. The presence of the insecticide had a significant influence on the dispersal capacity of *R. dominica* adults (*F* = 701; df = 1, 54; *P* < 0.0001), with not a single LLIN-exposed adult making it to the new food patch (Fig. 2-6). The distance that adults had to move to reach the new food patch did not significantly influence their ability to reach the destination (*F* = 2.31; df = 2, 54; *P* = 0.11). However, the post-exposure holding duration significantly affected the dispersal capacity of adults (*F* = 4.69; df = 2, 54; $P < 0.05$), with slightly fewer adults reaching the new food patch after being held for 10 min compared with being held for 24 h or being given the chance to disperse immediately, though this likely was not biologically meaningful. The interaction between post-exposure holding duration and insecticide was significant (*F* = 4.54; df = 2, 54; $P < 0.05$), but importantly, regardless of post-exposure holding time, no adult *R. dominica* reached the new food patch. The two-way interaction between distance and post-exposure holding duration (*F* = 0.10; df = 4, 54; $P = 0.98$) or the 3-way interaction between all the variables (*F* = 0.09; df = 4, 54; $P = 0.99$) did not significantly affect the dispersal capacity of adult *R. dominica*.

Across the other treatments, 7.5% and 77% of the adults were considered alive and affected, respectively, after exposure to LLIN, while 98% and 0% of adults were alive and affected after exposure to control netting. Overall, the total percentage of LLIN-exposed adult *R. dominica* considered dead at the end of the experiment was 61-fold greater than those exposed to control netting ($\chi^2 = 2714$; df = 1; $P < 0.0001$; Fig. 2-7). There was no significant difference in mortality among individuals in different distance treatments ($\chi^2 = 3.10$; df = 2; $P = 0.13$), and the interaction between distance and the presence of insecticide did not significantly affect the mortality of adults ($\chi^2 = 4.90$; df = 2; $P = 0.07$). By contrast, the post-exposure holding duration significantly affected the mortality of adults ($\chi^2 = 14.1$; df = 2; $P < 0.001$), with somewhat more affected and fewer dead *R. dominica* after a 10 min and 24 h holding duration, compared with those who were only held for 1 min (Fig. 2-7). There was a significant holding duration by distance interaction ($\chi^2 = 17.3$; df = 4; $P < 0.01$), but not holding duration by presence of insecticide in the netting ($\chi^2 = 4.03$; df = 2; $P = 0.13$) on mortality of adults. The three-way interaction between all the variables also did not significantly influence the mortality of adults ($\chi^2 = 0$; df = 4; $P = 1.0$).

Discussion

This is the first study to examine, in-depth, the sublethal effects of exposure to LLIN on any stored product insects. Specifically, we evaluated how LLIN may impact the movement and dispersal capacity of *T. castaneum* and *R. dominica*. Previously, LLINs were demonstrated to be lethal to the same two stored product species in this study (Scheff et al. 2018). However, the long exposure times required to induce mortality in that study and in another study using a different product (e.g. cypermethrin-coated netting) for control of *Lasioderma serricorne* (Fabricius) and *Ephestia elutella* (Hübner) (Rumbos et al. 2018), have raised questions about whether insecticide-netting could effectively prevent dispersal of adult stored product pests after contact, especially considering that they can potentially crawl through the holes in the mesh. We have shown here that even brief exposure times of 1-min are sufficient to induce the same dramatically decreased movement and increase in disorientation as longer 10-min exposures. In addition, a moderate exposure time of 5-min was sufficient to substantially reduce or effectively prevent the dispersal of adult stored product insects, with *R. dominica* the more susceptible of the two species studied. Vastly diminished dispersal capacity remained even after a 2–3 d period during which adult *T. castaneum* or *R. dominica* could have recovered, but did not. Even though two-thirds of the adults tested in the assays could be considered alive or affected after contact with insecticide netting, the behavioral data suggests that adults of both species are either incapable of dispersing to new food patches or show a marked reduction in dispersal capacity. This indicates that while mortality may be initially incomplete after brief periods of contact with the netting, brief exposure such as might occur if used in windows or vents in a food facility or bin may be adequate for halting immigrating insects.

Prior work with stored product insects has demonstrated a range of sublethal effects due to insecticide exposure. For example, the movement and velocity of the stored product psocids, *Liposcelis bostrychophila* Badonnel and *L. entomophila* (Enderlein), were reduced by approximately a third when exposed to concrete surfaces treated with β-cyfluthrin and pyrethrins (Guedes et al. 2008). Research evaluating the effects of sublethal exposure of phosphine on *R. dominica* found that it decreased the movement of three different populations of beetles in Brazil, including susceptible and resistant populations (Pimentel et al. 2012). Guedes et al. (2009) found that the resting time by adult *S. zeamais* increased by a third in deltamethrin-exposed individuals, but the total distance moved and velocity was not significantly different between insecticide-exposed and control individuals. In contrast, we found that the total distance moved by *T. castaneum* and *R. dominica* decreased by 3- to 4-fold after exposure to LLIN compared with the controls. Moreover, we found that *T. castaneum* exposed to LLIN quickly manifest multiple-fold reductions in movement within 2 h after exposure, while behavioral effects for *R. dominica* are even more immediate. Behavioral effects from LLIN-exposure for both species lasted up to 7 days after exposure, suggesting that exposure to this technology has both immediate and lasting effects on the dispersal of both species. As Guedes et al. (2011) pointed out, incorporating knowledge of the sublethal effects of insecticide exposure into insecticide evaluations is extremely important. When this information is included in evaluating the efficacy of LLINs as barriers, we find this technology to be potent at preventing dispersal of stored product insects that have been intercepted by the netting.

Stored product insects, including *R. dominica*, can immigrate into food facilities from the surrounding landscape during warmer periods in the year in the US (Toews et al. 2006). A variety of studies have documented their presence in and around stored product facilities (e.g. Semeao et al. 2013a, McKay et al. 2017). Many stored product facilities are insecurely sealed from the surrounding environment, with vents, windows, crevices, awnings, and other openings that render them vulnerable to infestation by immigrating insects. LLIN technology may potentially be useful for sealing these openings. While stored product insects may be sufficiently small to pass through the LLIN used in this study, we have shown that even brief contact can have immediate and lasting consequences to their ability to move on and disperse to new food resources. The wider mesh is an advantage over the fine screening needed to physically prevent insect movement because it may be less likely to be clogged by grain dust and small particulates and will allow greater air flow, while still reducing immigration of stored product insects from the surrounding landscape.

There are a variety of potential uses for LLINs in the setting of food facilities. For example, possible uses for LLINs include being incorporated as screening on vents and windows, and as a barrier covering pallets of food. However, one problem associated with LLINs is the incomplete dosing of insecticide that insects may receive after brief contact. This may be ameliorated by using netting that is paired with an attractant in an attract-and-kill context to control stored product insects. Attract-and-kill is based on the concept of attracting a pest to a spatially circumscribed area and eliminating them from the foraging population with a killing mechanism, usually an insecticide (e.g. Morrison III et al. 2016a; reviewed in Gregg et al. 2018). Pairing the netting with attractive semiochemicals may increase interception of immigrating stored product pests before they reach a commodity, and induce prolonged exposure to the insecticide-incorporated netting by exploiting their taxis towards pheromones, food cues, or other salient semiochemicals (e.g. Morrison III et al. 2017b). In the future, developing trapping devices that are compatible with the behavior of stored product insects and use of the netting should be a priority.

A related tactic is the use of long-lasting insecticide-treated bags to store grain for smallholders in developing countries. One such bag is Zerofly with 0.3% deltamethrin and produced by the same company that produced the nets in the current study. This technology is very promising, and was able to keep insect-damaged kernels below 5% over 4 months (Paudyal et al. 2017). However, our study highlights that the bags may also have substantial sublethal effects on stored product insects, which may potentially lead to a less robust pest population in an area where the bags are kept. Further research on insecticide-treated bags should incorporate sublethal effects in making an overall determination of effectiveness.

With increasing resistance to phosphine documented around the world (e.g. Zettler et al. 1989; Pimentel et al. 2009; Opit et al. 2012; Nguyen et al. 2016; Cato et al. 2017), it is increasingly important to provide alternative management tactics to food facility managers. LLINs appear effective at incapacitating *T. castaneum* and *R. dominica*, either through direct lethal effects or indirect sublethal changes to behavior. Future work should demonstrate: 1) the effectiveness of LLINs against the immature stages of stored product pests, 2) that LLINs have a long residual effectiveness under field conditions even in the presence of food dust, 3) LLINs function even when insects have direct access to food after exposure, and 4) that the use of LLINs can reduce populations of stored product insects under field conditions compared to standard management approaches. At the moment, LLINs are a very promising technology, and their eventual inclusion in IPM programs may help relieve the difficulty that food facility managers are under in ensuring stored products are free from infestation.

Figure 2-1. The mean distance moved by alive and affected adult *T. castaneum* (top) or *R. dominica* (bottom) after exposure to long-lasting insecticide netting (LLIN, red) or control netting (black) over 2 h in a movement assay after various exposure times

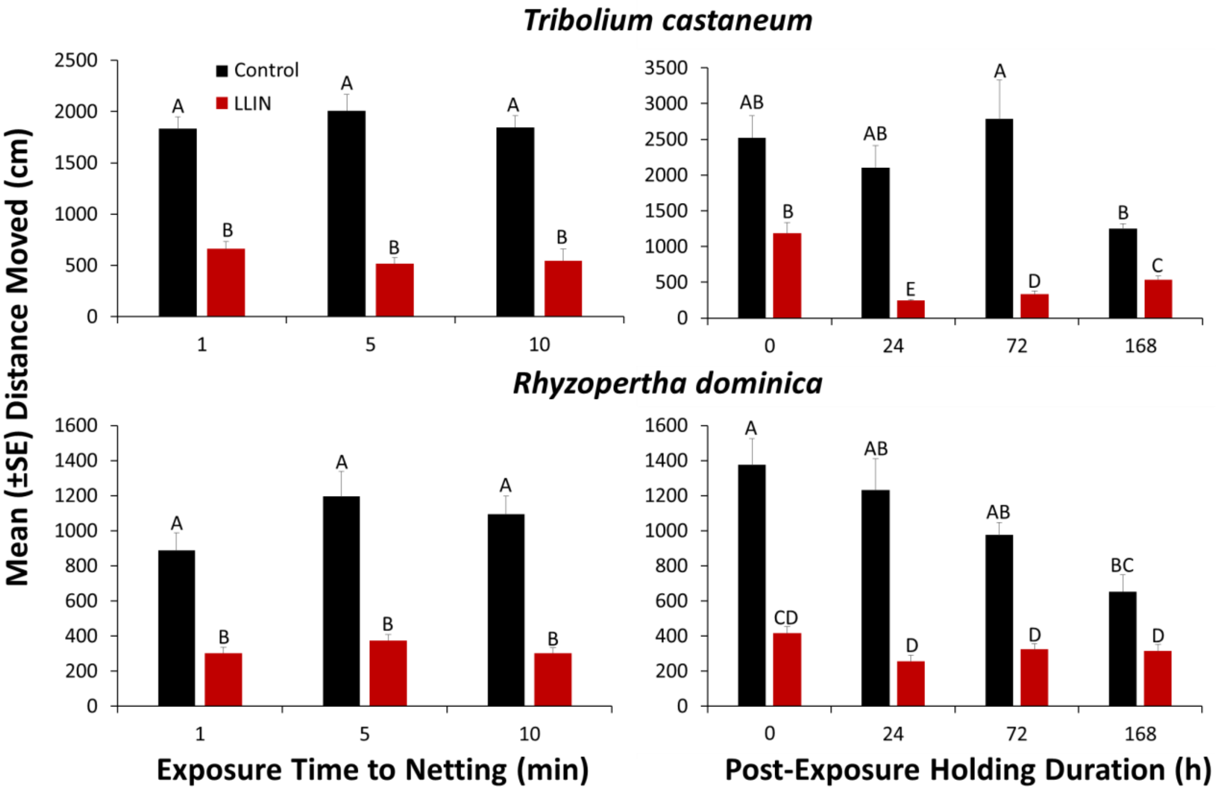


Figure 2-2. The mean distance moved by alive and affected adult *T. castaneum* (top) or *R. dominica* (bottom) after exposure to LLINs (red) or control netting (black) during four thirty-minute intervals, 0–30 (0), 30–60 (30), 60–90 (60), 90–120 min (90) in a 2-h movement assay after reclassification of variables into adults exposed and immediately run in the assay (dashed lines) or held for 24, 72, or 168 h and then tested (solid lines). Post-hoc contrasts were run between treatments at 0–30 and 90–120 min (Fisher's LSD, Bonferroni-corrected).

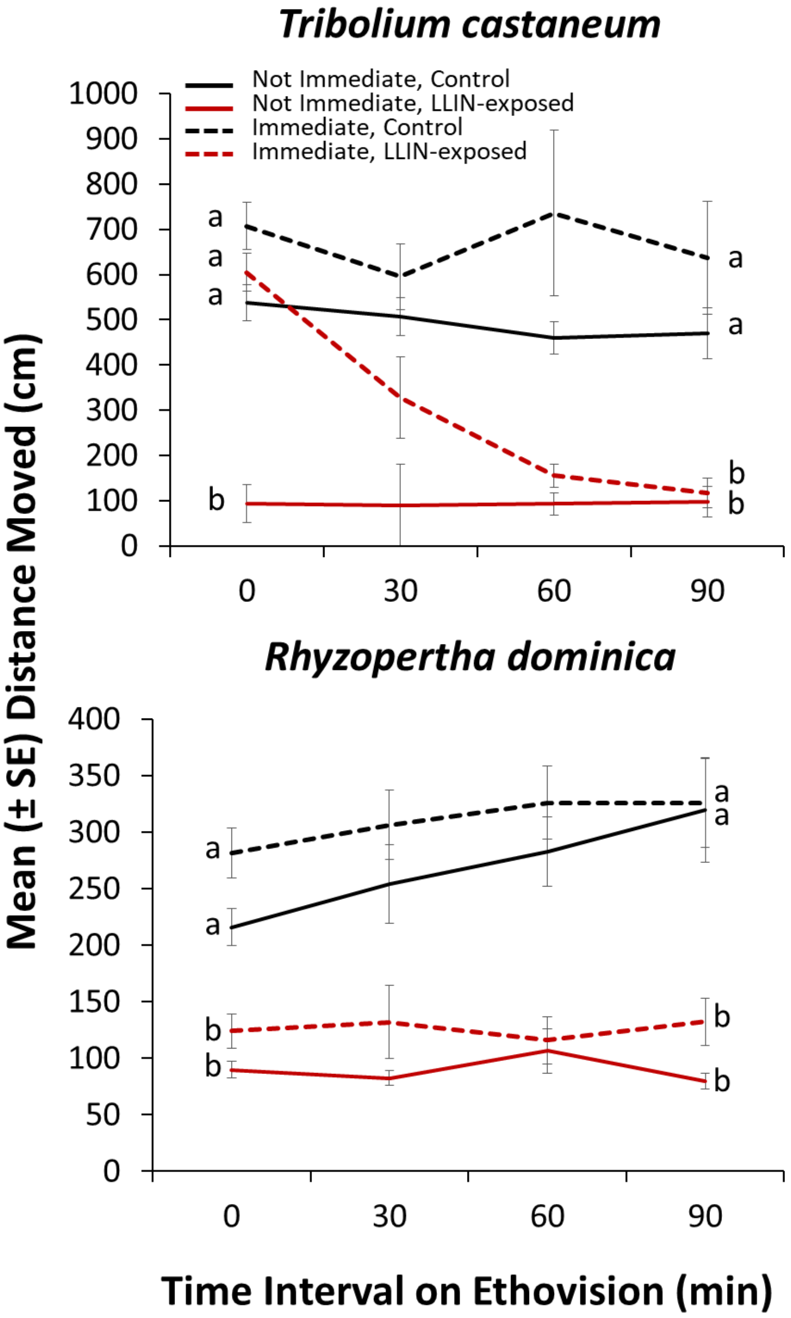


Figure 2-3. The mean angular velocity of alive and affected adult *T. castaneum* (top) or *R. dominica* (bottom) after exposure to long-lasting insecticide netting (LLIN, red) or control netting (black) over 2 h in a movement assay after various exposure times (left) and varying post-exposure holding durations (right). Bars that share a letter are not significantly different from each other (Tukey's HSD, α = 0.05).

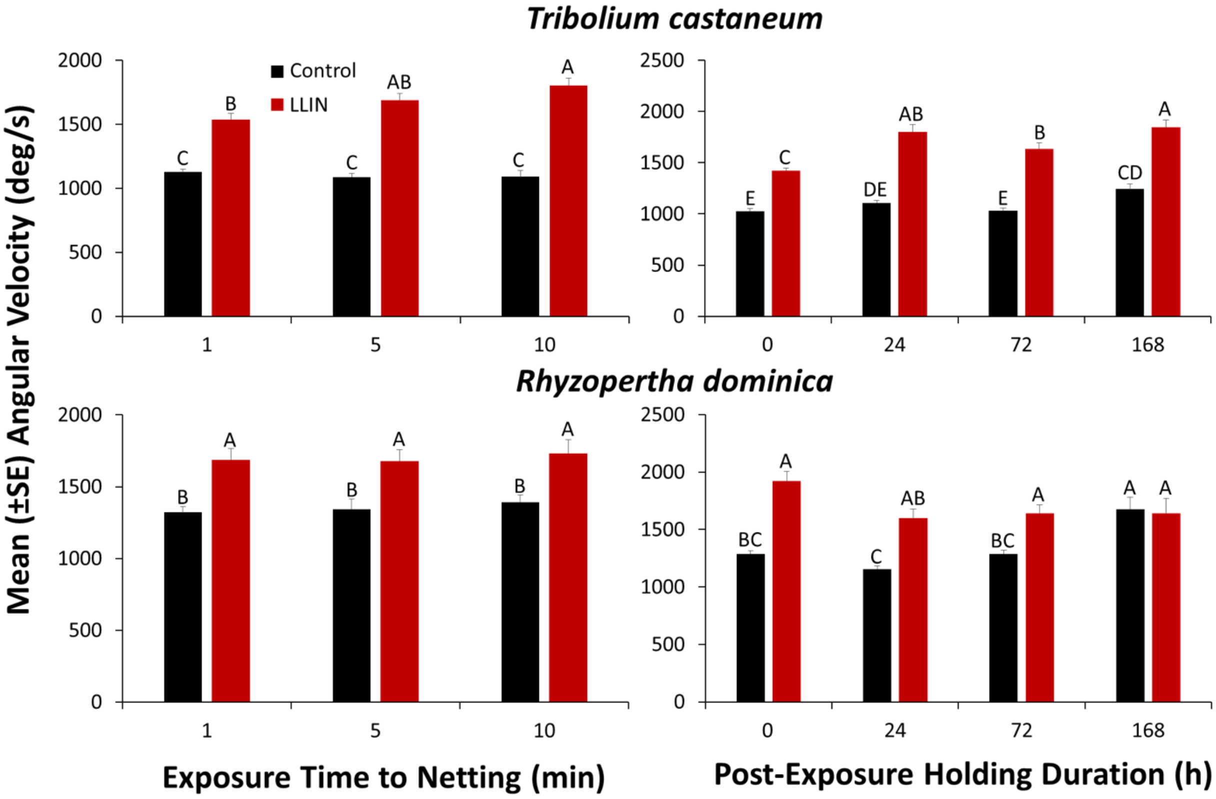


Figure 2-4. The mean angular velocity by alive and affected adult *T. castaneum* (top) or *R. dominica* (bottom) after exposure to LLINs (red) or control netting (black) during four thirty-minute intervals, 0–30 (0), 30–60 (30), 60–90 (60), 90–120 min (90) in a 2-h movement assay after reclassification of variables into adults exposed and immediately run in the assay (dashed lines) or held for 24, 72, or 168 h and then tested (solid lines). Post-hoc contrasts were run between treatments at 0–30 and 90–120 min (Fisher's LSD, Bonferroni-corrected).

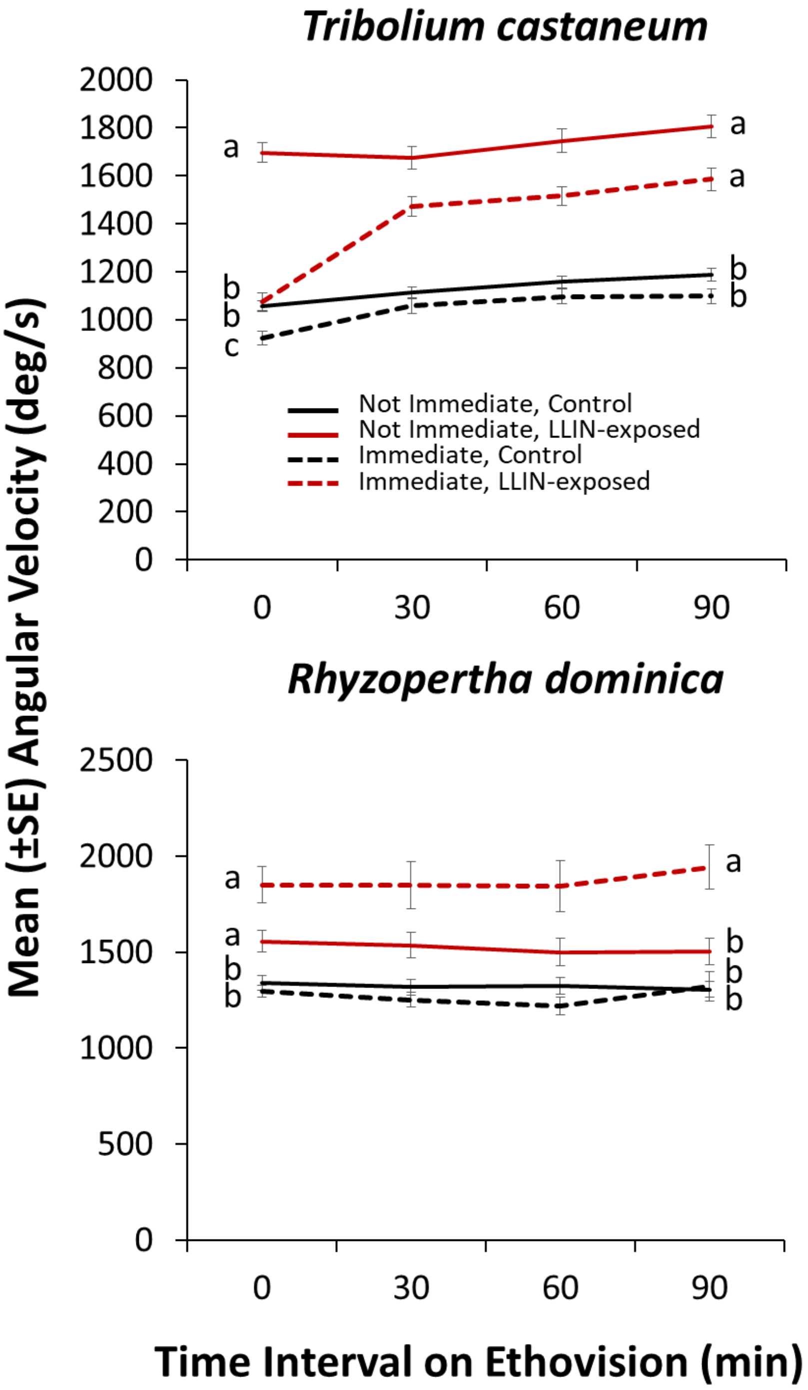


Figure 2-5. The percentage of adult *T. castaneum* (top) and *R. dominica* (bottom) after exposure to long-lasting insecticide netting (LLIN) or control netting that were alive (blue), affected (yellow), or dead (red) after various exposure times (left) and varying post-exposure holding durations (right) at the conclusion of a 2-h movement assay.

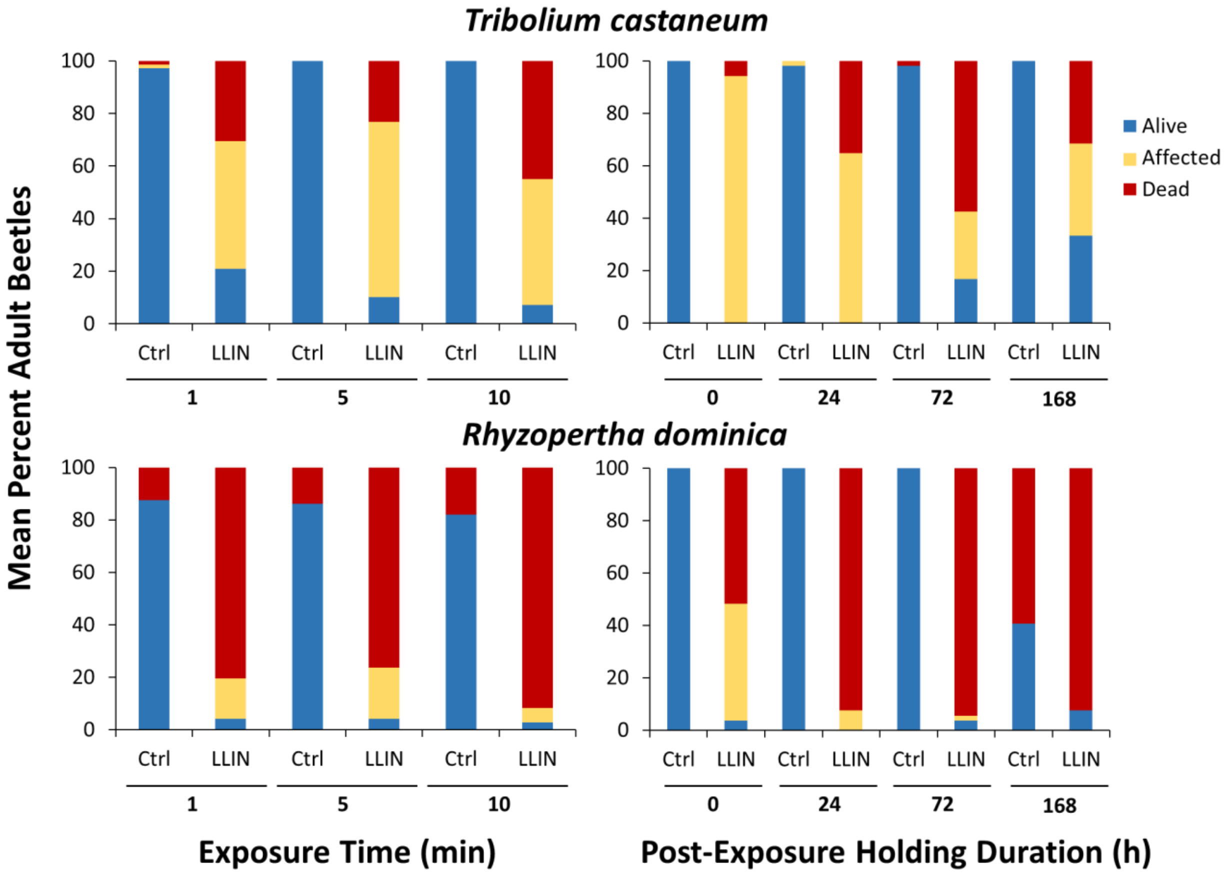


Figure 2-6 The mean number of adult *T. castaneum* (top) or *R. dominica* (bottom) that made it to the dispersal cup (new flour-filled food patch) at the end of 48 h after a 5-min exposure to long-lasting insecticide netting (LLIN: red) or control netting (black) depending on dispersal distance (left) or post-exposure holding duration (right). Bars with shared letters are not significantly different from each other (Tukey's HSD, α = 0.05).

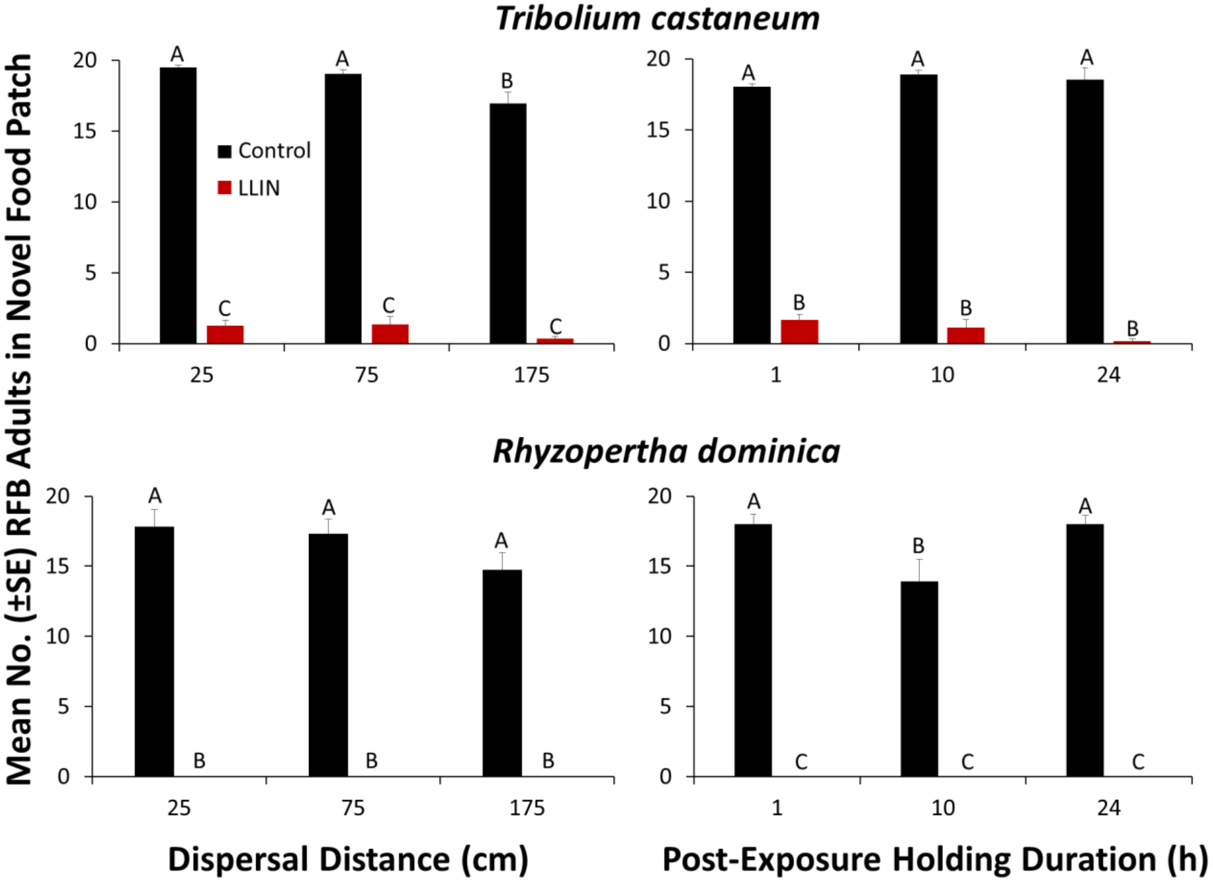
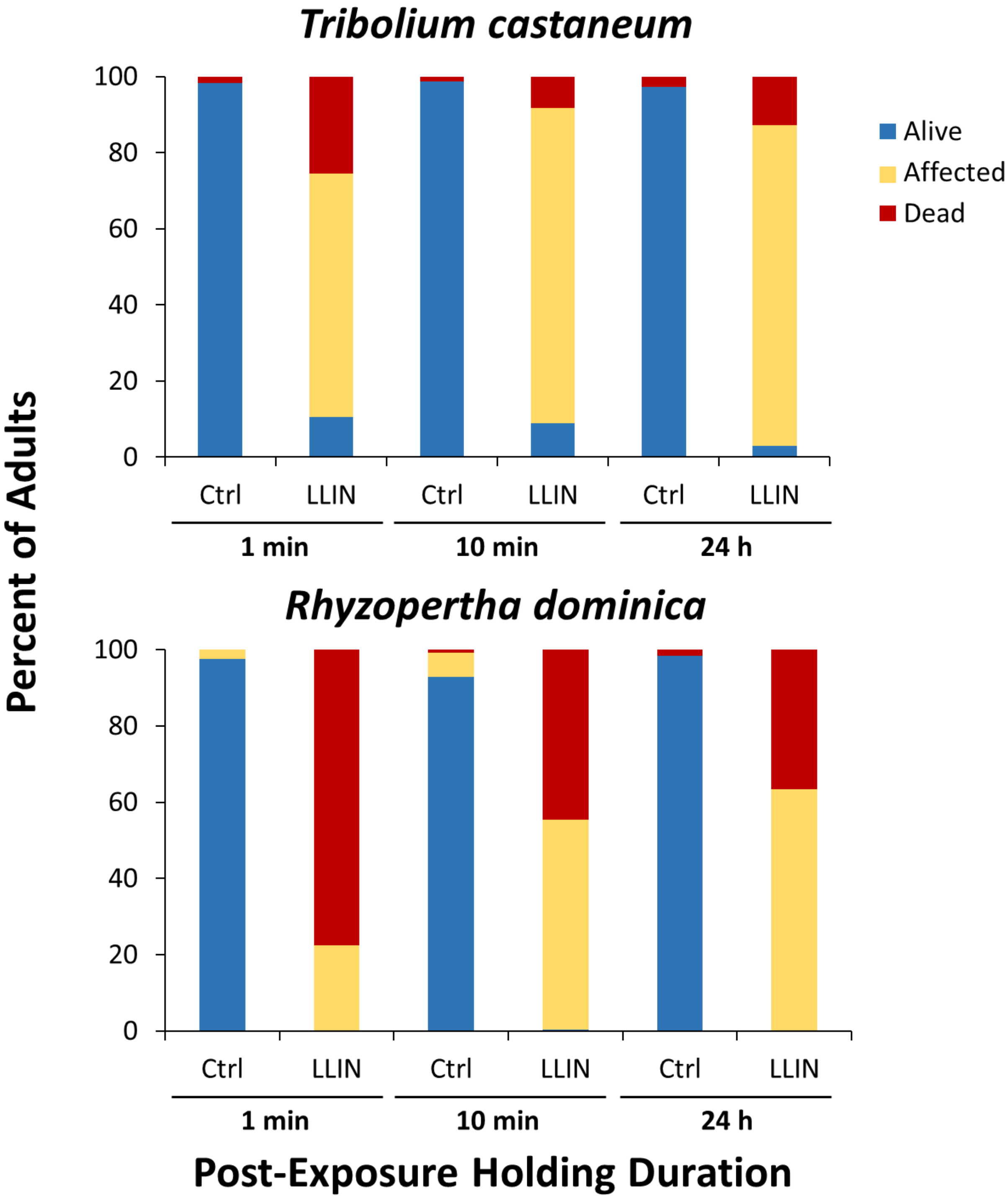


Figure 2-7. The percentage of adult *T. castaneum* (top) and *R. dominica* (bottom) after 5-min exposure to long-lasting insecticide netting (LLIN) or control netting that were alive (blue), affected (yellow), or dead (red) after varying post-exposure holding durations at the conclusion of a 2-h dispersal assay.



Chapter 3 - Mobility and dispersal of two cosmopolitan stored product insects are adversely affected by long-lasting insecticide netting in a life stage-dependent manner

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Introduction

Every year, the US produces and stores a large quantity of valuable commodities that contribute to global food security. For example, in 2017, the US stored $1 billion USD in beans, $2 billion in rice, and $8 billion in wheat (NASS 2019). As these commodities move through the postharvest supply chain from the farmer to the end user, they are vulnerable to insect infestation. Each link along the supply chain represents a new opportunity for insect infestation, with between 2–50% of commodities lost yearly after harvest (Davis 1991). This translates to roughly $100 billion worth of food products lost globally (Wacker 2018). Stored product insect infestations are often treated with fumigation of commodities or structures. Historically, methyl bromide and phosphine have been the preferred fumigants for treating infested structures and commodities, respectively. The use of the former has been largely phased out because it was labeled an ozone-depleting substance by the Montreal Protocol (Fields and White 2002). Phosphine has remained the most common fumigant used for treating infested commodities, but resistance is becoming a worldwide problem (Schlipalius et al. 2018; Huang et al. 2018;

Venkidusamy et al. 2018). Likewise, there has been increasing demand by consumers for low or no insecticide residues in the stored products throughout the postharvest supply chain (Batte et al. 2007). Since most grain and grain-based products start off free from insect infestation, avoidance of infestation by stored product insects is a major focus of integrated pest management programs (IPM).

A potential new IPM tactic is to use long-lasting insecticide-incorporated netting (LLIN) to serve as a barrier to prevent insect movement into food products (Morrison et al. 2018; Rumbos et al. 2018). Historically, LLIN has been used to reduce the spread of arthropod-borne diseases such as malaria by controlling mosquitoes and other vectors in tropical regions (Alonso et al 1991). LLIN generally has multi-year efficacy in other systems and is relatively inexpensive (Martin et al. 2006). Recently, LLIN has been used as a kill mechanism in a trap (Kuhar et al. 2017) and management strategy in specialty crops (Fernández et al. 2017), as well as to address nuisance pest issues for homeowners (Bergh and Quinn 2018). If netting is to be used effectively as a barrier, it needs to affect insects quickly before they can move from the point of contact into the food product. Prior work evaluating efficacy against stored product insects has found that even brief exposures to LLIN by adult red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), and lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), resulted in 2–3-fold reductions in movement as well as an inability to disperse to food patches that were in close proximity to the insects, including trivial distances of 25 cm (Morrison et al. 2018). However, it is unknown how other life stages such as immature stored product insects respond after exposure to LLIN.

One widespread and cosmopolitan stored product pest is *Trogoderma variabile* Ballion (Coleoptera: Dermestidae), commonly referred to as the warehouse beetle. As a secondary pest, *T. variabile* doesn’t readily infest whole grains, but is an important pest of milled grains, processed grains, animal feed, pet food, spices, nuts, as well as animal carcasses and museum artifacts (Hagstrum and Subramanyam 2006; Partida and Strong 1975; Arthur and Kelley 2015). Adults of this species are highly mobile and can be captured in high numbers outside whenever conditions are favorable (Campbell and Arbogast 2004; McKay et al. 2017) and can also move considerable distances within a facility, even moving between floors (Campbell et al. 2002). Pheromone trap captures indicated that *T. variabile* occurrences inside and outside a food facility are influenced by seasonal changes, and less influenced by fumigation treatments (Campbell and Arbogast 2004). Moreover, a 10-year study found that even after fumigation treatments decreased trap captures of *T. variabile*, captures of *T. variabile* quickly recovered inside a facility, suggesting that *T. variabile* readily immigrates into a facility from the outside (Gerken and Campbell 2019).

Another cosmopolitan, secondary pest in stored products is *T. castaneum* which feeds on a large variety of commodities and is an especially significant pest of flour (Hagstrum and Subramanyam 2006). Although adults tend to be less mobile than *T. variabile*, *T. castaneum* were found to move among floors within a flour mill (Semeao et al. 2013b). *Tribolium castaneum* females making short or long dispersal flights have already mated multiple times, with additional male beetle encounters only increasing progeny production (Gurdasani et al. 2019). It is also known that *T. castaneum* can fly at least 300 m during a dispersal flight (Gurdasani et al. 2019). Healthy adult *T. castaneum* have been documented to walk 20–25 m in a 2-h period (Morrison et al. 2018). Prior work has found that the proportion of *T. castaneum* locating a novel food patch follows a distance-decay function, though conspecifics seem to be anemotactic (Romero et al. 2010).

While adult insects are the primary dispersers among food patches, the larvae are also capable of moving among food patches within and outside facilities in order to find favorable conditions (Campbell and Arbogast 2004), potentially spreading infestations. Some species such as *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) undergo a wandering stage, which can result in spatially heterogenous populations (Campbell and Arbogast 2004, Mohandass et al. 2007). Larvae can cover considerable distance, with healthy immature *T. variabile* moving almost 15 m in a 2-h period, while *T. castaneum* larvae move 10 m in the same period (Morrison et al. 2019a). Developmental stages can often differ in their susceptibility to insecticides, so the stage dispersing might be differentially affected by exposure to treated surfaces. For example, wandering *P. interpunctella* larvae showed decreased adult emergence on methoprene-treated surfaces, whereas *P. interpunctella* eggs on the same surfaces showed little difference in adult emergence (Jenson et al. 2009). Additionally, stored product psocids ranged from very susceptible to very tolerant to sulfuryl fluoride fumigation, depending on life stage (Athanassiou et al. 2012). Differences in susceptibility between larvae and adults can be due to a variety of factors including physiology and degree of contact with treated surfaces. Pupae of *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae), were less susceptible to the chemical than other life stages, particularly the adults and young larval stages (Saglam et al. 2013). Other species of postharvest insects, particularly *Trogoderma* spp., have larvae that were less susceptible to deltamethrin than adults because larvae are able to feed and presumably recover whereas adults typically do not feed during their short lifespan (Ghimire et al. 2017). Thus, in evaluating the benefits of using LLIN in IPM programs it is important to also consider of how immature life stages respond to LLIN exposure.

Even in cases where individual insects are not outright killed by insecticides, there may be sublethal effects on behavior that contribute to a compound successfully managing a population. These indirect effects of insecticides have been an understudied area of entomology (Guedes et al. 2017), but a comprehensive understanding of their effects is vital to determine the full effectiveness of a control tactic. Indirect effects may result in sublethal changes to an insect's migration, movement, reproduction, or other fundamental life process (Desneux et al. 2007), and may be mediated by sanitation in a food facility (Morrison et al. 2019b). For example, previous studies have shown that certain insecticides upregulate or attenuate a species' rate of reproduction by 1.8–2.3-fold (Kerns and Stewart 2000; Bao et al. 2008; Wang et al. 2008), while flight capacity of a species may decrease by 20-60% after sublethal exposure (Morrison et al. 2017). These effects determine the extent of future infestations and damage caused by the surviving insects. Importantly, indirect effects may be particularly relevant when considering tactics designed to stop the movement of immigrating insects between food patches such as LLIN in the post-harvest environment. Therefore, the goals of this study were to evaluate the efficacy of LLIN against adult and immature *T. variabile* and as well as against immature *T. castaneum* (adult *T. castaneum* have been evaluated in an earlier study by Morrison et al. 2018) to determine whether there are life stage-specific differences in mortality, movement, and dispersal ability after exposure. Understanding how LLIN affects immatures will inform future management programs wanting to utilize this novel postharvest IPM approach.

Materials and Methods

Source Insects

Colonies of *T. castaneum* and *T. variabile* were obtained from the field in eastern Kansas in 2012 and 2016, respectively. They were reared in an environmental chamber under constant conditions (27.5˚C, 60% RH, 14:10 L:D). *Tribolium castaneum* were fed a prepared diet of 95% flour (100% organic, all-purpose, unbleached/unenriched flour) with 5% brewer's yeast, while *T. variabile* were fed ground dog food (Lamb & Chow, Purina One, St. Louis, MO) with a layer of oats and a moistened, crumpled paper towel on the surface.

To prepare immature individuals for use in each of the assays, the following procedure was performed. *Tribolium castaneum* larvae were raised on 40 g of prepared flour diet and kept in 118-mL jars. In each jar, 70–80 *T. castaneum* adults were added, then given 48 h to settle, mate, and lay eggs, before being removed using a #25 sieve (710 × 710 µm mesh, Fisher Scientific Co., Hampton, NH). Larvae were used in experiments 2.5–3 weeks after removal of adults. A similar procedure was performed for *Trogoderma variabile* larvae, with 60 adults given 48 h to reproduce before being removed by a similar-sized sieve. Given that the larvae may go through supernumerary molts based on food availability and density it is difficult to determine instars, so *T. variabile* larvae were classified as large, given 7–8 weeks to develop, or small, given 3–4 weeks, which conforms to prior size classification schemes for *Trogoderma* spp. (Domingue et al. 2020).

To compare the relative susceptibility of larvae to adults, the effects of exposure to LLIN on mixed sex *T. variabile* adults were evaluated. Adult *T. variabile* were collected directly from colony jars immediately prior to experimentation and were no more than 7 d old. Adult *T. castaneum* were previously evaluated by Morrison et al. 2018. To facilitate comparisons with larval *T. castaneum* collected in this study, some results from that prior study for adult *T. castaneum* are included for life stage-specific comparisons but are not otherwise re-analyzed.

Movement Assay

In order to assess mobility changes after exposure to netting, movement of adult or immature life stages for both species was tracked with a camera (GigE, Basler AG, Ahrenburg, Germany) centered and suspended 78 cm above the experimental arenas. Due to their size, small *T. variabile* larvae were unable to be video-tracked and thus are only used for the dispersal assay of this study. Prior to tracking movement, insects were exposed to LLIN (0.4% deltamethrin, D-Terrence, Vestergaard Inc., Lausanne, Switzerland) or control netting that was physically identical but lacked insecticide. Insects were exposed in a 24 × 24 cm square petri dish on a single layer of the netting for 1, 5, or 10 min. After exposure to netting, insect mobility was assessed immediately (approximately 1 min afterwards), or the insects were held separately in 4 × 4 cm (H:D) plastic cups for 24, 72, or 168 h in an environmental chamber with conditions identical to those used for rearing. These post-exposure durations were used to assess how quickly LLIN-exposure causes behavioral effects in the insects and to determine whether changes in behavior persisted over time.

Six individuals were video-tracked simultaneously, in separate filter paper-lined (85 mm D, Grade 1, GE Healthcare, Buckinghamshire, UK) petri dishes (100 × 15 mm D:H), with half consisting of control netting-exposed and half with LLIN-exposed individuals. Petri dishes and filter paper were discarded after use by each insect to avoid confounding effects from prior exposure. Average instantaneous velocity and total distance traveled over 2-h periods were calculated using Ethovision (v. 10.0.828, Noldus Inc., Leesburg, VA, USA). At the end of this period, the health conditions of control and treated individuals were recorded as either alive, affected, or dead under a dissecting stereomicroscope (Nikon, Inc.). Affected individuals were noted as having slow, irregular, or uncoordinated movements, sometimes with twitching, and may be unable to right themselves when flipped on their back. Completely immobile individuals, even after prodding, were classified as dead. Only alive or affected individuals were used for the subsequent analysis of movement. A total of n = 15 replicate individuals were tested per treatment combination for this assay, translating to a total of 360 individuals tested and 720 h of video per species and life stage.

Dispersal Assay

To test dispersal capacity to new food patches, a dispersal apparatus was employed. Species- and life stage-specific cohorts of 20 larvae (*T. castaneum* and *T. variabile*) or adults (*T. variabile*) were exposed to LLIN or control netting for 5 min, then given 48 h to disperse across 10, 25, or 75 cm standardized sections of PVC pipe (3.175 cm ID). Similar to the movement assay, insects were held for 1 min (immediate release), 10 min, or 24 h after exposure before placing them in the dispersal apparatus. The ends of both sides of the PVC pipe were sealed with Parafilm to prevent escape. At the far end of the pipe, a hole (2.22 cm D) was drilled and centered over a glass jar (5 × 6.5 cm D:H) to create a pitfall trap design. The glass jar contained 20 g of flour, representing a novel food patch, to entice insects to disperse. At the end of the sampling period, the number of insects in the jar and the insects remaining in the PVC pipe was recorded. The condition of each insect as alive, affected or dead was also recorded. A total of n = 12 replications each for *T. castaneum* larvae, *T. variabile* adults, and *T. variabile* larvae were completed per treatment combination, species, and life stage for this assay. In total, 3,600 *T. castaneum* and 3,960 *T. variabile* were tested in this experiment.

Statistical Analyses

The total distance traveled and average instantaneous velocity from the movement assay were analyzed separately using a 3-way factorial ANOVA. The independent explanatory variables included the type of netting (LLIN or control), exposure time (1, 5, or 10 min) and post-exposure holding duration (1 min, 24, 72, or 168 h). Separate models were used for each of the species and life stages. Residuals from each model were inspected to ensure that the assumptions of normality and homogeneity of variances were fulfilled. Where assumptions deviated, data were log-transformed, which corrected any issues. Upon a significant result from the overall model, Tukey HSD was employed for multiple comparisons. R Software was used (R Core Team, 2019), and tests were considered significant at $\alpha = 0.05$, unless otherwise noted.

For the dispersal assay, the number of insects reaching the novel food source was used as the response variable in a 3-way factorial ANOVA. The independent explanatory variables included the type of netting (LLIN or control), dispersal distance (10, 25, or 75 cm), and post-exposure holding duration (1 min, 10 min, or 24 h). Separate models were used for each life stage and species. Residuals were inspected to ensure that assumptions were fulfilled, and data were log-transformed in cases where there was deviation. Upon a significant result from the overall model, Tukey HSD was used for multiple comparisons.

To compare the susceptibility of life stages after LLIN exposure, which may exhibit vastly different overall mobility from each other, raw response variables (distance moved, velocity, number dispersing to new patches) were transformed into a percent of total movement relative to the corresponding control netting-exposed individuals for each assay (e.g. individual LLIN-exposed response divided by control netting-exposed response multiplied by 100). In addition, the relative percent reduction in movement was calculated as 100 minus the relative percent movement to controls. This information was calculated for major variables in each experiment, as well as a global mean, parsed by life stage. To determine whether life stages varied in susceptibility, the values for larvae and adults were compared with a chi-squared test using the null hypothesis of equal movement and changes in movement between larvae and adults. A Bonferroni correction was used for the alpha threshold in these post-hoc tests.

Results

Movement Assay: Distance Moved

Larval *T. castaneum* exposed to LLIN experienced significant reductions in distance moved compared to control-netting exposed individuals (ANOVA: $F = 69.8$; df = 1, 330; $P < 0.0001$; Fig. 3-1), with the former moving 3.6-fold less than the distance that controls moved (Fig. 3-1). Regardless of exposure time ($F = 2.55$; df = 2, 330; $P < 0.08$), LLIN-exposed *T. castaneum* larvae moved 2.7–10-fold less than control larvae. Post-exposure holding duration also had a significant effect on the distance moved by larvae ($F = 41.9$; df = 3, 330; $P < 0.0001$). Immediately to 72 h after exposure, LLIN-exposed *T. castaneum* larvae moved 2.4–3.8-fold less distance than the controls did. After 168 h, LLIN-exposed larvae moved 54-fold less than control larvae (Fig. 3-1). There was no 2-way interaction between netting treatment and exposure time ($F = 0.79$; df = 2, 330; $P < 0.37$), but there was an interaction between exposure time and holding duration ($F = 9.31$; df = 3, 330; $P < 0.0001$), as well as a three way interaction ($F = 12.2$; df = 6, 330; $P < 0.0001$), though these were likely quantitative and not qualitative.

The distance moved by *T. variabile* larvae was significantly reduced by exposure to LLIN compared to control netting (ANOVA: $F = 134$; df = 1, 336; $P < 0.0001$; Fig. 3-1), with LLIN-exposed larvae moving 3-fold less of the distance moved by controls. Likewise, exposure time to LLIN significantly reduced movement to a greater degree with increasing time ($F = 7.01$; df = 2, 336; $P < 0.001$); LLIN-exposed larvae moved 1.8-, 3.8-, and 6.1-fold less than the distance moved by their control netting-exposed counterparts at 1, 5, and 10 min, respectively. The movement of *T. variabile* larvae was more severely affected 24, 72, and 168 h later compared to immediately after exposure (*F* = 21.8; df = 3, 336; *P* < 0.0001). LLIN-exposed larvae moved 1.7-fold less than the distance moved by control netting-exposed individuals immediately after exposure, while they moved 3.1–11.6-fold less than the controls at 24, 72, and 168 h later (Figure 3-1). There was a significant two-way interaction between netting type and exposure time (*F* = 3.29; df = 2, 336; *P* < 0.05), due to a change in the direction of the effect size. The two-way interaction between netting type and post-exposure holding duration (*F* = 1.08; df = 3, 336; *P* = 0.35) and the three-way interaction between all variables (*F* = 1.16; df = 6, 336; *P* = 0.33) were not significant.

The distance that *T. variabile* adults moved was dramatically affected by exposure to LLIN (*F* = 89.5; df = 1, 176; *P* < 0.0001; Fig. 3-1), and was 9.2-fold less than the distance that the control netting-exposed adults moved. The exposure time to netting did not significantly affect the distance moved (*F* = 0.08; df = 2, 176; *P* = 0.78), demonstrating that even brief bouts of exposure were sufficient to elicit strong reductions in insect movement. At every post-exposure holding duration, the movement of LLIN-exposed individuals were impaired compared to the controls (*F* = 1.88; df = 3, 176; *P* = 0.13); LLIN-exposed adults moved 6–14-fold less than the distance of the controls regardless of duration after exposure. None of the 2-way interactions were significant (netting type × exposure time: *F* = 1.80; df = 2, 176; *P* = 0.18; netting type × post-exposure holding duration: *F* = 0.33; df = 3, 176; *P* = 0.81; exposure time × post-exposure holding duration: *F* = 0.34; df = 6, 176; *P* = 0.91), but the 3-way interaction was significant (*F* = 4.20; df = 6, 176; *P* < 0.01).

Both LLIN-exposed *T. castaneum* larvae and adults moved 3.3-fold less than the distance that the control insects moved on average. There were no life stage-based differences in distance that insects moved by exposure time to LLIN except for 10-min exposure periods where movement of larvae was almost three times less than for adults (Chi-squared test, Table 3-1). Importantly, the reduced movement between *T. castaneum* larvae and adults was similar, hovering at about 70% for each (Table 3-1). Larvae and adults moved similar distances relative to the controls immediately after exposure to LLIN, but larvae moved twice as much as adults at 24 h post-exposure, while by contrast they moved 8- to 1.6-fold less compared to adults at 72 h and 168 h post-exposure, respectively (Chi-squared tests, Table 3-2).

Overall, there was a more dramatic reduction in the distance that *T. variabile* adults moved than larvae, with adults moving almost 3-times less than larvae (Chi-squared test, Table 3-1). Depending on exposure time, adults moved 1.6–8-times less than larvae after contact with LLIN (Table 3-1). Adults were more susceptible to LLIN immediately after exposure and 168 h later, where they moved 5-times and 2-times less than larvae, respectively (Chi-squared tests, Table 3-2).

Movement Assay: Velocity

Larval *T. castaneum* exposed to LLIN exhibited a significantly reduced instantaneous velocity over 2-h trial periods ($F = 37.8$; df = 1, 330; $P < 0.0001$; Fig. 3-2), with insecticide-netting exposed individuals 3.4-fold slower than the velocity of controls. Exposure time of larvae on netting did not significantly affect velocity ($F = 1.89$; df = 2, 330; $P = 0.17$), indicating that in each case the velocity for LLIN-exposed larvae was 2.7–8.1-fold less than the controls. By contrast, the holding duration after exposure significantly affected velocity ($F = 26.0$; df = 3, 330; $P < 0.0001$), with velocity for LLIN-exposed larvae decreased by 2.3–55-fold of the velocity for the controls between 1 h–168 h after exposure. There was a significant 2-way interaction between type of netting and exposure time ($F = 14.5$; df = 2, 330; $P < 0.001$), type of netting and post-exposure holding duration ($F = 8.81$; df = 3, 330; $P < 0.0001$), and exposure time and post-exposure holding duration ($F = 7.69$; df = 3, 330; $P < 0.0001$). The 3-way interaction between all the variables on velocity was also significant ($F = 11.2$; df = 6, 330; $P < 0.0001$), but in each case the LLIN-exposed individuals moved less than controls.

The velocity of *T. variabile* larvae was significantly reduced when exposed to LLIN compared to control netting ($F = 142$; df = 1, 336; $P < 0.0001$), traveling 3-fold slower than the controls (Fig. 3-2). Exposure time to netting also significantly affected the velocity of larvae ($F = 7.42$; df = 2, 336; $P < 0.001$); individuals moved 1.8-fold slower than the controls after 1 min exposure to LLIN, while larvae moved 3.7- and 6.1-fold slower than controls after 5 min and 10 min, respectively. The post-exposure holding duration also significantly affected velocity of *T. variabile* larvae ($F = 33.2$; df = 3, 336; $P < 0.0001$). In particular, immediately after exposure to LLIN, larvae moved 1.8-fold slower than the controls, while at 24 h–168 h post-exposure the velocity decreased by 3.1–12-fold compared to the controls. None of the interactions were significant (netting type × exposure time: $F = 3.20$; df = 2, 336; $P = 0.06$; netting type × post-exposure holding duration: $F = 0.97$; df = 3, 336; $P = 0.42$; exposure time × post-exposure holding duration: $F = 1.84$; df = 6, 336; $P = 0.08$; 3-way interaction: $F = 0.99$; df = 6, 336; $P = 0.43$).

Similarly, the velocity of *T. variabile* adults was significantly reduced after contact with LLIN relative to control netting ($F = 94.8$; df = 1, 176; $P < 0.0001$), which traveled 10-fold slower. The exposure time to LLIN also significantly affected the velocity of adults ($F = 5.91$; df = 2, 176; $P < 0.01$). Specifically, adult velocity declined with LLIN exposure time: at 1, 5, and 10 min exposure, LLIN-exposed adults traveled 6.1-, 6.6-, and 48-fold slower than the control netting-exposed ones. The post-exposure holding duration significantly affected the velocity of *T. variabile* adults ($F = 3.17$; df = 3, 176; $P < 0.05$), but regardless of time, the velocity for

LLIN-exposed adults was decreased by 6–14-fold compared to controls. There was a significant two-way interaction between netting type and post-exposure holding duration ($F = 25.6$; df = 3, 176; $P < 0.0001$), with a more pronounced decrease in velocity at 24 and 72 h than immediately or 168 h after exposure. There was also a significant netting type by exposure time interaction ($F = 17.7$; df = 2, 176; $P < 0.0001$); with a numerical, but nonsignificant decrease in velocity at 1 min exposure compared to significantly decreased velocity at 5 and 10 min. The interaction between exposure time and post-exposure holding duration on velocity was not significant ($F = 2.65$; df = 6, 176; $P = 0.07$). Finally, the 3-way interaction between all variables on velocity was also not significant ($F = 0.99$; df = 6, 176; $P = 0.43$).

Both *T. castaneum* larvae and adults were equally susceptible to LLIN exposure. For each life stage, the velocity was decreased by 3-fold compared to controls, with a corresponding reduction in movement of about 70% (Chi-squared tests, Table 3-3). The life stages responded similarly to varying exposure time (Table 3-3), but differently for duration after exposure where larvae and adults behaved similarly 1 min and 168 h after LLIN exposure, while larvae moved 2-times faster than adults at 24 h after LLIN exposure and 8-fold slower at 72 h (Chi-squared tests, Table 3-4).

Trogoderma variabile adults were much more susceptible to LLIN exposure than larvae, with adults moving 2.9-fold slower than larvae overall (Table 3-3). At 1 min and 10 min of LLIN exposure, adults moved 3-fold and 8-fold slower than larvae, respectively (Chi-squared tests, Table 3). After 1 min and 168 h after LLIN exposure, adults moved 5-fold and 2-fold slower than larvae, while velocity of the life stages was equivalent 24 h and 72 h after exposure (Table 3-4).

Dispersal Assay

Exposure to LLIN significantly affected the number of *T. castaneum* larvae reaching a novel food resource (*F* = 89.9; df = 1, 54; *P* < 0.0001; Fig. 3-3), with 2.2-fold fewer individuals successfully dispersing after LLIN exposure compared to controls. The dispersal distance also significantly reduced dispersal of larvae (*F* = 36.3; df = 2, 54; *P* < 0.0001). Specifically, compared to controls, the percent of individuals dispersing was decreased by 1.3-, 2.5-, and 15-fold at 10, 25, and 75 cm, respectively. The post-exposure holding duration, by comparison, did not significantly affect dispersal of *T. castaneum* larvae (*F* = 3.01; df = 2, 54; *P* = 0.06). There was a significant two-way interaction between type of netting and dispersal distance on the number of dispersing larvae (*F* = 7.21; df = 2, 54; *P* < 0.01), with equivalent numbers of larvae dispersing at 10 cm, but 2.5- and 15-fold fewer LLIN-exposed larvae dispersing compared to controls at 25 and 75 cm. The two-way interaction between type of netting and post-exposure holding duration on dispersing larvae was not significant (*F* = 0.36; df = 2, 54; *P* < 0.70).

By contrast, the dispersal of small *T. variabile* larvae were not as strongly affected overall by exposure to LLIN netting (*F* = 1.73; df = 1, 54; *P* < 0.19; Fig. 3-4). Dispersal distance did significantly affect the number that successfully reached a novel food patch (*F* = 16.6; df = 2, 54; *P* < 0.0001), with 2.6- and 6.2-fold fewer small larvae making it across at 25 and 75 cm, respectively, compared with 10 cm. The post-exposure holding duration also significantly affected dispersal (*F* = 8.81; df = 2, 54; *P* < 0.001); 3.5-fold and 2-fold fewer larvae were able to successfully disperse 10 min and 24 h after exposure, respectively, compared to immediately being given the opportunity to disperse. Importantly, the 2-way interaction between type of netting and dispersal distance significantly affected the percentage of dispersing insects (*F* = 3.38; df = 2, 54; *P* < 0.05), with 6- and 4-fold fewer dispersing small *T. variabile* larvae after LLIN exposure compared to controls at 25 and 75 cm (Fig. 3-4). However, the two-way interaction between type of netting and post-exposure holding duration was not significant (F = 3.55; df = 2, 54; $P < 0.06$).

Similar to *T. castaneum* larvae, the dispersal of large *T. variabile* larvae was significantly affected by LLIN exposure (F = 73.5; df = 1, 54; $P < 0.0001$; Fig. 3-4), with 2-fold fewer LLIN-exposed individuals dispersing compared to controls. The dispersal distance also significantly affected the number of large larvae reaching a novel food patch (F = 87.5; df = 2, 54; $P < 0.0001$). Two-fold and 4-fold large larvae reached a novel food patch when placed 25 and 75 cm away, respectively, compared to 10 cm (Fig. 3-4). While the post-exposure holding duration also significantly affected dispersal of large larvae (F = 4.48; df = 2, 54; $P < 0.05$), there was an 8-fold reduction in the number reaching a novel food resource at 10 min and 24 h after exposure compared to if they were given the opportunity to disperse immediately. Importantly, the two-way interaction between type of netting and dispersal distance did not significantly affect the number of dispersing large larvae (F = 0.22; df = 2, 54; $P = 0.80$), with a 1.5-, 2.4-, and 8.1-fold reduction in the number of LLIN-exposed larvae reaching a novel food source compared to controls at 10, 25, and 75 cm, respectively. There was also a significant effect of the post-exposure holding duration on the number of dispersing larvae (F = 9.35; df = 2, 54; $P < 0.001$), with fewer large larvae able to disperse immediately after exposure and 24 h later compared with 10 min later.

LLIN-exposure to *T. variabile* adults had a pronounced negative effect on dispersal to novel food patches (F = 1185; df = 1, 36; $P < 0.0001$; Fig. 3-4), with a 22-fold decrease in the number of adults making it across after exposure compared to controls. Likewise, the increasing dispersal distances negatively affected the number successfully reaching a novel food patch (F = 63.7; df = 2, 36; $P < 0.0001$), with 1.2-fold and 2.2-fold fewer adults successful at 25 and 75 cm compared to 10 cm. The post-exposure holding duration did not affect the number of dispersing adults (*F* = 1.66; df = 2, 36; *P* < 0.20). There was a significant interaction between type of netting and distance on the number of dispersing individuals (*F* = 37.8; df = 2, 36; *P* < 0.0001), but primarily manifested as a decreasing number of control netting-exposed adults reaching a novel food patch with increasing distance; importantly, there were 14-fold and 24-fold fewer LLIN-exposed adults that made it across compared to control netting-exposed adults at 10 and 25 cm, while not a single LLIN-exposed adult was able to reach the novel food patch at 75 cm. The two-way interaction between type of netting and post-exposure holding duration on dispersal was not significant (*F* = 1.46; df = 2, 36; *P* < 0.25).

Overall, the number of dispersing *T. castaneum* adults after contact with LLIN was 8-fold lower than larvae (Chi-squared test, Table 3-5). With increasing dispersal distance, adults maintained at a relatively constant 93-98% reduction in movement, while the dispersal of LLIN-exposed larvae was progressively reduced by 21% at 10 cm to 93% at 75 cm (Table 3-5). Regardless of holding duration after exposure to LLIN, adults moved 6–44-fold less compared to larvae (Table 3-6).

Likewise, the number of dispersing *T. variabile* adults was 10-fold lower than large and small larvae (Chi-squared test, Table 3-5). While there were 10-fold fewer adults that successfully dispersed to a novel food patch after LLIN exposure than larvae at 10 and 25 cm, not a single LLIN-exposed adult dispersed at 75 cm. Regardless of holding duration after LLIN exposure, 6–45-fold fewer adults than larvae dispersed to novel food patches (Table 3-6).

Discussion

Prior work has documented the utility of pyrethroid-incorporated LLIN (e.g. tradename: Carifend ®, α-cypermethrin; D-Terrence ®, deltamethrin) in laboratory tests to induce mortality in multiple species of adult stored product pests (Rumbos et al. 2018; Paloukas et al. 2020; Morrison et al. 2018). Adult *T. castaneum* and *R. dominica* exhibited multiple-fold decreases in movement and dispersal after exposure to deltamethrin-based LLIN (Morrison et al. 2018). Further, traps comprised of the α-cypermethrin-based LLIN were effective at protecting the long-term storage of tobacco against adult insects in semi-field and commercial facilities (Athanassiou et al. 2019). Notably, these prior studies have restricted their evaluation to adult stored product insects. Ours is the first study to assess differential susceptibility to LLIN against stored product insect larvae. Overall, we found that LLIN exposure more dramatically reduced adult movement and dispersal capacity compared to larvae.

Specifically, we have evaluated total distance moved and mean velocity as two measures of movement for adults and larvae after exposure to LLIN. These are important variables that mediate immigration into food facilities, foraging, mate-finding, dispersal, and other important biological processes. At 30°C, *T. castaneum* only spends 4% of its life cycle in the larval stage (Brown et al. 2009), while *T. variabile* spends 85% of its life cycle as larvae (Partida and Strong 1975). However, regardless of specific differences in life history, adults are considered the dispersing stage for both species (Hagstrum and Subramanyam 2006). Because adults of both species were generally more susceptible than larvae, targeting with LLIN thus has the potential to disrupt important events in the life cycle, including colonization of grain stores and food facilities from the landscape.

In a more realistic test of how LLIN may affect dispersal capacity to novel food patches, our data from the dispersal assay mimicked exposure to LLIN as insects enter a food facility with the potential food source being located a relatively trivial distance away. It is likely that in many cases inside food facilities the resources might be much further away, because food facilities often represent sprawling complexes with multiple buildings. However, despite the trivial distance, both LLIN-exposed larvae and adults, but especially the latter, had difficulty reaching the novel food source even though many or most of the control netting-exposed individuals made it across. In prior work, although knockdown of stored product adults was not immediate (D. S. Scheff, personal communication), LLIN-exposed *R. dominica* adults were found to be incapable of dispersing 25–175 cm in an equivalent assay (Morrison et al. 2018). Thus, it appears that the dispersal capacity of stored product insects will be significantly impaired after brief contact with LLIN.

Differences in behavioral responses to LLIN after contact by insects may be species-specific and life stage-specific. For example, *T. variabile* adults appeared to experience greater reductions in mobility compared to *T. castaneum* adults (e.g., Table 1 and 2). Other work has found that there may be species-specific responses to insecticides, for example with greater larval mortality for the khapra beetle, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) than *T. variabile* after contact with an insecticide (Ghimire et al. 2017), despite the fact that both are closely related (Castalanelli et al. 2012). In addition, there may be life stage-specific differences in response to stressors. In this study, for example, we found *T. variabile* larvae were less susceptible than adults to LLIN exposure. Previous work in evaluating life stage-based susceptibility to other pyrethrin or pyrethroid products is consistent with our findings, but these studies typically provided a food source for the individuals after insecticide exposure (Kharel et al. 2014; Athanassiou et al. 2015; Ghimire et al. 2016). For example, Ghimire et al. (2016) found that *T. variabile* adults are over 3-fold more susceptible to deltamethrin, the active ingredient in the LLIN used in the current study, in residual contact trials compared to larvae. Similarly, Arthur and Fontenot (2012) determined that *T. castaneum* larvae were more susceptible than adults when exposed to chlorfenapyr residual on partially treated concrete arenas. However, those authors concluded that the mobility of life stages contributed to their differential susceptibility. Our study has shown that *T. variabile* larvae are actually more mobile than adults, while *T. castaneum* larvae are far less mobile than adults. Nevertheless, in the current study, the biological importance of these life stage-specific differences may be minimal, because the dispersal capacities for both species were significantly and similarly impaired. Thus, it is likely for both species that the reduced movement will lead to reduced ability to infest stored products.

There are a variety of ways that LLIN may be integrated into the IPM programs of food facilities. For example, LLIN may be deployed over external vents, eaves, and openings of food facilities as a barrier against insects immigrating from the landscape, particularly adult stages. Our research shows that brief exposure to only a single layer of LLIN is sufficient to significantly affect dispersal ability. Additionally, LLIN may be deployed over windows, doorways, and other internal partitions inside food facilities. This LLIN deployment method will help localize the infestation to one area of the facility. Finally, pallets of product that are brought into warehouses may be wrapped with LLIN. This will prevent insect dispersal into and out of pallets of goods. However, future work should assess whether there are differences in efficacy associated with each of these deployment procedures. At a minimum, our current work provides insight into how dispersing life stages may be controlled by LLIN.

While we have demonstrated that the use of LLIN is very effective at reducing movement and dispersal of adults and even larvae, to a lesser extent, under controlled conditions, there are still several outstanding questions about the implementation. For example, it is currently unknown whether LLIN deployment method affects efficacy (e.g., on a vent, covering a pallet directly, etc.). In addition, there is little information about whether the systematic use of LLIN is able to intercept immigrating insects from the landscape to reduce commodity infestation in bins or infestation of structures such as warehouses or food facilities, though the data from this study and related work suggests that it is likely. Future work investigating these avenues will be relevant for determining the widescale applicability of LLIN for use in food facilities. In the meantime, LLIN is a promising new IPM tactic that warrants further consideration in the post-harvest environment.

Table 3-1. Relative susceptibility in distance moved by *T. castaneum* and *T. variabile* adults and larvae depending on holding duration after exposure to long-lasting insecticide incorporated netting (LLIN) in the movement assay. Lowercase letters represent comparisons between larvae and adults for percent distance moved compared to controls, while uppercase letters represent comparisons between larvae and adults in percent reduction in distance moved (Chi-Square, α = 0.05). All comparisons are within a specific post-exposure holding duration.

	LLIN-exposed Larvae		**LLIN-exposed Adults**	
Exposure Time	Relative % Distance Moved[1]	Relative % Reduction in Distance Moved[2]	Relative % Distance Moved[1]	Relative % Reduction in Distance Moved[2]
T. castaneum				
1	35.7a	64.3A	36.1a	63.9A
5	37.7a	62.3A	25.7a	74.3A
10	9.9b	90.1A	29.5a	70.5A
Overall	27.8a	72.2A	30.5a	69.5A
T. variabile				
1	55.9a	44.1B	16.5b	83.5A
5	26.1a	73.9A	16.2a	83.8A
10	16.5a	83.5A	2.1b	97.9A
Overall	32.8a	67.2A	11.6b	88.4A

[1] The distance that LLIN-exposed individuals moved as a percent of the distance moved by control netting-exposed individuals in the Movement Assay.

[2] The percent reduction in distance moved by LLIN-exposed individuals relative to control netting-exposed individuals in the Movement Assay.

Table 3-2. Relative susceptibility in distance moved by *T. castaneum* and *T. variabile* adults and larvae depending on holding duration after exposure to long-lasting insecticide incorporated netting (LLIN) in the movement assay. Lowercase letters represent comparisons between larvae and adults for percent distance moved compared to controls, while uppercase letters represent comparisons between larvae and adults in percent reduction in distance moved (Chi-Square, α = 0.05). All comparisons are within a specific post-exposure holding duration.

	Larvae		**Adults**	
Post-Exposure Holding Duration	Relative % Distance Moved[1]	Relative % Reduction in Distance Moved[2]	Relative % Distance Moved[1]	Relative % Reduction in Distance Moved[2]
T. castaneum				
1	40.9a	59.1A	52.6a	47.4A
24	26.6a	73.4A	13.4b	86.6A
72	1.8b	98.2A	14.6a	85.4A
168	26.1b	73.9A	42.9a	57.1A
Overall	23.9a	76.1A	30.9a	69.1A
T. variabile				
1	56.8a	43.2B	11.9b	88.1A
24	8.6a	91.4A	7.1a	92.9A
72	23.9a	76.1A	16.7a	83.3A
168	32.4a	67.6A	15.4b	84.6A
Overall	30.4a	69.6A	12.8b	87.2A

[1] The distance that LLIN-exposed individuals moved as a percent of the distance moved by control netting-exposed individuals in the Movement Assay.

[2] The percent reduction in distance moved by LLIN-exposed individuals relative to control netting-exposed individuals in the Movement Assay.

Table 3-3. Relative susceptibility in velocity of *T. castaneum* and *T. variabile* adults and larvae depending on exposure time to long-lasting insecticide incorporated netting (LLIN) in the movement assay. Lowercase letters represent comparisons between larvae and adults for relative percent velocity compared to controls, while uppercase letters represent comparisons between larvae and adults for percent reduction in velocity (Chi-Square, α = 0.05). All comparisons are within a specific exposure time.

	LLIN-exposed Larvae		**LLIN-exposed Adults**	
Exposure Time	Relative % Velocity[1]	Relative % Reduction in Velocity[2]	Relative % Velocity[1]	Relative % Reduction in Velocity[2]
T. castaneum				
1	36.0a	64.0A	36.1a	63.9A
5	37.7a	62.3A	25.7a	74.3A
10	12.3b	87.7A	29.5a	70.5A
Overall	28.7a	71.3A	30.5a	69.5A
T. variabile				
1	55.3a	44.7B	16.4b	83.6A
5	26.6a	73.4A	15.2a	84.8A
10	16.5a	83.5A	2.1b	97.9A
Overall	32.8a	67.2A	11.2b	88.8A

[1] The velocity that LLIN-exposed individuals moved as a percent of the velocity moved by control netting-exposed individuals in the Movement Assay.

[2] The percent reduction in velocity moved by LLIN-exposed individuals relative to control netting-exposed individuals in the Movement Assay.

Table 3-4. Relative susceptibility in velocity of *T. castaneum* and *T. variabile* adults and larvae depending on holding duration after exposure to long-lasting insecticide incorporated netting (LLIN) in the movement assay. Lowercase letters represent comparisons between larvae and adults for relative percent velocity compared to controls, while uppercase letters represent comparisons between larvae and adults for percent reduction in velocity (Chi-Square, α = 0.05). All comparisons are within a specific post-exposure holding duration.

	Larvae		**Adults**	
Post-Exposure Holding Duration	Relative % Velocity[1]	Relative % Reduction in Velocity[2]	Relative % Velocity[1]	Relative % Reduction in Velocity[2]
T. castaneum				
1	43.2a	56.8A	52.6a	47.4A
24	26.1a	73.9A	13.4b	86.6A
72	1.8b	98.2A	14.6a	85.4A
168	26.7a	73.3A	42.9a	57.1A
Overall	23.7a	76.3A	26.9a	73.1A
T. variabile				
1	56.5a	43.5B	11.3b	88.7A
24	8.6a	91.4A	7.0a	93.0A
72	23.9a	76.1A	16.7a	83.3A
168	32.2a	67.8A	15.3b	84.7A
Overall	29.6a	70.4A	11.7b	88.3A

[1] The velocity that LLIN-exposed individuals moved as a percent of the velocity moved by control netting-exposed individuals in the Movement Assay.

[2] The percent reduction in velocity moved by LLIN-exposed individuals relative to control netting-exposed individuals in the Movement Assay.

Table 3-5. Relative susceptibility in dispersal ability of *T. castaneum* and *T. variabile* adults and larvae depending on dispersal distance after exposure to long-lasting insecticide incorporated netting (LLIN) in the dispersal assay. Lowercase letters represent comparisons between larvae and adults for relative percent dispersal compared to controls, while uppercase letters represent comparisons between larvae and adults for percent reduction in dispersal, while (Chi-Square, α = 0.05). All comparisons are within a specific dispersal distance.

	Small Larvae		**Large Larvae**		**Adults**	
Dispersal Distance	Relative % Dispersal[1]	Relative % Reduction in Dispersal[2]	Relative % Dispersal[1]	Relative % Reduction in Dispersal[2]	Relative % Dispersal[1]	Relative % Reduction in Dispersal[2]
T. castaneum						
10	-	-	79.0[3]	21.0[3]	-	-
25	-	-	39.2a	60.8B	6.6b	93.4A
75	-	-	6.7a	93.3A	7.0a	93.0A
175	-	-	-	-	1.97[3]	98.0[3]
Overall	-	-	41.6a	58.4B	5.2b	94.8A
T. variabile						
10	127.7a	-27.7C	69.6b	30.4B	7.2c	92.8A
25	16.7b	83.3A	41.4a	58.6B	4.1c	95.9A
75	26.3a	73.7B	12.4b	87.6AB	0.0c	100A
Overall	72.a	27.3C	41.1b	58.9B	3.8c	96.2A

[1] The number of dispersing individuals that made it to novel food patches after LLIN-exposure as a percent of the number of dispersing individuals after control netting-exposure in the Dispersal Assay.

[2] The percent reduction in successfully dispersing individuals after LLIN-exposure relative to control netting-exposed individuals in the Dispersal Assay.

[3] Statistical comparisons not possible between these life stages for dispersal distance.

Table 3-6. Relative susceptibility in dispersal ability of *T. castaneum* and *T. variabile* adults and larvae depending on post-exposure holding duration after contact with long-lasting insecticide incorporated netting (LLIN) in the dispersal assay. Lowercase letters represent comparisons between larvae and adults for relative percent dispersal compared to controls, while uppercase letters represent comparisons between larvae and adults for percent reduction in dispersal, while (Chi-Square, α = 0.05). All comparisons are within a specific post-exposure holding duration.

	Small Larvae		**Large Larvae**		**Adults**	
Post-exposure Holding Duration	Relative % Dispersal[1]	Relative % Reduction in Dispersal[2]	Relative % Dispersal[1]	Relative % Reduction in Dispersal[2]	Relative % Dispersal[1]	Relative % Reduction in Dispersal[2]
T. castaneum						
1 min	-	-	55.7a	44.3B	9.2b	90.8A
10 min	-	-	40.4a	59.6B	5.9b	94.1A
24 h	-	-	40.0a	60.0B	0.9b	99.1A
Overall	-	-	45.3a	54.7B	5.3b	94.7A
T. variabile						
1 min	47.1a	52.9BC	63.6a	36.4C	11.5b	88.5A
10 min	94.7a	5.3C	67.7b	32.3B	1.5c	98.5A
24 h	14.2a	42.3B	23.3a	76.7A	0.8b	99.2A
Overall	72.7a	27.3C	51.5a	48.5B	4.6b	95.4A

[1] The number of dispersing individuals that made it to novel food patches after LLIN-exposure as a percent of the number of dispersing individuals after control netting-exposure in the Dispersal Assay.

[2] The percent reduction in successfully dispersing individuals after LLIN-exposure relative to control netting-exposed individuals in the Dispersal Assay.

Figure 3-1. The distance moved (± SE) by *Tribolium castaneum* (top) or *Trogoderma variabile* (bottom) adults and large larvae after varying exposure times (1–10 min, left column) and over time (1–168 h after exposure, right column) to control or long-lasting insecticide-incorporated netting during 2 h trials in the laboratory. Uppercase letters indicate pairwise comparisons among treatments for adults, while lowercase letters represent comparisons among treatments for larvae (Tukey HSD, α = 0.05). Bars with shared letters are not significantly different from each other.

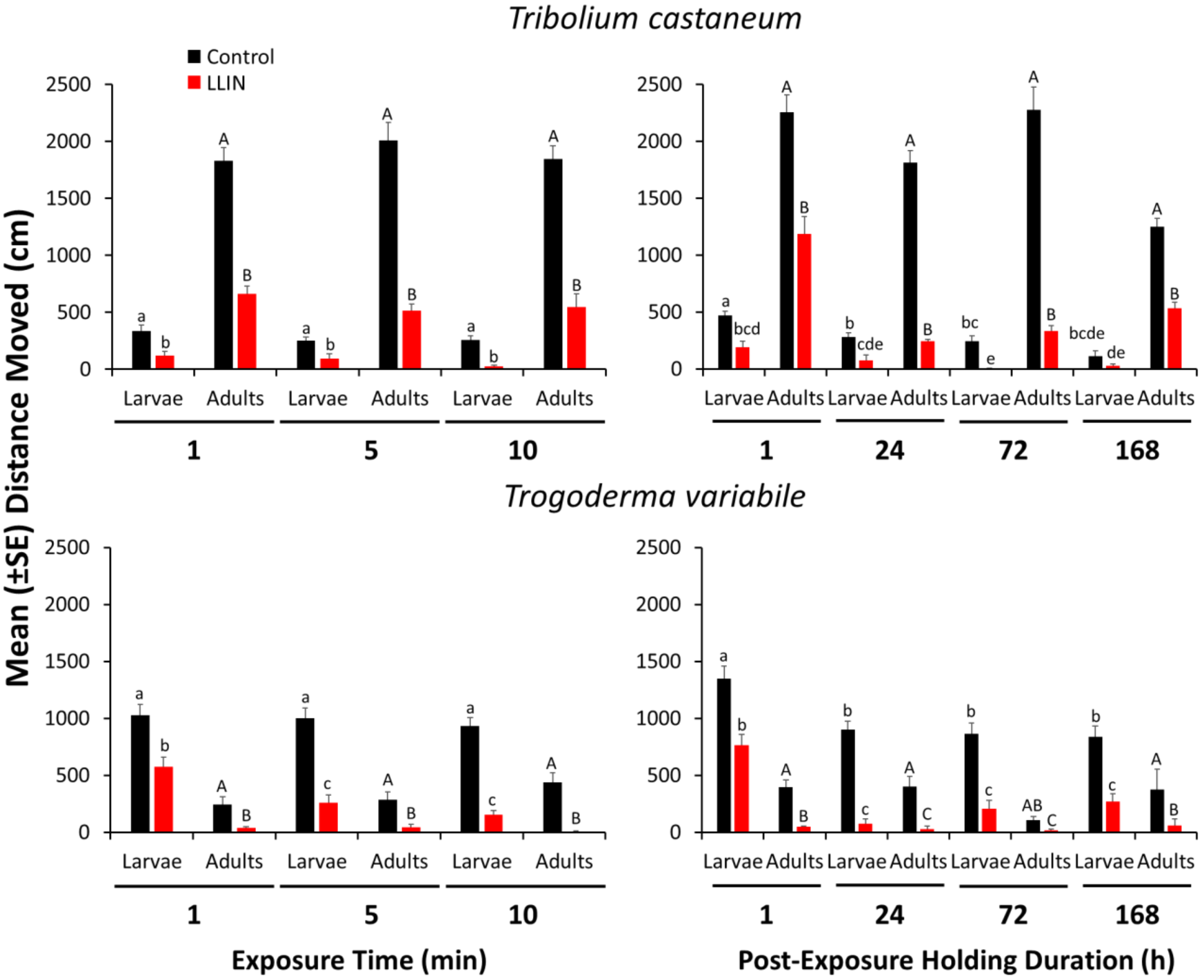


Figure 3-2. The mean instantaneous velocity (± SE) by *Tribolium castaneum* (top) or *Trogoderma variabile* (bottom) adults and large larvae after varying exposure times (1–10 min, left column) and over time (1–168 h after exposure, right column) to control or long-lasting insecticide-incorporated netting during 2 h trials in the laboratory. Uppercase letters indicate pairwise comparisons among treatments for adults, while lowercase letters represent comparisons among treatments for larvae (Tukey HSD, α = 0.05). Bars with shared letters are not significantly different from each other.

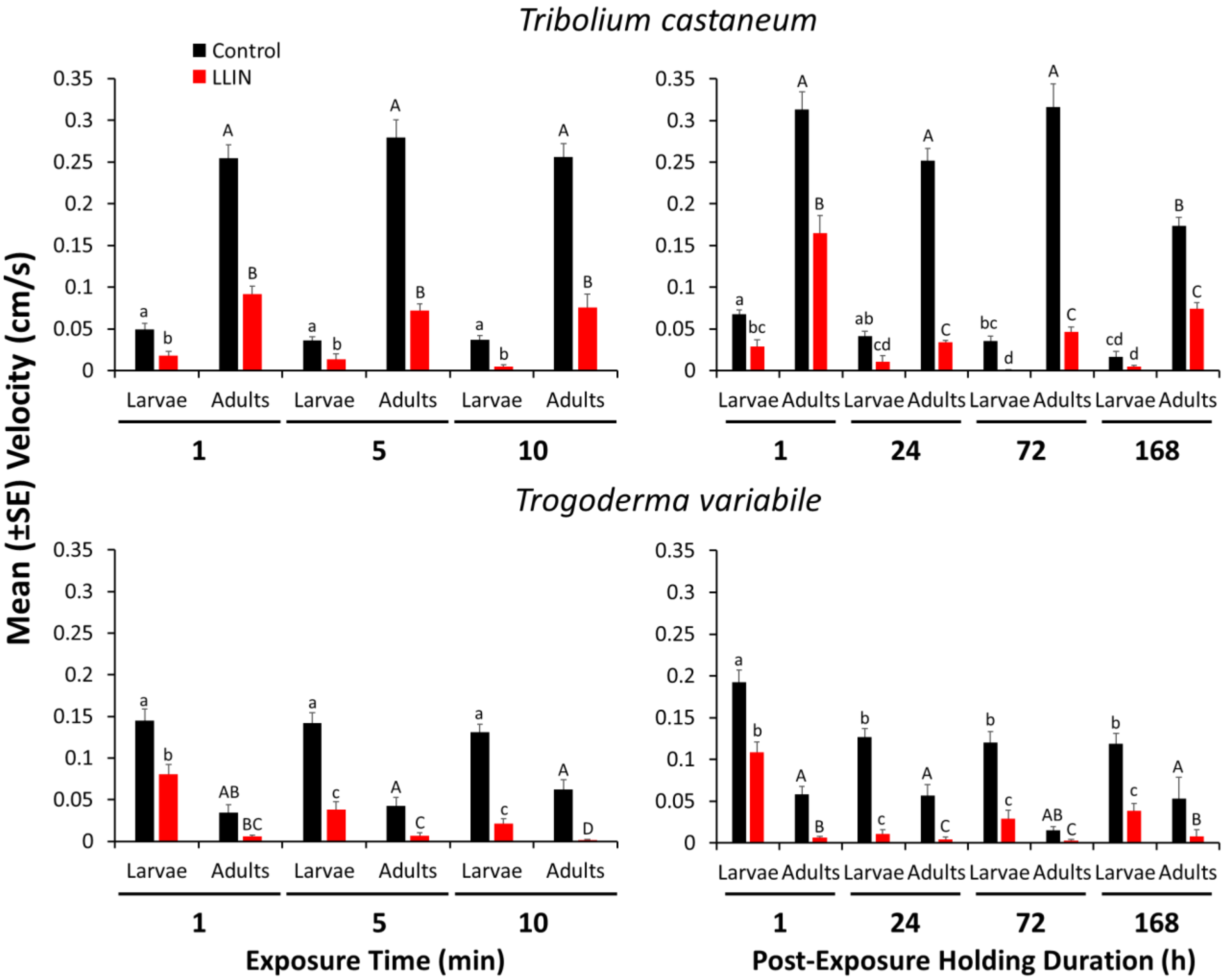


Figure 3-3. The mean percentage of *Tribolium castaneum* adults and larvae reaching a novel food resource after 48 h, depending on distance that they had to travel (top) or the post-exposure holding duration prior to dispersal opportunity (bottom) after cohorts of 20 adults were exposed for 5 min exposure to control or long-lasting insecticide-incorporated netting in environmental chambers at 30°C and 65% RH. Each bar represents the mean of n = 12 (larvae) or n = 18 (adults) replicates. Uppercase letters indicate pairwise comparisons among treatments for adults, while lowercase letters represent comparisons among treatments for larvae (Tukey HSD, α = 0.05). Bars with shared letters are not significantly different from each other. Larvae were given the opportunity to disperse at 10, 25, and 75 cm, while adults were given the opportunity to disperse at 25, 75, 175 cm based on prior information about the dispersal capacity of each life stage.

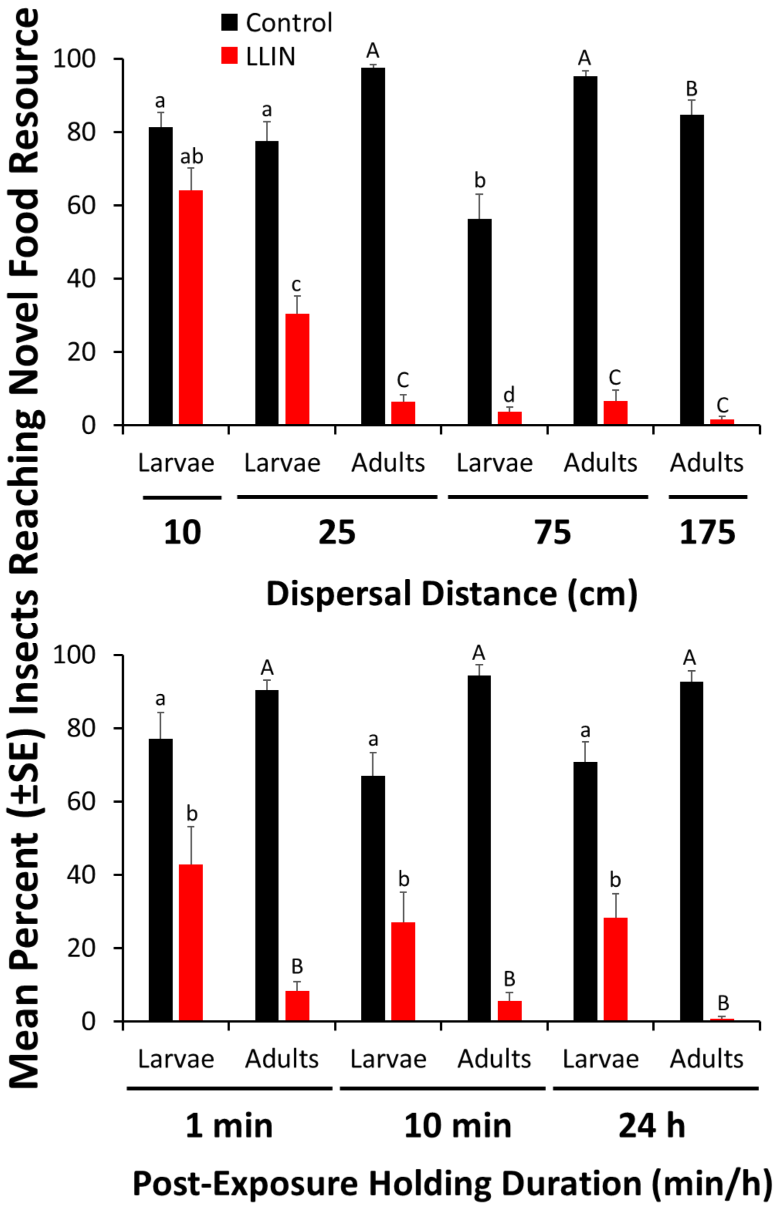
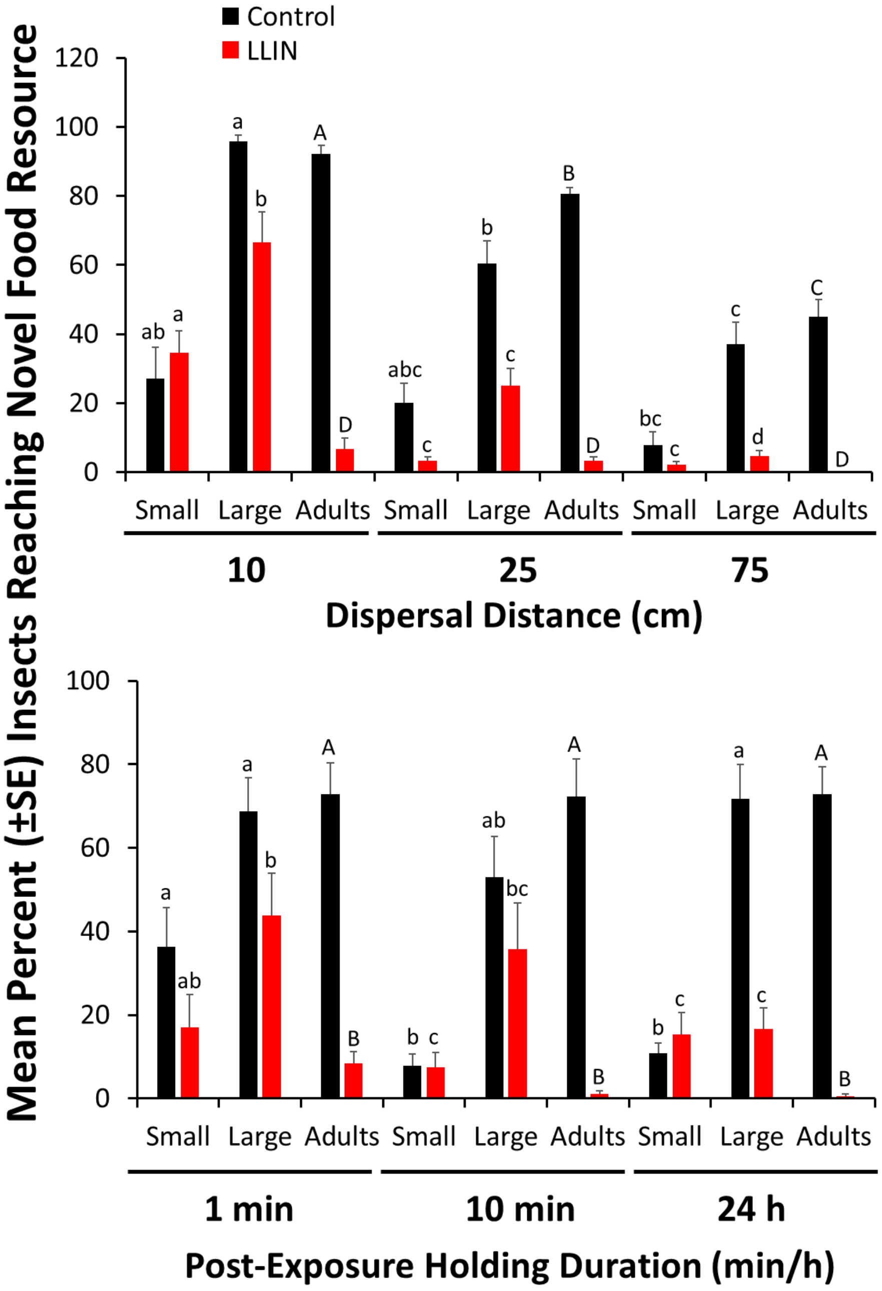


Figure 3-4. The mean percentage of *Trogoderma variabile* adults and small or large larvae reaching a novel food resource after 48 h, depending on distance that they had to travel (top) or the post-exposure holding duration prior to dispersal opportunity (bottom) after cohorts of 20 adults were exposed for 5 min exposure to control or long-lasting insecticide-incorporated netting in environmental chambers at 30°C and 65% RH. Each bar represents the mean of n = 12 (small larvae, large larvae, or adult) replicates. Uppercase letters indicate pairwise comparisons among treatments for adults, while lowercase letters represent comparisons among treatments for larvae (Tukey HSD, α = 0.05). Bars with shared letters are not significantly different from each other.



Chapter 4 - The use of long-lasting insecticide-incorporated netting and interception traps at pilot-scale warehouses and commercial facilities to prevent infestation by stored product beetles

(This chapter was originally submitted as follows prior to disposition of thesis: Wilkins, R. V., J. F. Campbell, K. Y. Zhu, L. A. Starkus, T. McKay, and W. R. Morrison III. 2020. The use of long-lasting insecticide-incorporated netting and interception traps at pilot-scale warehouses and commercial facilities to prevent infestation by stored product beetles. Agric. Ecosys. Env., submitted.)

Introduction

Stored product integrated pest management (IPM) ideally attempts to holistically combine different management tactics to control insects as commodities are harvested, transported, stored, processed, and marketed to end consumers. At any point along this supply chain, commodities are vulnerable to insect infestation (Kumar and Kalita 2017). Globally, insect feeding and damage accounts for approximately $100 billion USD in postharvest losses (Wacker 2018), thus, developing an effective management strategy to reduce these economic losses is crucial. Fumigation is the most common chemical control tactic once insects have entered and infested commodities, and food facilities, including bulk storage, are routinely fumigated on a calendar basis (Espino et al. 2014). Methyl bromide, historically one of the most common structural fumigants, was banned in 2005 by the Montreal Protocol after being declared an ozone-depleting substance (Fields and White 2002). Phosphine has remained a commonly used fumigant for product fumigations, but it is highly corrosive against electrical equipment which limits its application for structural fumigations. Meanwhile, insects are becoming increasingly resistant to phosphine worldwide (Zhao et al. 2015; Huang et al. 2018; Schlipalius et al. 2018). On the other side of the post-harvest supply chain, there is a high demand for organic or low-insecticide products by consumers, who are willing to pay a price premium (Batte et al. 2007). Thus, a central drive in stored product IPM has been increasing the efficiency of preventative management tactics to avoid insect infestations, while reducing the need for remedial chemical control tactics.

Insect movement and dispersal in the landscape around food facilities presents a serious challenge to existing chemical control tactics. For example, fumigation only kills insects currently present in a grain mass or structure, but efficacy may be short-lived as insects quickly disperse into the facility from refugia in the surrounding landscape (Roesli et al. 2003; Campbell and Arbogast 2004). Insect abundance outside storage bins full of grain has been found to be greater than bins that are empty, suggesting that the abundant quantity of commodities in a spatially circumscribed space makes bulk storage and food facilities a strong attractant for insects in the landscape (Vela-Coiffier et al. 1997). Prior trapping studies have indicated high species diversity outside facilities, yet due to the numerous landscape features present and commodities handled, the patchy distribution of these species makes monitoring for them particularly challenging (Semeao et al. 2013a; McKay et al. 2017).

Prior work has made it clear that stored product insects are highly mobile. For example, release-recapture studies have found that *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) have an average dispersal capacity of 380 m in the field (Ching'oma et al. 2006). Other work documenting dispersal in different landscapes found *R. dominica* dispersed 337–375 m and were more often recaptured in wooded sites, whereas they typically dispersed 261–333 m in open sites (Mahroof et al. 2010). In Australia, *R. dominica* was found in traps throughout the landscape, while *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) was localized around grain storage facilities (Daglish et al. 2017). Even where resources are abundant, *T. castaneum* apparently leaves and returns to the same area, suggesting regular, and frequent exchange of individuals between food facilities and the surrounding landscape (Rafter et al. 2019). Pheromone trap captures of *T. castaneum* outside a food facility were reduced after fumigation treatments, and mark-recapture data found that *P. interpunctella* (Hübner) (Lepidoptera: Pyralidae) from outside had immigrated inside facilities, further suggesting that inside populations are connected to those outside (Campbell and Arbogast 2004; Buckman et al. 2013). Additionally, *T. castaneum* had low genetic differentiation between field and storage facility captures, indicating that populations inside facilities are readily dispersing outside (Ridley et al. 2011). Thus, it appears that insect movement to and from food facilities may be common, and it may occur over long distances.

Even inside a food facility, there may be significant movement of stored product insects. For instance, *Trogoderma variabile* Ballion (Coleoptera: Dermestidae) is capable of moving between floors and can travel an average distance of 21 m (Campbell et al. 2002). *Tribolium castaneum* is highly mobile between floors as well, typically moving downward even in a relatively well-sealed facility (Semeao et al. 2013b). Therefore, developing and optimizing methods to intercept these insects as they disperse and move into and around facilities is key for preventative stored product IPM.

Long-lasting insecticide-incorporated netting (LLIN) may be a particularly efficacious tactic for intercepting stored product insects prior to entering food facilities or even halting movement of insects between different parts of facilities, which may contribute to decreased infestation of commodities. Historically, insecticide-treated netting has been successfully used as bed nets to kill mosquitoes and reduce the spread of malaria in tropical regions of Africa (Alonso et al. 1991; Martin et al. 2007). The efficacy of the netting has typically spanned multiple years and is relatively inexpensive to replace (Dev et al. 2010; Hailu et al. 2018). Recent studies in apple production have incorporated LLIN as a kill mechanism in monitoring traps against the invasive *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (Kuhar et al. 2017; Peverieri et al. 2017).

The netting has also been used as a preventative measure against pests of specialty crops and ornamental trees (Marianelli et al. 2018; Ranger et al. 2020). These studies support the idea that LLIN can be used to intercept insects prior to reaching their destination and causing damage. In a postharvest setting, recent work has shown that LLIN significantly causes mortality of stored product insects, including *Lasioderma serricorne* (F.) (Coleoptera: Ptinidae) (Rumbos et al. 2018), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Anaclerio et al. 2018), *R. dominica*, *T. castaneum* (Morrison et al. 2018), *T. variabile*, and other species (Paloukas et al. 2020), including different life stages (Wilkins et al. 2020). Further, LLIN has been documented to reduce movement and dispersal ability by multiple-fold over long periods even after just brief exposure times compared to untreated netting controls (Morrison et al. 2018). Importantly, only a single field study with LLIN has been performed in the postharvest environment, which showed that an α-cypermethrin-incorporated LLIN could be successfully deployed as a cube encasing tobacco to protect against *Ephestia elutella* (Hübner) (Lepidoptera: Pyralidae) and *L. serricorne* (Rumbos et al. 2018). Thus, there has been relatively little semi-field or field research regarding the application method of LLIN for intercepting stored product insects.

There are several possible ways to imagine LLIN being deployed in the post-harvest environment. One method may be to cover external vents and openings on a building, which would provide a large distance between the LLIN deployment location and the commodity. Another deployment method may be as a wrap for a pallet of goods in a warehouse, which would necessitate close contact with the product. A final alternative method may be as a screen on windows, partitions, and doors between different parts of a facility, which may provide an intermediate amount of distance between the LLIN and the commodity. However, to date, no study has assessed whether deployment in these different ways affects LLIN efficacy at preventing infestation and/or progeny production in stored products.

One concern with the use of LLIN at food facilities is that insects that are exposed may be insufficiently dosed to induce knockdown or kill. Nonetheless, this limitation may be potentially circumvented by exploiting kairomones, pheromones, and other semiochemicals to attract stored product insects and help ensure prolonged or repeated exposures (e.g. multiple dosings) to LLIN. However, food facility managers are generally concerned about deploying attractive compounds adjacent to where commodities are stored, so an ideal option would be to include an efficient kill mechanism (e.g., LLIN) and attractive stimuli in an interception trap that can be deployed on the perimeter of the facility to divert immigrating insects from the landscape. An interception trap may provide an added layer of protection to a food facility if used in combination with LLIN on vents and openings along with other ongoing IPM protocols. Previous research has already documented a plethora of volatile compounds that elicit positive responses from multiple stored product species, including *T. castaneum*, *R. dominica*, *Trogoderma* spp., *Sitophilus* spp. (Coleoptera: Curculionidae), and moth pests (Burkholder 1985; Cox 2004; Balakrishnan et al. 2016; Morrison et al. 2020). These interception traps may function as miniature attract-and-kill (AK) traps, whereby the pest population is attracted to a spatially circumscribed area and removed from the foraging population with a kill mechanism (Gregg et al. 2018). In other agricultural systems, AK traps have been employed to intercept a multitude of pest insect species before they contact valuable specialty crops (Charmillot et al. 2000; Kroschel and Zegarra 2009; Morrison et al. 2016b, 2019). Whether used alone or in combination with other control tactics, AK may be successful in reducing insect damage to commodities (El-Sayed et al. 2009). AK traps are most efficient when used against small to moderate population sizes (Charmillot et al. 2000; El-Sayed et al. 2009), which is likely the case for diffuse populations of stored product insects in the landscape. As a result, interception traps, like attract-and-kill, may be a strong perimeter management tool that pairs well with other uses of LLIN in food facilities, as part of a comprehensive IPM program.

Therefore, the goal of the current study was to 1) examine the ability of interception traps to capture stored product insects at commercial wheat and rice food facilities, 2) assess whether LLIN deployment method affected efficacy in preventing infestation by stored product insects in pilot-scale warehouses, and 3) determine the success of using LLIN alone, interception traps alone, or both together to prevent infestations.

Materials and Methods

Source Insects

Insects used for these experiments were reared in an environmental chamber held under constant conditions (27.5°C, 60% RH, 14:10 L:D). In particular, *T. castaneum* (field-derived strain from central Kansas in 2012) was reared on 95% organic flour and 5% brewer’s yeast, while *R. dominica* (field-derived strain from north-central Kansas in 2012) was reared on organic whole wheat, and *T. variabile* (field-derived strain from eastern KS in 2016) was reared on ground dog food (Lamb & Rice, Purina One, St. Louis, MO) and whole grain oats with a crumpled, damp paper towel placed on the surface of the diet in 828-ml containers filled two-thirds full with diet. For the assays below, 1–8-week old individuals were used.

Interception Trap Lure Treatments

A suite of potential attractants was tested in order to determine which were the most promising for inclusion in an interception trap to deploy at the perimeter of commercial food facilities. The behavioral response of *T. castaneum* and *R. dominica* to two kairomones and one commercial pheromone lure was assessed in two separate assays. The specific treatments included: 0.76 g of dried distillers' grains (DDGS), 950 µl wheat germ oil (WGO), and one, two, or three Stored Product Beetle tab lures (SPB tab; a broad-spectrum attractant for 20 species of stored product beetles, IL-2800-10, Insects Limited, Westfield, IN, USA) and were tested in the wind tunnel and release-recapture assays as described below.

Interception Trap Lures: Wind Tunnel Assay

To assess attraction among potential interception trap attractants, a laminar flow wind tunnel assay was conducted in an environmental chamber set at constant conditions (25°C; 65% RH). Either DDGS, WGO, or a single SPB tab were placed in a 100 mm × 10 mm petri dish without a lid and located 13.5 cm upwind of a release 21.6 × 27.9 cm release arena. An empty dish with no attractant was designated as the negative control. A single adult insect was released in the center of the arena and given 2 min to exit the arena. Insects leaving on the edge of the arena closest to the stimulus source (e.g. upwind edge) were denoted as a positive stimulus response while insects exiting from one of the other three sides of the arena were denoted as a non-stimulus response. Insects that did not exit the arena within the allotted time were excluded from the analysis. For each treatment, there were a total of n = 30 replicate individuals tested.

Interception Trap Lures: Release-Recapture Assay

To evaluate whether the deployment of the attractants above would result in higher captures in a trap, we employed a release-recapture assay conducted in large walk-in environmental chambers under constant conditions (4.88 × 5.81 × 2.43 m; 25ºC, 65% RH, 16:8 L:D). Treatments were placed in commercial pitfall traps (e.g. Storgard® Dome™ Trap, Trécé Inc., Adair, OK). An empty trap acted as a negative control. For *T. castaneum*, four pitfall traps were placed equidistant along the perimeter of the chamber. A total of 300 *T. castaneum* adults (1:1 M:F) were removed from colony jars 24 h before release and allowed to settle in an 8 × 8 cm square of corrugated cardboard. The following day, the cardboard refuge was placed in the center of the chamber and the adults were given 24 h to respond to the traps. The number of insects captured per trap treatment was recorded. There were a total of n = 8 replicates per treatment. Because *R. dominica* are not as mobile (e.g., Morrison et al. 2018), an altered release-recapture assay was performed as follows. In the same large walk-in chambers, each treatment was placed in a large individual plastic bin (86.3 × 39.4 × 30.5 cm L:W:H; Sterilite Corp., Townsend, MA, USA) whose bottom surface had been systematically scuffed up with sandpaper to allow for easy movement by insects. *R. dominica* adults were pulled from colony jars 24 h prior to the beginning of the experiment and allowed to settle in an 8 x 8 cm square of cardboard. A single pitfall trap was placed in a randomized opposite corner from where 20 *R. dominica* adults (mixed sex) were released. The adults were given 24 h to respond to the trap, and at the end, the number of insects captured by each trap was recorded. A total of n = 2 replicates with all treatments represented were run at a time, and over the course of the experiment there were a total n = 12 replicates per treatment.

Dose-Dependency in Attraction to Stimuli in Interception Trap

Following the assessment of attractants above, the SPB tab was deemed the most attractive lure for *T. castaneum* and *R. dominica* and a strong candidate for use as the primary attractant in interception traps. A follow-up experiment was conducted to assess whether increasing the number of SPB tabs would result in a dose-dependent increase in attraction by stored product insects to interception traps. For these assays, one, two, or three SPB tab lures were incorporated into petri dishes (wind tunnel assay) or commercial pitfall traps (release-recapture experiment) as described above. There were n = 30 replicate individuals tested for each treatment and species in the wind tunnel assay, and there were n = 24 replicates per treatment and species for the release-recapture assay.

Field Interception Trap Assay

To understand whether interception traps could be developed to prevent insects from immigrating into food facilities, spillage traps from prior work (e.g., Campbell et al. unpublished data) were constructed and modified as follows. The spillage traps (henceforth termed interception traps) were constructed with PVC pipe (5 cm length × 5.2 cm I.D.) filled with 60 g of crimped wheat kernels (Kansas) or a mixed variety of brown rice (Arkansas) as a kairomone source. LLIN (0.4% deltamethrin-incorporated, Vestergaard-Frandsen Inc., Lusanne, Switzerland) or control netting without insecticide acted as the kill mechanism in the interception traps. The attractant included in the interception traps was a single SPB tab placed on the surface of the grain mass. Two pieces of perforated metal plates held together by a screw, washer, and wingnut were arranged on both openings of the PVC pipe to hold in the grain (Figure 1). Two pieces of control netting or LLIN (6.4 cm diameter) were placed between the metal plates and the openings of the PVC pipe. Both the netting (2 mm I.D.; 49 holes/cm^2) and perforated metal plates (2 mm I.D.; 9 holes /cm^2) had openings large enough for insects from the surrounding refugia to enter the trap. Each trap corresponded to one of four treatments: control netting only (no insecticide) without attractant, control netting with attractant, LLIN only without attractant, LLIN with attractant. These four treatments made up one transect, and three transects were placed around the perimeters of each of the six food facilities in Kansas (n = 3) and Arkansas (n = 3) (Table 1). Traps were left out for 48 h, then retrieved, and the number of stored product insects found in each trap and their health conditions (alive, affected, or dead) were recorded according to prior definitions in the literature (Morrison et al. 2018). Trapping occurred roughly once every two weeks from 17 August 2018 to 21 September 2018 and 25 April 2019 to 18 September 2019 in Kansas, and from 31 May 2019 to 30 September 2019 in Arkansas. There were a total of n = 8–12 deployments at each site. In some cases, trapping dates were altered to account for adverse weather conditions. All captured insects were individually placed on 20 g of fresh wheat or brown rice, held at the above environmental chamber conditions, and their health condition was recorded a week later. The original amount of wheat was held for 6 weeks under the same conditions and checked for progeny production.

LLIN Deployment Assay

To understand whether the method by which LLIN was deployed affected subsequent commodity infestation and progeny production, pilot-scale warehouses (5.85 × 2.81 m) in Manhattan, KS were used. The temperature of the warehouse was monitored with a datalogger (HOBO UX-100, Onset Computers, Bourne, MA) at hourly intervals, with average temperature and RH at 24.5 ± 0.13˚C and 62.3 ± 0.57%, respectively, during the course of the experiment. At the far end of the warehouse against the back wall, a commodity consisting of a mixture of 210 mL organic, whole wheat kernels and 210 mL of organic, unbleached flour was placed in a lid-less plastic container (14 × 24 cm) with eight holes (0.32 cm diameter) drilled and equally placed around the bottom circumference of the container to allow for insect dispersal into the commodity. A total of 100 individuals each of *T. castaneum*, *R. dominica*, and *T. variabile* were released at the opposite end of the warehouse (approx. 5.25 m away). There were n = 12 replicate releases per treatment from 26 April 2019 to 16 August 2019, comprising a total of 3,600 released insects.

There were four LLIN deployment methods that were tested (Figure 2). In the "hanging" treatment, LLIN (2.72 × 2.41 m) was affixed to the warehouse ceiling and allowed to hang down to the floor, completely bisecting the room. This represented deployment of LLIN to partition two areas of a food facility, or as a screen for doors and windows. In the "cover" deployment method, LLIN was directly laid over the commodity, representing LLIN application on a pallet as a wrap to protect final products. In the "pipe" deployment method, a PVC pipe (91 cm length, 5.1 cm I.D.) was bisected halfway with LLIN to represent insects immigrating into a food facility through small openings such as vents, eaves, or crevices. These were compared with a control that used the same PVC pipe design, but without netting. For the pipe treatments, insects were released directly into one end of the pipe, and the release end was sealed off with parafilm. For the hanging and cover treatments, insects were released at an identical location but on the floor of the warehouse, 0.5 m away from the netting.

Insects were given 72 h to disperse across the warehouse to the commodity. After this period, insects were collected by pre-designated zones in the warehouse (Figure 2). The zones were noted respective to the location of the commodity, and included Zone 1 (inside the commodity), Zone 2 (0.5 m radius around commodity), Zone 3 (1 m radius around the commodity), Zone 4 (1–2.7 m), Zone 5 (2.7–4.5 m; approx. halfway), and Zone 6 (4.5 m–5.6 m, e.g. the release zone). For statistical analysis and discussion, the zones were reclassified as in commodity (e.g. Zone 1), partial dispersal (Zones 2–5), and no dispersal (Zone 6). The insects were retrieved, and then brought back to the lab where their health condition was assessed as alive, affected, or dead. The commodity was sieved (#10 sieve, 2.0 × 2.0 mm mesh, W.S. Tyler, Mentor, OH; then #25 sieve, 0.71 × 0.71 mm mesh, Fisher Scientific Co., Hampton, NH) for adult insects, whose number and health condition were recorded. The commodity was held for 6 weeks after deployment under the previously described environmental chamber conditions to evaluate progeny production. The species and health conditions of the progeny were recorded.

Combined Use of Interception Traps and LLIN

The final assay in this study also occurred in pilot-scale warehouses as described above and was intended to evaluate the efficacy of LLIN deployment and interception traps alone or together. There were four treatments in total applied to warehouses for this experiment: LLIN alone, AK-based interception trap alone, both together (AK + LLIN), or neither (e.g. a control treatment that had no netting or interception trap). The zones were similar to the descriptions above, but a Zone 7 was introduced which described insects captured inside the interception traps (Appendix A). For analysis, the zones were collapsed to their new definitions as above (e.g., in commodity, partial dispersal, no dispersal). White butcher paper was affixed to the floor of the warehouse to encourage mobility of the insects. To simulate the inside and outside environment of a food facility at a single warehouse, two wooden planks (1.7 m long) projected into the warehouse from the two corners of Zone 6 at a 50° angle in a funnel arrangement, leaving a 10 cm-wide gap between them in the center of the warehouse floor (Figure 3). No netting (e.g., control, or AK alone) or LLIN (for LLIN alone, or AK + LLIN) bridged the two wooden planks to create an unprotected or protected entrance for released insects to pass through. The boards were affixed to the floor and fluon (polytetrafluoroethylene, MilliporeSigma, Burlington, MA) was applied to the vertical sides of the wooden planks to prevent circumventing the netting by climbing insects. This presents a realistic scenario of an imperfectly sealed food facility, because flying insects in the experiment could circumvent the LLIN. In the interception trap only treatment or treatment with both tactics, the interception traps (as described above with LLIN and attractant) were placed 0.76 m in front of the simulated entrance, giving the insects the option to enter the warehouse or be diverted to the interception trap, as may happen under field conditions. For each treatment, insects were released in the two corners of Zone 6, approximately 1.5 m from the netting, and given 72 h to reach the commodity. Afterwards, the insects were collected by zone and their health condition was recorded. There were n = 12 replicate releases per treatment from 23 August 2019 to 8 November 2019. As in the previous assay, the commodity was sieved for adults and held for 6 weeks to check for progeny production.

Statistical Analysis

A generalized linear model with exit edge (stimulus or non-stimulus) for the wind tunnel or percentage of adults recaptured in the release-recapture assay was used as the response variables. Models were checked for overdispersion, which was found to be a problem, thus a quasibinomial (wind tunnel) or quasipoisson (release-recapture) with a logit-link function was used as the underlying distribution. The R package *multcomp* was used for multiple comparisons with a call to the *glht* function. R software was used for this and all analyses (R Core Team, 2019) with $\alpha = 0.05$, unless otherwise specified.

The total captures from interception traps were expressed as a percentage of total captures and compared using a χ^2-test with a Bonferroni correction to the α-threshold for significance. These were based on the main explanatory factors, including year (2018 or 2019), state of collection (KS or AR), interception trap configuration (LLIN only, Ctrl only, LLIN + lure, and Ctrl + lure). The null hypothesis assumed equal percentages among levels within categories. If preliminary analysis indicated no significant differences between years or states, the data were collapsed for the final analysis.

The number of adults found in each collapsed dispersing zone (in commodity, partial dispersal, and no dispersal) from the LLIN deployment assay were analyzed with a multivariate analysis of variance (MANOVA) using the health status (alive, affected, or dead) and treatment (control, hanging, cover, or pipe) as fixed, explanatory factors. Release date was used as a random blocking variable. Upon a significant result from the overall model, sequential ANOVAs were performed with the same model structure followed by Tukey HSD for multiple comparisons. In addition, a generalized linear model based on a quasipoisson distribution (to account for overdispersion) was used to determine changes in progeny production among the LLIN deployment treatments, followed by Tukey HSD upon a significant result from the overall model for multiple comparisons. The data from the combined tactic assay was analyzed in a similar manner with the exception of using tactic (LLIN alone, interception trap alone, both, or neither) as a fixed explanatory variable. Inspection of residuals and quantile-quantile plots confirmed that there were no significant deviations from normality or homogeneity of variances for normality-based tests.

Results

Interception Trap Attractant Assessment

In the wind tunnel, lures had a significant effect on attraction by *T. castaneum* ($\chi^2 = 27.5$; df = 3; $P < 0.0001$), with 2.2-fold more adults exiting on the stimulus edge of the SPB tab than for the negative control (Fig. 4-4). Likewise, the traps baited with different lures had a significant effect on recapture of *T. castaneum* ($\chi^2 = 24.8$; df = 3; $P < 0.0001$), with the SPB tab-baited traps capturing 2.8-fold more adults in the release-recapture experiment than control traps (Fig. 4-5).

Similar to *T. castaneum*, the lures had a significant effect on attraction by *R. dominica* in the wind tunnel ($\chi^2 = 27.1$; df = 3; $P < 0.0001$). In particular, the SPB tab resulted in 2.2-fold more *R. dominica* adults exiting on the stimulus edge compared to the negative control. The same pattern was observed in the release-recapture assay, where traps with lures had a significant effect on recapture of *R. dominica* ($\chi^2 = 54.3$; df = 3; $P < 0.0001$), and the greatest recapture was in traps baited with the SPB tab lure (Fig. 4-5).

Assessing for Dose-Dependency in Attraction to Interception Trap Lures

When including the negative control with no lures, the number of lures significantly affected attraction by *T. castaneum* ($\chi^2 = 13.6$; df = 3; $P < 0.01$) and *R. dominica* ($\chi^2 = 30.8$; df = 3; $P < 0.0001$) adults in the wind tunnel. In particular, between the negative control and a single lure, there were 1.5-fold and 2.2-fold increases in attraction by *T. castaneum* and *R. dominica,* respectively (Table 4-2). Importantly, there was no statistically significant benefit of adding additional lures beyond a single one (Table 4-2).

Likewise, the number of lures had a significant effect on capture of *T. castaneum* ($\chi^2 = 9.07$; df = 3; $P < 0.05$) and *R. dominica* ($\chi^2 = 10.0$; df = 3; $P < 0.05$) in baited traps in a release-recapture experiment. There were 3-fold and almost 6-fold more *T. castaneum* and *R. dominica* adults captured, respectively, in traps baited with a single lure compared to no lures. Importantly, there was no significant benefit from adding more lures to a trap (Table 4-2, χ^2-tests).

3.3 Field Interception Trap Assay

Captures of stored product insects in interception traps at the perimeter of facilities were significantly different by state ($\chi^2 = 6.55$; df = 1; $P < 0.05$). Thus, each state was analyzed separately for the main analysis. However, there was no significant effect of year on captures in interception traps ($\chi^2 = 0.82$; df = 1; $P = 0.37$), as a result the sampling years were collapsed for the final analysis. In total, over 3,000 insects were collected over the two years, representing 14 stored product insect taxa (Table 4-3). The interception trap configuration had a significant effect on captures in both Arkansas ($\chi^2 = 46.6$; df = 3; $P < 0.0001$) and Kansas ($\chi^2 = 94.5$; df = 3; $P < 0.0001$; Fig. 4-6). In Arkansas, there were 2.5–2.8-fold more stored products insects captured in interception traps with lures than without lures, while there were 89–100-fold more insects in Kansas interception traps. The use of LLIN appeared not to impede the colonization of traps by stored product insects. From 2018 to 2019, there were 20 weeks of insect captures, with average numbers of captures more variabile on a week-to-week basis in Arkansas than Kansas (Appendix A).

Progeny production after 6 weeks in interception traps was significantly different by state ($\chi^2 = 8.33$; df = 1; $P < 0.01$), so each was analyzed separately for the final analysis. Sampling year did not significantly affect progeny production in interception traps ($\chi^2 = 3.83$; df = 1; $P = 0.06$), thus year was collapsed for the final analysis. The configuration of the interception trap significantly affected progeny production for those deployed in both Kansas ($\chi^2 = 93.0$; df = 3; $P < 0.0001$), and Arkansas ($\chi^2 = 33.2$; df = 3; $P < 0.0001$). In Kansas, deployment of LLIN reduced progeny production by 99% in traps with stimuli compared to when control netting was used that lacked insecticide (Fig. 4-7). In Arkansas, LLIN deployment in interception traps reduced progeny production by 57% in traps with lures compared to when interception traps contained control netting.

Pilot-Scale Warehouse Trial 1: LLIN Deployment Assay

Overall, the deployment of long-lasting insecticide netting in pilot-scale warehouses had a significant effect on the percentage of insects that were able to disperse (Table 4-5; Fig. 4-8). The released insect species also affected dispersal (Table 4-5). Warehouses that employed LLIN demonstrated an 89–93% reduction in the number of insects making it to the commodity compared to the control warehouses, which lacked LLIN. There was a significant interaction between treatment and species type, with far more *T. castaneum* infesting the commodity in controls, and more individuals partially dispersing in the hanging and pipe deployments than either of the other two species.

A sequential ANOVA indicated that species significantly affected the percentage of insects reaching the commodity (Fig. 4-8). In particular, there were 33-fold more *T. castaneum* that made it to the commodity than either *R. dominica* or *T. variabile*. In addition, while the treatment did not have an overall effect, there was a significant species by treatment interaction (Table 4-5). Regardless of method, deployment of LLIN resulted in an 88–94% reduction in the percentage of *T. castaneum* making it to the commodity, while it had no significant effect for the other two species, which both had uniformly low success in reaching the commodity regardless of treatment.

By contrast, species did not significantly affect the number of insects partially dispersing, but the LLIN deployment method did (Table 4-5). There were 60–74% fewer individuals that partially dispersed across the pilot-scale warehouse in the hanging and pipe deployment of LLIN compared to the control without LLIN and the cover treatment. The cover treatment likely did not have as much impact on individuals partially dispersing because it was located so close to the commodity (e.g., far from the release point of the insects). Further, there was a significant interaction between species and LLIN deployment method (Table 4-5). The hanging and pipe deployment of LLIN reduced the percentage of partially dispersing *R. dominica* and *T. variabile* by 89–96% and 78–98%, respectively, while it increased the percentage of partially dispersing *T. castaneum*, a much stronger walker, by 152–160% (Fig 4-8).

Species, LLIN deployment method, and the interaction between the two all significantly affected the percentage of insects that did not disperse. There were 2.2–2.3-fold more *R. dominica* and *T. variabile* that did not disperse compared to *T. castaneum*. Furthermore, there were 20–72% more individuals that did not disperse across the pilot-scale warehouse in the hanging and pipe deployment of LLIN compared to the control without LLIN, likely because the release point was so close to the plane of deployed LLIN. Additionally, the cover treatment allowed a greater number of individuals to disperse, because the LLIN was located so close to the commodity (e.g. far from the insect release point in the warehouse), with 1.5–2.1-fold more individuals dispersing compared to the hanging and pipe treatments. The LLIN deployment method had a much stronger effect on the percentage of *T. castaneum* that did not disperse compared to either *R. dominica* or *T. variabile*.

The LLIN deployment method significantly affected progeny production 6 weeks after bringing the commodity back to an environmental chamber from the pilot-scale warehouse (Table 4-5; Fig. 4-8). Warehouses that deployed LLIN had a 98–100% reduction in progeny production compared to control warehouses without LLIN ($\chi^2 = 21.4$; df = 3; $P < 0.0001$). The only appreciable number of progeny from the commodities was from *T. castaneum*, but was confined to the controls (Fig. 4-8). There were no significant differences between the three types of LLIN deployments, suggesting that infestation and contamination of commodities could be reduced through multiple LLIN application methods.

Pilot-Scale Warehouse Trial 1: Health Conditions After Exposure

Overall, the MANOVA indicated that treatment, species, and their interaction had a significant effect on the percentage of affected insects recaptured throughout a warehouse (Table 4-5). However, neither the species, LLIN deployment method, nor their interaction significantly changed the percentage of affected individuals in the commodity. In addition, the released species did not significantly alter the percentage of affected individuals found to be partially dispersing in the warehouse, but the LLIN deployment method and its interaction with species did (Table 4-5). For instance, there was a 44% reduction in the percentage of insects partially dispersing when LLIN was deployed in the pipe treatment compared to the control treatment. However, species responded differently to the LLIN deployment method, with the percentage of partially dispersing *R. dominica* reduced by 7.7–9.3-fold compared to controls without LLIN, while partially dispersing *T. castaneum* and *T. variabile* increased by 9–15-fold and 3–5-fold.

The LLIN deployment method, species, and their interaction significantly changed the number of individuals that did not disperse in pilot-scale warehouses that were affected (Table 4-5). There were 8–13-fold more affected individuals among the insects that did not disperse in the pipe and hanging LLIN deployment compared to the control, likely due to the proximity of treated netting to the release location. On average, 1.4-fold more *R. dominica* did not disperse compared to either of the other two species. The interaction between the two variables was likely quantitative, as there were 5–7-, 20–49-, and 14–18-fold fewer affected *R. dominica*, *T. castaneum*, and *T. variabile*, respectively, that did not disperse in the hanging and pipe deployment of LLIN compared to controls without LLIN.

By contrast, the LLIN deployment method, species, and their interaction strongly affected the number of recaptured dead insects within the group of insects that did not disperse (Table 4-5). For example, there was a 1.7–1.8-fold more dead insects found in the no dispersal group in the hanging and pipe deployments compared to controls, again likely a result of the proximity of the LLIN to the release point. There were 3.9- and 20-fold more dead *T. variabile* that did not disperse than either *R. dominica* or *T. castaneum*, respectively. While there were 6–10-fold and 1.4–1.5-fold more dead *R. dominica* and *T. variabile*, respectively, recaptured in the no dispersal group for the hanging and pipe deployments than the controls, there was 8–67% fewer *T. castaneum* (Fig. 4-9). However, none of the variables affected the percentage of dead insects in the commodity at the far end of the warehouse, nor the percentage of dead insects that were able to partially disperse prior to mortality (Fig. 4-9).

Pilot-Scale Warehouse Trial 2: Combined Use of LLIN and Interception Traps

An overall MANOVA demonstrated no significant effect of single or combined tactics on the dispersal dynamics of insects in pilot scale warehouses, while species significantly altered dispersal, though not the interaction between the two (Table 4-6). Species affected the percentage of individuals making it to the commodity, partially dispersing, and not dispersing at all. Single or combined tactics, by contrast, had no effect on the dispersal of insects in warehouses, nor did its interaction with species. For example, only *T. castaneum* were able to reach the commodity (Fig. 4-10), while there were 2.9- and 3.3-fold more *T. variabile* that did not disperse compared to *R. dominica* and *T. castaneum*, respectively. By contrast, there were 5- and 7-fold more *T. variabile* and *T. castaneum*, respectively, that partially dispersed compared to *R. dominica*. Only *R. dominica* and *T. castaneum* were captured by interception traps, but at low levels (Fig. 4-11).

Only *T. castaneum* progeny were recorded in the commodities after six weeks ($\chi^2 = 57.0$; df = 2; $P < 0.0001$). The management tactic significantly affected progeny production ($\chi^2 = 17.2$; df = 3; $P < 0.001$), with an 83% and 19% reduction in the number of progeny produced in the commodity when LLIN alone or both LLIN and interception traps together were used, respectively (Fig. 4-10). Conversely, there was 2.3-fold more progeny in the commodity where AK-based interception traps alone were used (Fig. 4-10). There was no significant interaction between tactic and species ($\chi^2 = 0.01$; df = 6; P = 0.99). Therefore, LLIN, alone or with other IPM tools such as interception traps, can effectively reduce progeny production within stored products.

Pilot-Scale Warehouse Trial 2: Health Conditions After Exposure

The management tactic and species changed the percentage of affected insects recaptured in the partial dispersal area of warehouses, but not their interaction (Table 4-6). On average, there was an 82% increase in the number of affected individuals recaptured in the partial dispersal area with interception traps alone compared to the control. For LLIN alone or both LLIN and interception traps, there were 1.6- and 1.5-fold more affected individuals recaptured in the partial dispersal area relative to the control where no tactics were deployed (Fig. 4-12). There were 5.6-fold and 13.4-fold more affected *T. castaneum* than *T. variabile* or *R. dominica* recaptured in the partial dispersal area of the warehouse. Only the management tactic changed the percentage of affected individuals that did not disperse in warehouses, with 3, 5-, 2-, and 5-fold more affected individuals recaptured in the no dispersal group when using the interception trap only, LLIN only, or both combined, respectively, compared to warehouses without either. However, there were no affected insects recorded from the commodity, thus none of the variables had a significant effect on the percentage recaptured.

By contrast, only species identity affected the percentage of dead insects recaptured in the partial or no dispersal zones, but not the management tactic used, nor their interaction (Table 4-6). No dead insects were found in the commodity (Fig. 4-12). There was a 5.2-fold greater percentage of dead *T. castaneum* and *T. variabile* that partially dispersed prior to death compared to *R. dominica*. There were 3-fold and 11-fold more dead *R. dominica* and *T. variabile*, respectively, that did not disperse compared to *T. castaneum*.

Discussion

Overall, we have found that LLIN was highly effective when used alone or in interception traps to halt immigrating stored product insects. It appears likely that food facilities with LLIN deployed will have less insect colonization and fewer infestations. Many insects that contact LLIN while moving through facilities will be affected, through reduced mobility and increased mortality, and the net result is that individuals will be unable to successfully infest commodities.

While the findings from this study found no dose-dependency using the commercial SPB tab lure in AK-based interception traps, interception traps were still able to intercept naturally-occurring insects immigrating toward commercial food facilities. This aligns with previous literature, where AK traps were used to successfully intercept insects and monitor for or reduce infestations in other agricultural settings (Morrison et al. 2016b; Camelo et al. 2007; Navarro-Llopis et al. 2013). Deploying these traps around the perimeters of food facilities could be effective at capturing insects, but it is currently unknown what percentage of immigrating insects in the vicinity would be ensnared by the traps (e.g. their trapping efficiency). Future work should investigate the density of traps needed and the distance these traps should be placed from each other and the food facility for optimal effectiveness. Optimizing deployment could increase the efficiency of trapping for stored product pests and avoid unnecessary costs or loss of product (Hossain et al. 2010; Sargent et al. 2014). Additionally, effectiveness of AK could be improved through the identification of more effective attractants and different trap designs. Other novel cues should be investigated that may elicit a stronger attraction from insects than the lure or trap design used in this study. For example, in other work, aggregation pheromones or other sensory stimuli deployed in AK settings are commonly synergized by the presence of host plant volatiles (Morrison et al. 2019c, Wallingford et al. 2018; Morrison et al. 2016a), while trap type for another agricultural pest, *H. halys*, significantly affected successful capture (Morrison et al. 2015). Ideally, in AK-based approaches, the goal is to attract a large number of insects to a circumscribed area; however, we were not able to increase captures by increasing the number of lures in the trap, despite prior work showing this to be a promising method for other species (Kroschel and Zegarra 2010). Despite the very short deployment periods (~48 h) over the course of two summers, these interception traps were able to attract 3,800 insects, suggesting that these traps were fairly effective. Importantly, the inclusion of LLIN eliminated progeny production, but did not hinder trap captures by interception traps, suggesting that it acted as an effective kill mechanism for insects that were captured while not reducing trap attractiveness. Finally, perhaps the largest limitation of using interception traps as they are currently designed is the fact that they cannot be deployed for long periods. This may be improved if provided with a small rain shelter or overhang built into the top of the trap, or by using a more durable kairomone source than grain, which easily molds under unprotected field conditions.

Furthermore, we found that deploying LLIN in pilot-scale warehouses significantly reduces the dispersal ability and commodity colonization by three species of stored product insects. These findings expand on a previous study that showed a significant decrease in the movement and dispersal of adult and immature *T. castaneum* and *R. dominica* after exposure to LLIN in the laboratory (Morrison et al. 2018; Wilkins et al. 2020). Regardless of deployment method, infestations and progeny production decreased by 89–93% and 98–100%, respectively, in warehouses that incorporated the netting. The impact of LLIN deployment primarily affected *T. castaneum*; *R. dominica* and *T. variabile* exhibited low dispersal and colonization in the pilot-scale warehouse. Even laying LLIN directly over a commodity reduced the ability of insects to infest the product. This is consistent with an earlier study that used an α-cypermethrin-based LLIN against *L. serricorne* and *E. elutella* to surround a carton of tobacco in a commercial tobacco facility (Athanassiou et al. 2019). Trees wrapped with LLIN also exhibited significantly reduced beetle attacks (Ranger et al. 2020). Another study found that LLIN reduced *S. oryzae* infestations of maize in mini-bag bioassays by 98–100%, however, there were varying amounts of permethrin residues on the maize (Anaclerio et al. 2017). Thus, applications of LLIN deployed farther from the commodity, like the hanging and pipe treatments used in this study, may be preferred for commercial implementation.

Interestingly, when interception traps were deployed together with LLIN, there was actually a significant decrease in efficacy and an increase in progeny production inside the commodity relative to deploying LLIN alone in pilot scale warehouses or even nothing at all. This may have arisen as a result of attractive stimuli in the traps promoting flight initiation (e.g., Cox and Dolder 1995); thus, individuals may have been able to circumvent the LLIN at ground-level, which did not reach to the ceiling. Further, due to the size constraints of the pilot-scale warehouses, the area of arrestment around the trap may have been large enough to attract insects to the opening of the warehouse where they may have wandered in the vicinity of the trap until “accidentally” entering the warehouse. While sex pheromones typically attract individuals to a point-source emission, aggregation pheromones, by contrast, attract individuals only until they reach some threshold level of pheromone, after which they wander in a delimited area of arrestment around the trap (Morrison et al. 2016b). Combined with the tight spatial arrangements, the interception trap was not actually placed in a realistic location if the setup were adjusted to scale. Instead of being placed on the equivalent of the perimeter of a food facility where the area of arrestment would not intersect buildings, the interception trap was placed at the equivalent of the front door for the facility in our experiment. Thus, the net result was greater commodity colonization when interception traps were used alone compared to other treatments. Nonetheless, we have successfully shown the utility of LLIN at pilot-scale, and future work should assess both the specific area of arrestment around the interception traps and the use of LLIN against insect immigration in commercial-scale food facilities and in bulk storage commodity bins. While our results suggest that LLIN is an effective, preventative IPM tool that can work along with other tactics in a comprehensive IPM program, it is unlikely to completely replace the need for fumigations. However, it could reduce the number of treatments required. Therefore, the ability of LLIN to reduce fumigation events should also be further evaluated in future studies to confirm these predictions.

Exposure to LLIN does not always result in mortality, but may instead manifest as indirect toxicity through reduced movement and dispersal. In this study, there was an extensive number of affected individuals recaptured inside the warehouses. These were most often the insects that contacted the netting as they attempted to move through the warehouse towards the commodity. However, it was clear that the netting was successfully acting as a barrier to dispersal because most of these insects were found near the LLIN, but situated on the opposite side of the LLIN relative to the food source. Thus, while insects can physically pass through the netting, the knockdown effects are immediate enough that most insects are unable to crawl through the netting and advance farther into the warehouse to colonize commodities. Importantly, this portends success in the use of LLIN in the ways that we are describing here for food facilities. These results are also in line with a previous study that found both brief, multiple and continuous, longer exposures to LLIN by *T. castaneum* resulted in equally poor recovery (Gerken et al. 2020; Arthur et al. 2020). Additionally, in the AK-based interception trap field study and Trial 1 semi-field study, progeny production in the traps with LLIN and the commodities of pilot-scale warehouse where LLIN was deployed was minimal, which is consistent with past research on the sublethal effects of deltamethrin on progeny production (Athanassiou et al. 2004). Accounting for both these lethal and non-lethal effects of insecticide-incorporated netting provides a fuller picture of the efficacy of the netting as an IPM tool (Guedes et al. 2016).

Finally, there is more research needed on the efficacy of LLIN and interceptions traps together at a commercial level, especially if improvements to AK-based interception trap design or stimuli are made. For example, it would be of interest to know from what distance stored product insects are attracted to interception traps, and whether the use of LLIN can result in decreased numbers of insects inside facilities or bins and thus reduce the need for suppressive management tactics such as fumigation. Previous studies show that integrating additional tools like AK improved the overall efficacy of pest management programs by lowering total crop loss (Rahman and Broughton 2016; Hafsi et al. 2015). Additionally, deploying an interception trap incorporating LLIN will likely result in multiple exposures to the netting, decreasing the chances of insects like *T. castaneum* recovering (Gerken et al. 2020). Furthermore, incorporating novel insecticide active ingredients into the netting will help mitigate insecticide resistance in insect pest populations and should also be investigated. Building upon and implementing these novel tactics and IPM programs will help conserve the efficacy of current fumigant tools for years to come.

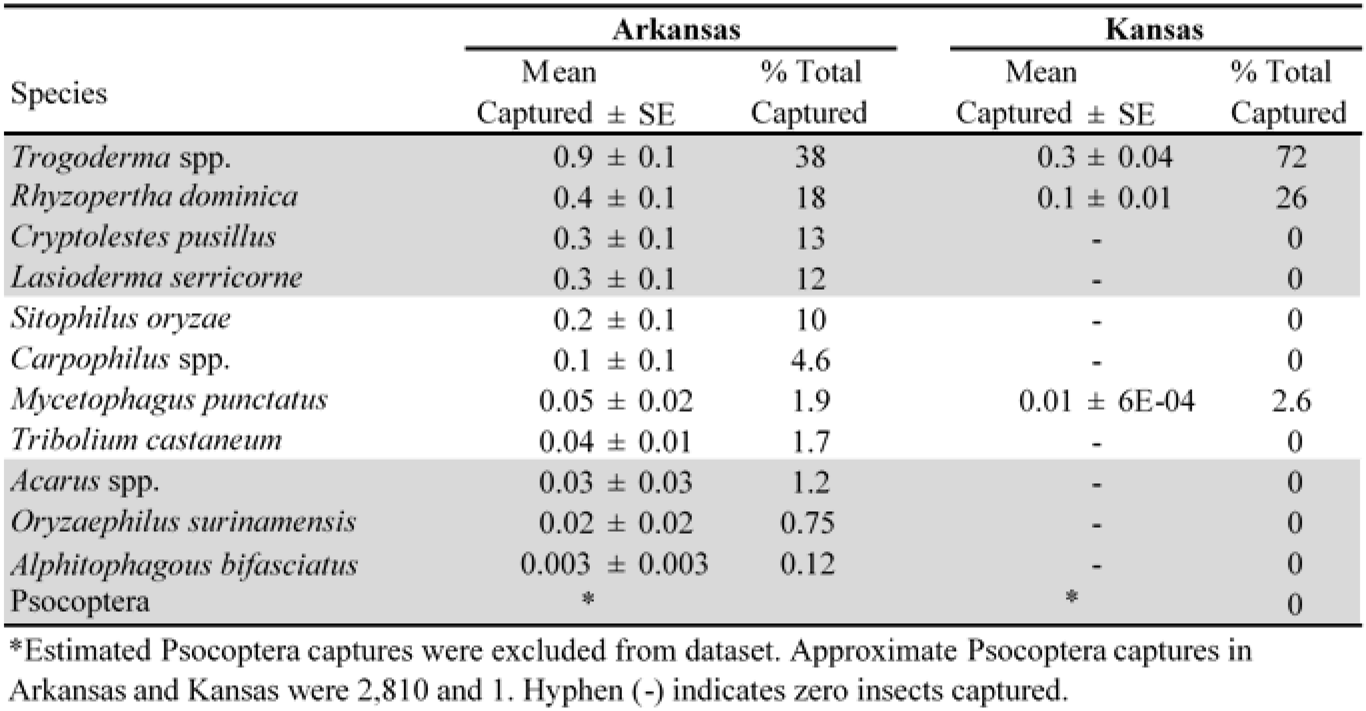
Table 4-1. Summary of field sites used for the interception trap assays in 2018 and 2019 in Kansas and Arkansas.

Site ID#	County	State	Facility Type	Commodities Handled	# Dates of Deployment
1	Riley	Kansas	Pilot	Whole Wheat	12
2	Riley	Kansas	Pilot	Flour, Corn, Sorghum, Legumes	12
3	Riley	Kansas	Commercial	Wheat, Corn, Soybean	12
4	Craighead	Arkansas	Commercial	Rice	8
5	Craighead	Arkansas	Commercial	Rice	10
6	Craighead	Arkansas	Commercial	Rice	10

Table 4-2. Assessing lure number-dependent attraction to Stored Product Beetle (SPB) tab lures in the wind tunnel by individuals exiting on the stimulus edge of the arena and recapture in traps in a release-recapture assay by *T. castaneum* and *R. dominica* adults. Lower case letters represent multiple comparisons among different numbers of lures for *T. castaneum*, while upper case letters represent multiple comparisons among different numbers of lures for *R. dominica* (χ^2-test, Bonferroni Correction).

	Wind Tunnel				Release-Recapture			
# of Lures	% Responding	±	SE		% Recaptured	±	SE	
T. castaneum								
0	53	±	6.6	b	4.6	±	2.2	b
1	77	±	14.5	a	14	±	4.2	a
2	93	±	6.7	a	13	±	3.2	a
3	93	±	3.3	a	14	±	2.8	a
R. dominica								
0	33	±	5.6	B	3.0	±	1.8	B
1	73	±	3.3	A	17	±	3.5	A
2	77	±	4.8	A	14	±	4	A
3	97	±	0.0	A	16	±	4.7	A

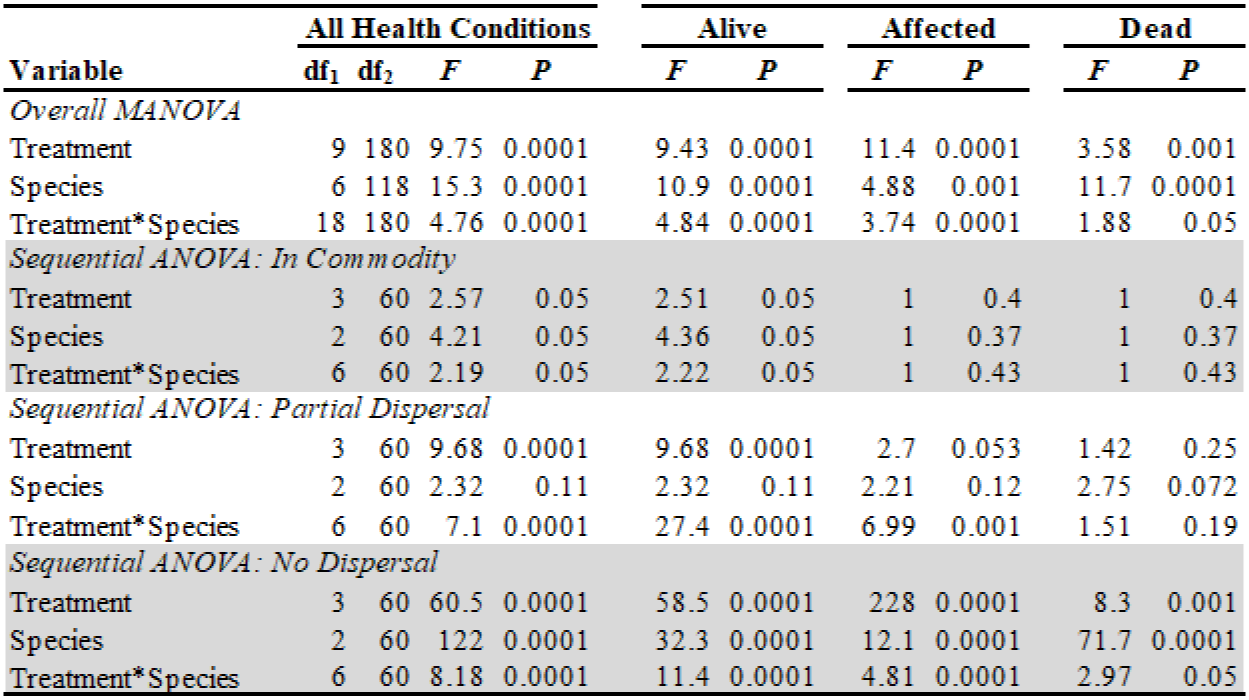
Table 4-3. The community composition of stored product insects captured in interception traps deployed 17 August 2018 at three sites in KS, and from 25 April 2019 to 18 September 2019 at three commercial sites in KS and 31 May 2019 to 30 September 2019 at three commercial sites in AR. There were three replicates of each treatment per site, with each trap deployed for an approximate 48-h period.



Species	**Arkansas** Mean Captured ± SE	**Arkansas** % Total Captured	**Kansas** Mean Captured ± SE	**Kansas** % Total Captured
Trogoderma spp.	0.9 ± 0.1	38	0.3 ± 0.04	72
Rhyzopertha dominica	0.4 ± 0.1	18	0.1 ± 0.01	26
Cryptolestes pusillus	0.3 ± 0.1	13	-	0
Lasioderma serricorne	0.3 ± 0.1	12	-	0
Sitophilus oryzae	0.2 ± 0.1	10	-	0
Carpophilus spp.	0.1 ± 0.1	4.6	-	0
Mycetophagus punctatus	0.05 ± 0.02	1.9	0.01 ± 6E-04	2.6
Tribolium castaneum	0.04 ± 0.01	1.7	-	0
Acarus spp.	0.03 ± 0.03	1.2	-	0
Oryzaephilus surinamensis	0.02 ± 0.02	0.75	-	0
Alphitophagous bifasciatus	0.003 ± 0.003	0.12	-	0
Psocoptera	*		*	0

*Estimated Psocoptera captures were excluded from dataset. Approximate Psocoptera captures in Arkansas and Kansas were 2,810 and 1. Hyphen (-) indicates zero insects captured.

Table 4-4. Statistical model results for recapture of individuals in the commodity, partially dispersing and not dispersing in Trial 1 examining efficacy of LLIN deployment method in a pilot-scale warehouse release-recapture assay deployed in 2019 in Manhattan, KS. Treatments included no LLIN, or LLIN deployed in pipe, cover, or hanging applications.



	All Health Conditions				**Alive**		**Affected**		**Dead**	
Variable	**df$_1$**	**df$_2$**	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Overall MANOVA										
Treatment	9	180	9.75	0.0001	9.43	0.0001	11.4	0.0001	3.58	0.001
Species	6	118	15.3	0.0001	10.9	0.0001	4.88	0.001	11.7	0.0001
Treatment*Species	18	180	4.76	0.0001	4.84	0.0001	3.74	0.0001	1.88	0.05
Sequential ANOVA: In Commodity										
Treatment	3	60	2.57	0.05	2.51	0.05	1	0.4	1	0.4
Species	2	60	4.21	0.05	4.36	0.05	1	0.37	1	0.37
Treatment*Species	6	60	2.19	0.05	2.22	0.05	1	0.43	1	0.43
Sequential ANOVA: Partial Dispersal										
Treatment	3	60	9.68	0.0001	9.68	0.0001	2.7	0.053	1.42	0.25
Species	2	60	2.32	0.11	2.32	0.11	2.21	0.12	2.75	0.072
Treatment*Species	6	60	7.1	0.0001	27.4	0.0001	6.99	0.001	1.51	0.19
Sequential ANOVA: No Dispersal										
Treatment	3	60	60.5	0.0001	58.5	0.0001	228	0.0001	8.3	0.001
Species	2	60	122	0.0001	32.3	0.0001	12.1	0.0001	71.7	0.0001
Treatment*Species	6	60	8.18	0.0001	11.4	0.0001	4.81	0.0001	2.97	0.05

Table 4-5. Statistical model results for recapture of individuals in the commodity, partially dispersing and not dispersing in Trial 2 examining management tactic efficacy in a pilot-scale warehouse release-recapture assay deployed in 2019 in Manhattan, KS. Treatments included LLIN alone, AK alone, both together, or neither (control).

			All Health Conditions		Alive		Affected		Dead	
Variable	**df$_1$**	**df$_2$**	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Overall MANOVA										
Treatment	9	180	0.718	0.69	1.01	0.44	4.25	0.0001	0.993	0.45
Species	6	118	28.1	0.0001	18.9	0.0001	9.38	0.0001	11.5	0.0001
Treatment*Species	18	180	0.801	0.7	0.778	0.72	1.09	0.36	1.36	0.16
Sequential ANOVA: In Commodity										
Treatment	3	60	0.259	0.85	0.259	0.85	1	0.4	1	0.4
Species	2	60	21.1	0.0001	21.1	0.0001	1	0.37	1	0.37
Treatment*Species	6	60	0.259	0.95	0.259	0.95	1	0.43	1	0.43
Sequential ANOVA: Partial Dispersal										
Treatment	3	60	1.05	0.38	0.728	0.54	3.91	0.05	0.71	0.55
Species	2	60	15.6	0.0001	5.74	0.01	48.8	0.0001	3.06	0.054
Treatment*Species	6	60	1.81	0.11	1.64	0.15	1.66	0.15	1.53	0.19
Sequential ANOVA: No Dispersal										
Treatment	3	60	0.852	0.47	1.77	0.16	14.4	0.0001	0.965	0.42
Species	2	60	58.6	0.0001	26.7	0.0001	0.089	0.91	44.3	0.0001
Treatment*Species	6	60	0.481	0.82	0.527	0.79	0.59	0.74	1.02	0.42

Figure 4-1. Exploded-view picture of interception traps (top), and field-deployed interception trap (bottom). In order from left to right, each trap included a screw (to hold all parts together), a bottom perforated metal plate, a piece of netting (control or LLIN), cut PVC pipe holding 60 g of whole wheat kernels as kairomone and SPB tab lure, a second piece of netting, a top perforated metal plate, and a washer and wingnut on the end of the screw to tightly hold each piece together (top). Interception trap assembled and deployed in the field (bottom).

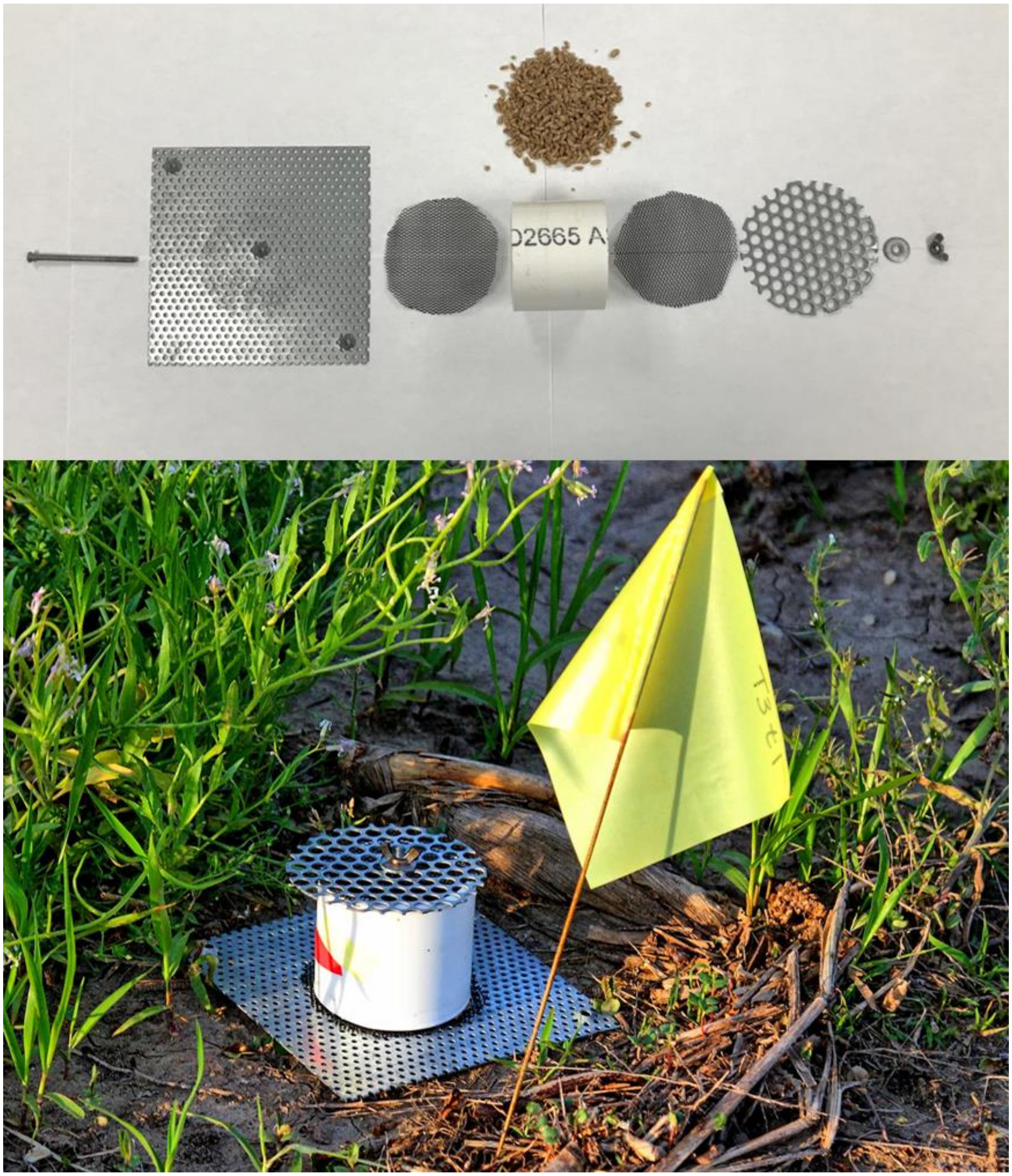


Figure 4-2. Schematic of the designated recapture zones inside the pilot-scale warehouses where insects were released (top, left). Insects were released in Zone 6, given 72-h to travel across the warehouse through Zones 2–5, and collected in the commodity (Zone 1). Outside habitus image of the pilot-scale warehouses used for the LLIN and interception trap deployment assays (bottom, left). Treatments are schematically represented on the right, showing the three LLIN deployment methods for Trial 1, including a control treatment with no LLIN.

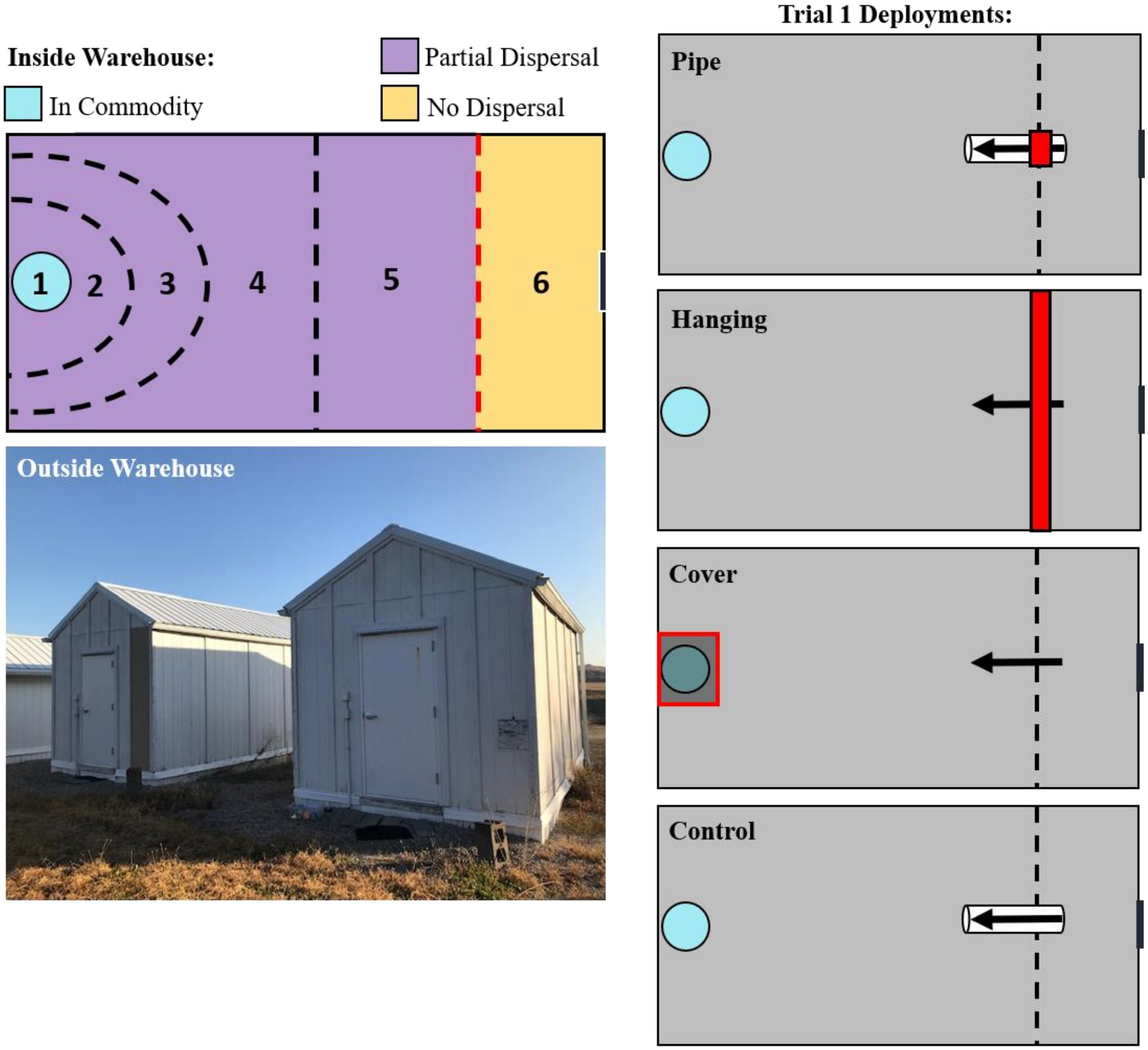


Figure 4-3. Simulated inside and outside of a warehouse in Trial 2. Two wooden planks acted as a funnel for the insects to enter inside the warehouse. The gap (see arrow) between the two planks was the point of entrance. Either control netting (without insecticide) or LLIN bridged the gap between the two planks. Netting was affixed to a white test-tube rack and held in place at the entrance point. Insects that did not pass through the gap were considered outside the warehouse.

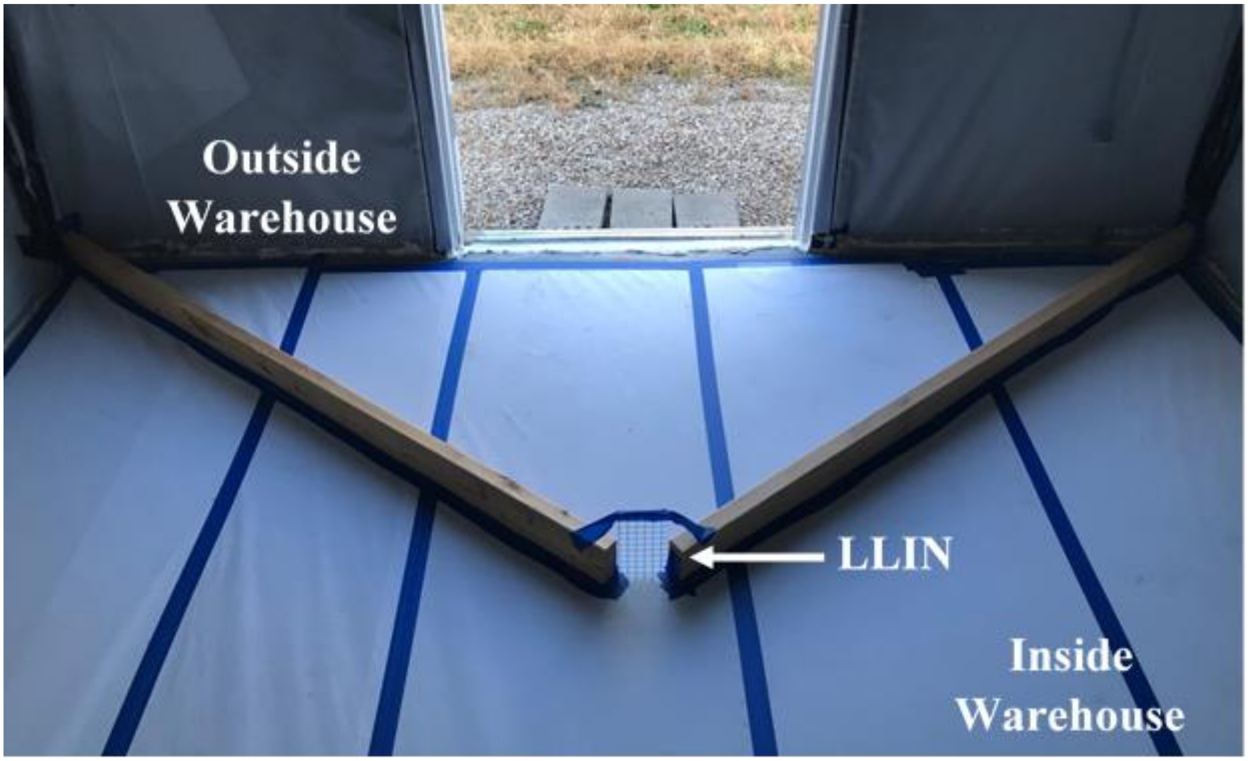


Figure 4-4. The percentage of *T. castaneum* and *R. dominica* exiting the release arena on the stimulus (upwind) edge in a wind tunnel assay. These stimuli included dried distillers' grains with solubles (DDGS), wheat germ oil (WGO), the commercial lure Stored Product Beetle Tab (SPB Tab), and ambient air (Ctrl). Bars with shared letters are not significantly different from each other ($\chi 2$-tests, Bonferroni correction).

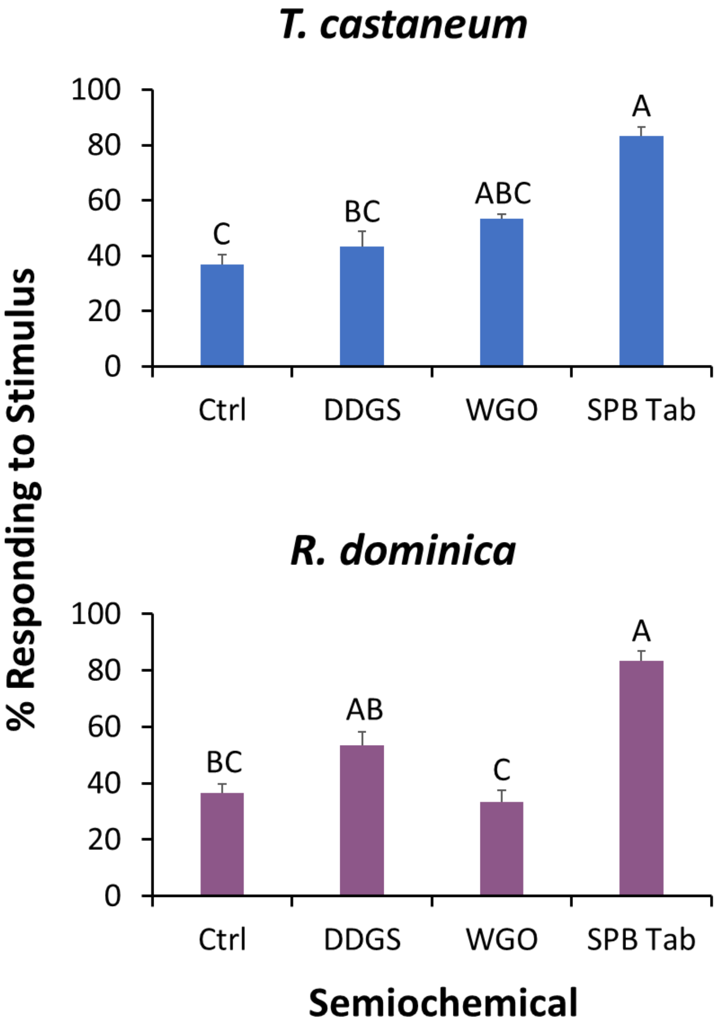


Figure 4-5. The percentage of *T. castaneum* and *R. dominica* captured in Trécé Storgard Dome® pitfall traps in a release-recapture assay. Each dome trap contained the dried distillers' grains with solubles (DDGS), wheat germ oil (WGO), the commercial lure Stored Product Beetle Tab (SPB Tab), or ambient air (Ctrl). Bars with shared letters are not significantly different from each other (Tukey HSD, α= 0.05).

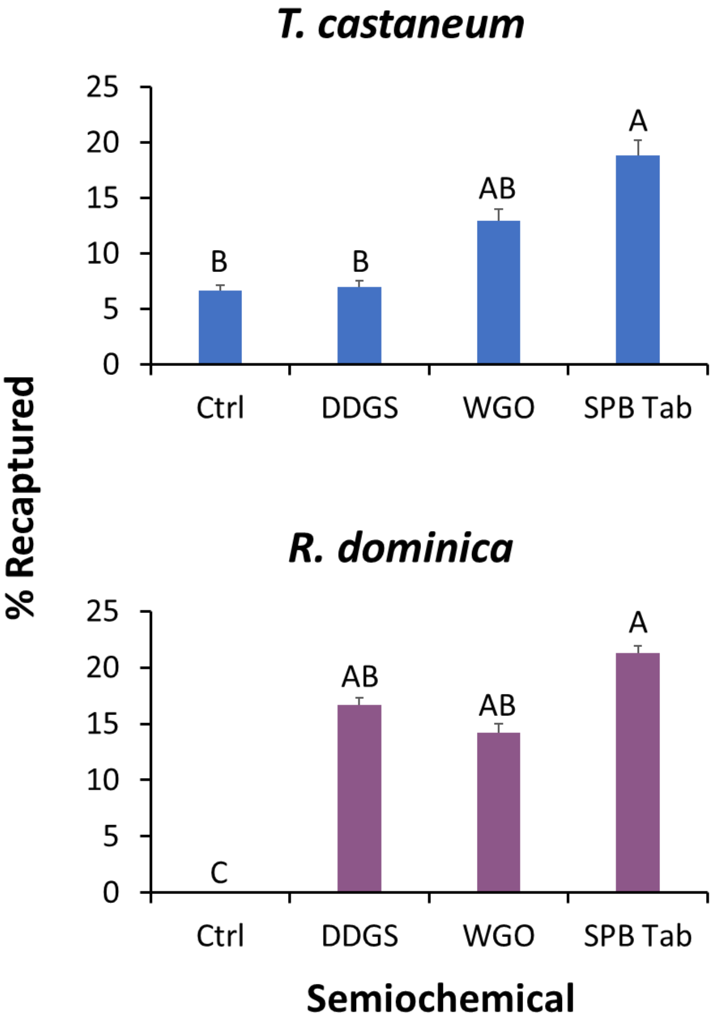


Figure 4-6. Mean (± SE) number of stored product insects captured by interception configuration. Traps were deployed for 48-h periods once every other week at six sites during 2018 and 2019 in in Kansas and Arkansas at commercial food facilities. Treatments included interception traps 1) with control netting and no lure (Ctrl), 2) control netting + SPB Tab (Ctrl + Lure), 3) with LLIN and no SPB Tab (LLIN), 4) with LLIN + SPB Tab (LLIN + Lure). Bars with shared letters are not significantly different from each other ($\chi 2$-tests, Bonferroni correction). Traps with lures captured more insects, regardless of netting type.

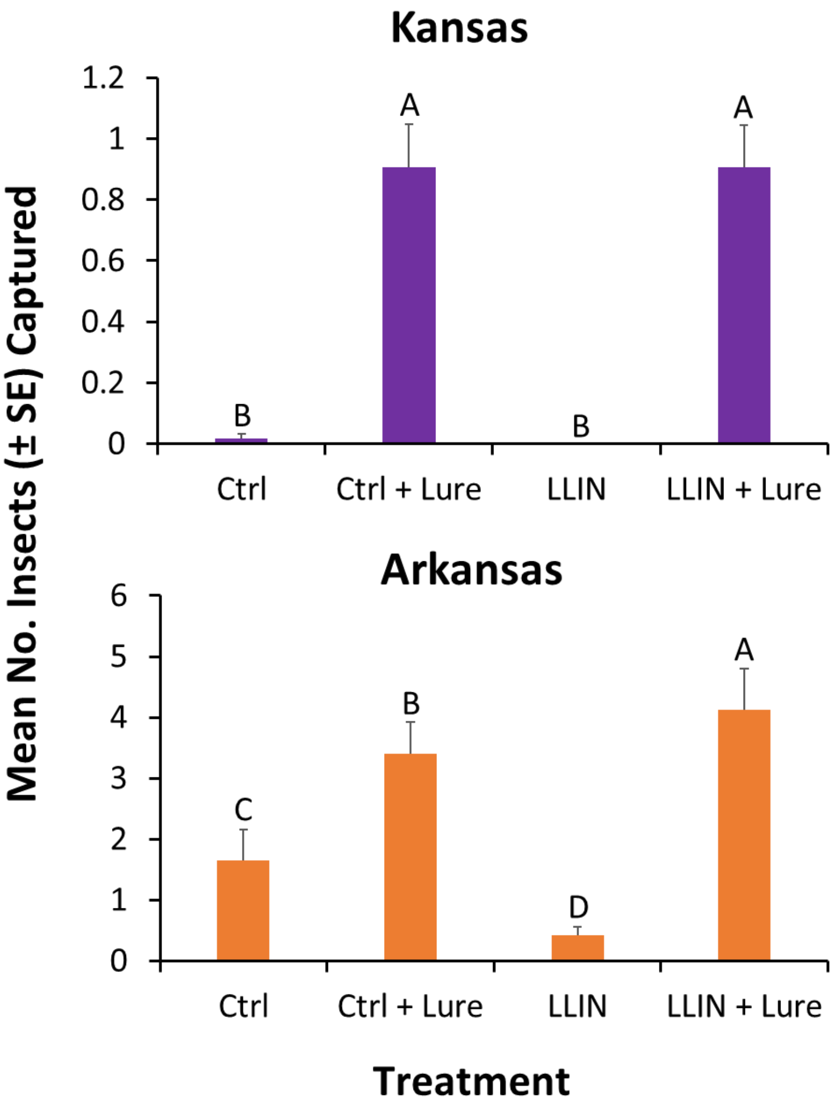


Figure 4-7. Mean progeny (± SE) produced after 6 weeks from grain inside traps deployed for 48 h periods once every other week at six sites during 2018 and 2019 in in Kansas and Arkansas at commercial food facilities. Bars with shared letters are not significantly different from each other (χ2-test, Bonferroni correction). At both locations, traps with lures, and LLIN resulted in significantly less progeny produced.

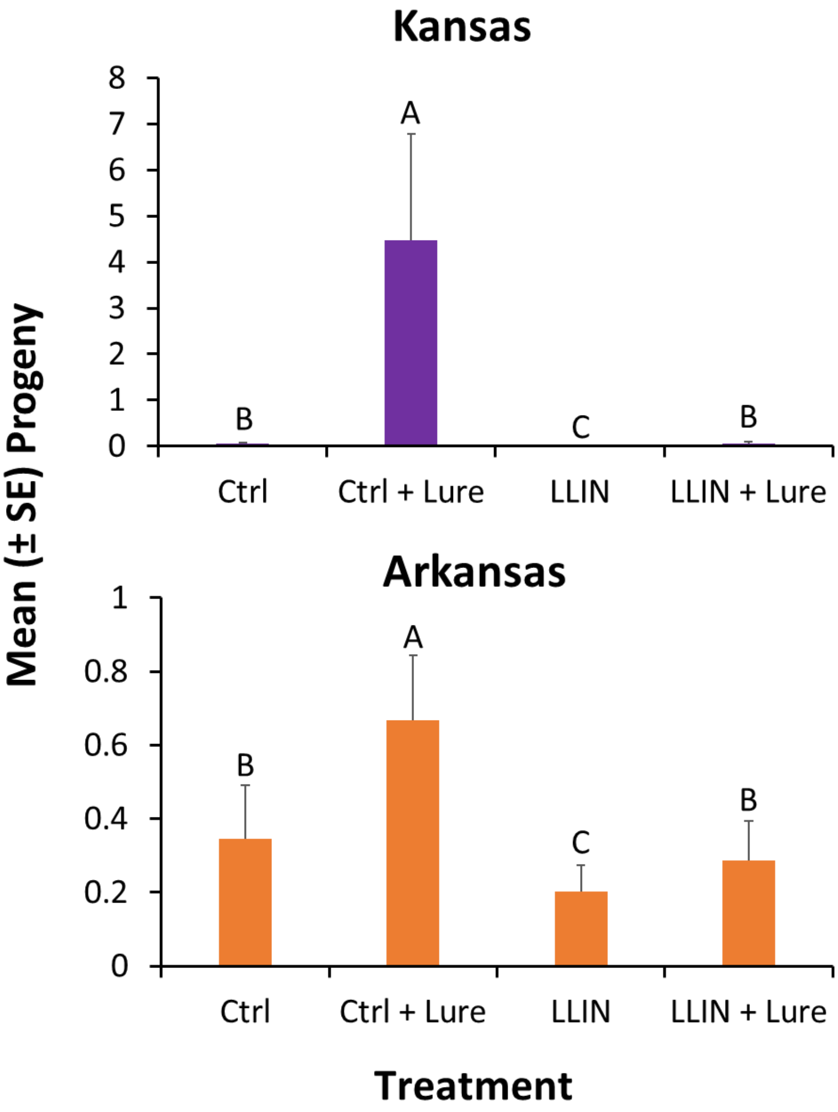


Figure 4-8. Mean (± SE) percentage of 100 *T. castaneum* (light blue bars), *R. dominica* (pink bars), and *T. variabile* (dark blue bars) adults released in pilot-scale warehouses in Manhattan, KS during 2019 recaptured after 72 h for Trial 1 to assess relative efficacy of different LLIN deployment methods. Individuals were recorded as not dispersing (Zone 6, top row), partially dispersing (Zones 2-5, second row), in the commodity (Zone 1, third row), or progeny production after a 6-week holding period (bottom row). Deployment methods included: Cover – covering the commodity directly with LLIN, Hanging – a single piece of LLIN bisecting the warehouse, Pipe – a piece of LLIN bisecting a PVC pipe with adults released in the pipe, and Ctrl – no deployment of LLIN. Bars with shared letters are not significantly different from each other (Tukey HSD, α = 0,05).

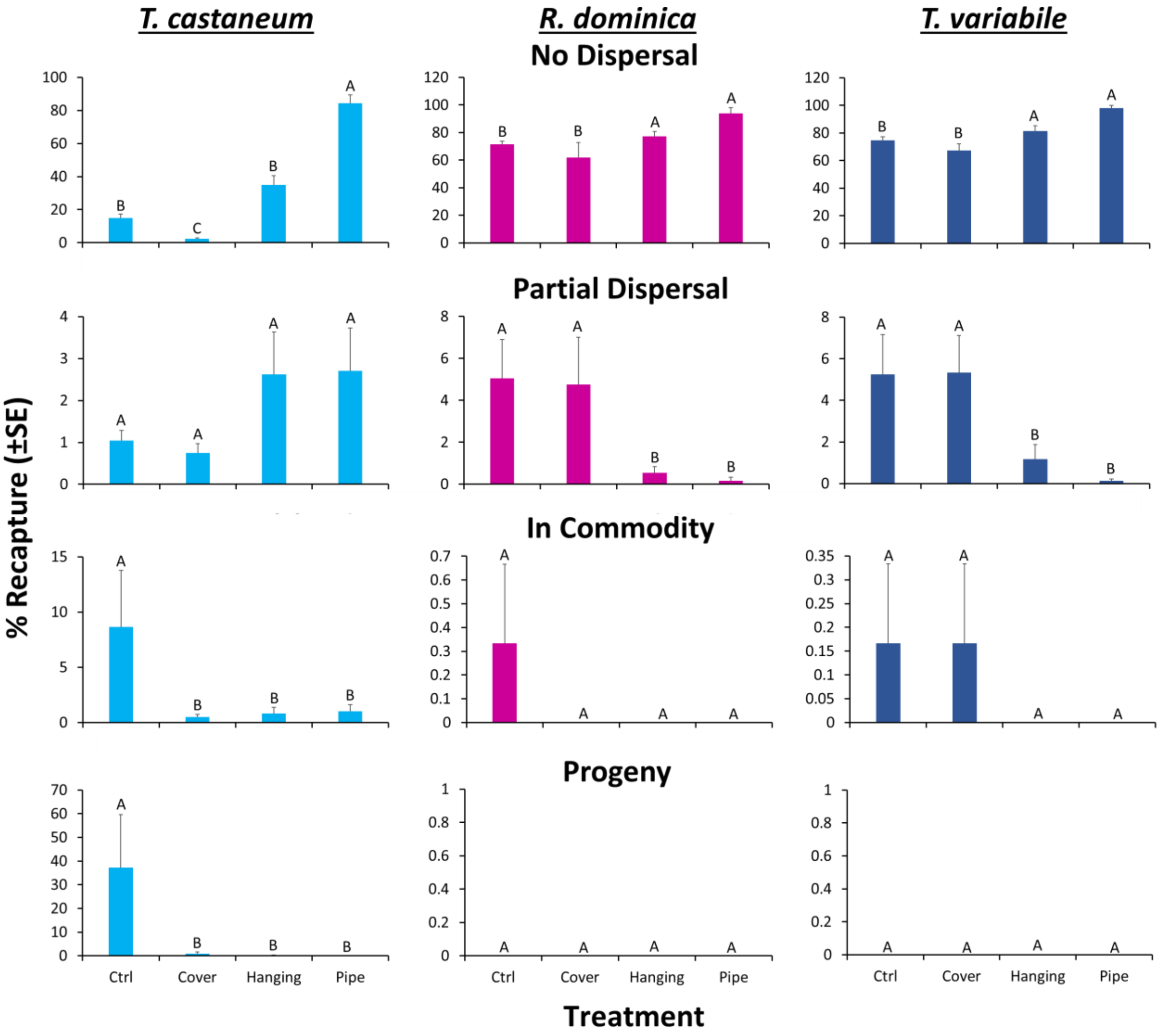


Figure 4-9. Mean (± SE) percentage of 100 *T. castaneum* (left column), *R. dominica* (middle), and *T. variabile* (right) recaptured that were classified as alive (blue), affected (yellow), or dead (red) after dispersing 72 h in pilot-scale warehouses with different methods of LLIN deployment in Manhattan, KS during 2019. Individuals were recorded as not dispersing (Zone 6, top row), partially dispersing (Zones 2-5, second row), in the commodity (Zone 1, third row), or progeny production after a 6-week holding period (bottom row). Deployment methods included: Cover – covering the commodity directly with LLIN, Hanging – a single piece of LLIN bisecting the warehouse, Pipe – a piece of LLIN bisecting a PVC pipe with adults released in the pipe, and Ctrl – no deployment of LLIN. Bars with shared letters are not significantly different from each other (Tukey HSD, α = 0,05).

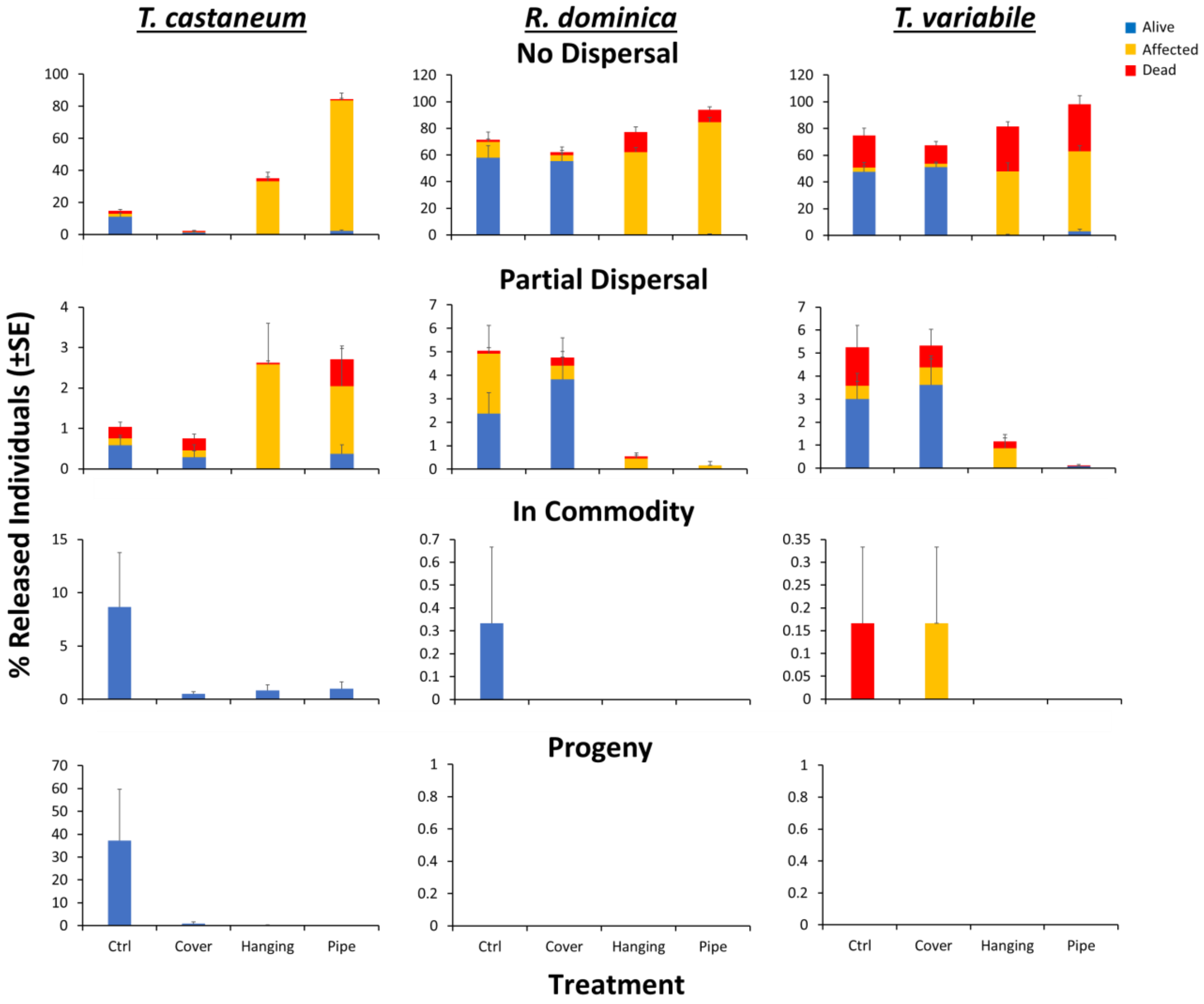


Figure 4-10. Mean (± SE) percentage of 100 *T. castaneum* (light blue bars), *R. dominica* (pink bars), and *T. variabile* (dark blue bars) adults released in pilot-scale warehouses in Manhattan, KS during 2019 recaptured after 72 h for Trial 2 to assess relative efficacy of LLIN alone (LLIN alone), AK-based interception traps alone (AK alone), both together (AK + LLIN), or neither (Ctrl). Individuals were recorded as not dispersing (Zone 6, top row), partially dispersing (Zones 2-5, second row), in the commodity (Zone 1, third row), or progeny production after a 6-week holding period (bottom row). Bars with shared letters are not significantly different from each other (Tukey HSD, α = 0,05).

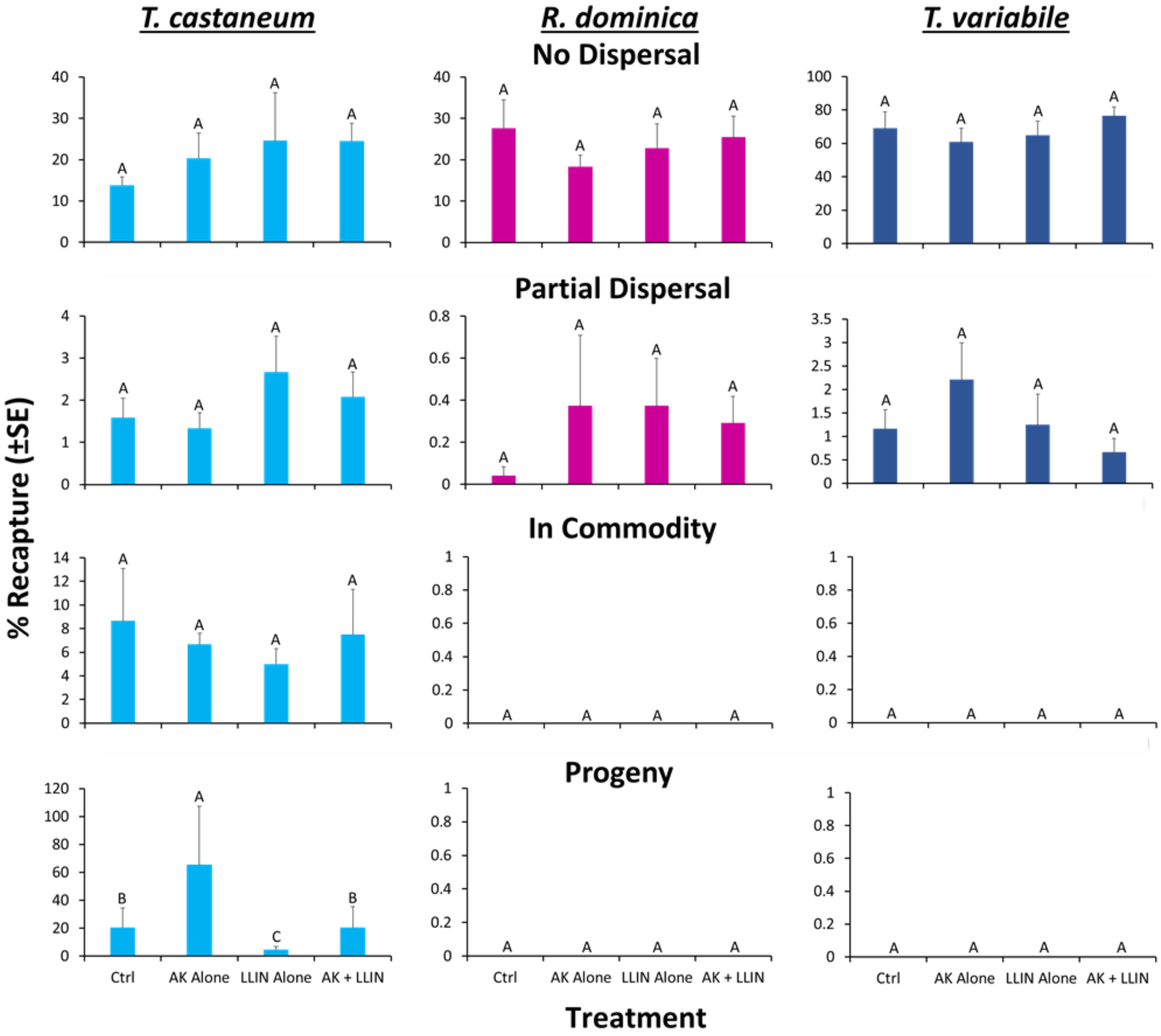


Figure 4-11. Mean (SE) percentage of 100 *T. castaneum* (light blue bars), *R. dominica* (pink bars), and *T. variabile* (dark blue bars) recaptured in AK-based interception traps deployed in pilot-scale warehouses during Trial 2 in Manhattan, KS in 2019. Lower case letters represent pairwise comparisons within *R. dominica*, while upper case letters represent pairwise comparisons within *T. castaneum*. Bars with shared letters are not significant different from each other (Tukey HSD, α = 0.05).

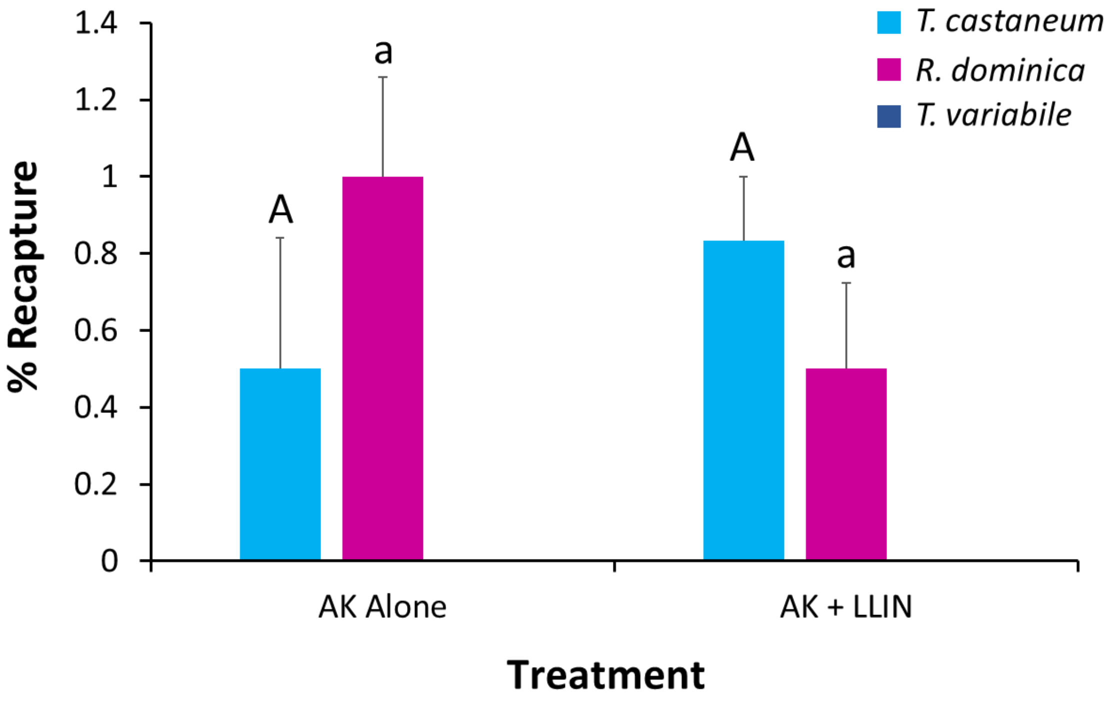
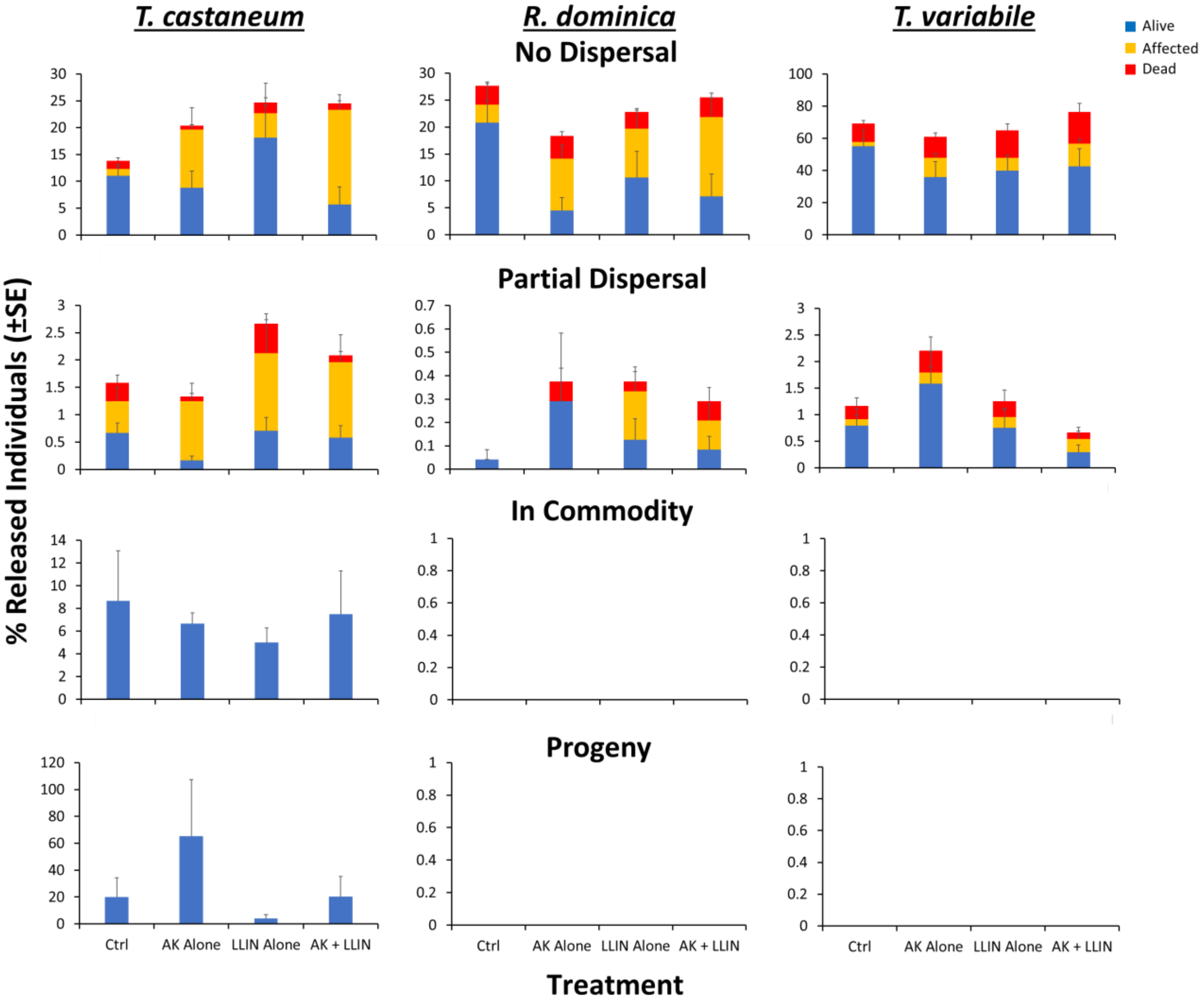


Figure 4-12. Mean (± SE) percentage of 100 *T. castaneum* (left column), *R. dominica* (middle), and *T. variabile* (right) recaptured that were classified as alive (blue), affected (yellow), or dead (red) after dispersing 72 h for Trial 2 in pilot-scale warehouses in Manhattan, KS during 2019 to assess the relative efficacy of LLIN alone (LLIN alone), AK-based interception traps alone (AK alone), both together (AK + LLIN), or neither (Ctrl). Individuals were recorded as not dispersing (Zone 6, top row), partially dispersing (Zones 2-5, second row), in the commodity (Zone 1, third row), or progeny production after a 6-week holding period (bottom row). Bars with shared letters are not significantly different from each other (Tukey HSD, α = 0,05).



Chapter 5 - Summary and Future Directions

The findings from this thesis strongly support long-lasting insecticide netting as an effective preventative tool for stored product IPM programs. This netting, with incorporated deltamethrin, ensures quick knockdown of at least three key insect pests upon brief contact with the netting. With significantly reduced movement and an impaired or absent ability to disperse, the likelihood of insects infesting postharvest commodities is greatly minimized. Immature stages of *T. castaneum* and *T. variabile*, which are mobile and can travel within an infested facility or out of infested products, showed significantly reduced movement and dispersal after contacting LLIN as well. While larvae of these insects are more tolerant than their adult counterparts, larvae that are exposed to LLIN are significantly affected compared to larvae that do not contact LLIN at all. Importantly, because adults are usually the primary dispersing stage, the use of LLIN to halt the immigration of stored product insects seems very likely.

LLIN may also be leveraged as a kill mechanism successfully in AK-based interception traps. When combined with a single, commercial lure, these traps were able to intercept multiple thousands of stored product insects, including almost 15 taxa, on the perimeter of commercial food facilities before they were able to immigrate into commodities and infest products. Significantly reduced progeny counts from interception traps with LLIN supports the use of insecticide netting as an effective kill mechanism.

Deploying LLIN in pilot-scale warehouses successfully prevents the dispersal of insects into commodities, regardless of the specific way in which it is deployed. The method of deployment can vary and still be highly effective. However, deploying LLIN near areas where insects may enter the facility will prevent these pests from moving any further towards the commodity. That being said, LLIN can still reduce commodity infestations by wrapping the netting around a pallet of product. Overall, the use of LLIN in warehouses was able to reduce progeny production in commodities by 98–100% compared to controls without LLIN.

While our research has validated use of LLIN in the laboratory and semi-field, future work should implement LLIN to understand if it can reduce infestations on a commercial scale. LLIN is an effective kill mechanism in AK traps, but novel attractants, trap designs, and trap spacing and density should also be investigated to enhance the performance of these traps. LLIN deployment in pilot-scale warehouses produced promising results, therefore, the performance of this netting should be evaluated at a larger scale, particularly within commercial facilities as well as for bulk storage conditions. Additionally, studies should determine how the incorporation of LLIN into an IPM program affects other management practices in and around commercial food facilities, such as frequency of fumigations or insect abundance in monitor traps.

LLIN is an effective tool on its own, but there are still limitations to the netting that can be alleviated through continued research. For example, insects may develop resistance to insecticides and management practices, and this could happen with LLIN over time if only one active ingredient in the netting is used. Thus, novel insecticides should be tested with the netting, and the netting should be incorporated into management programs that deploy multiple techniques at a time. Further, LLIN has multi-year efficacy within peoples' homes and outside, even in adverse weather conditions. However, inside commercial facilities, food dust accumulations on the netting could potentially disrupt the netting's efficacy. As a result, future work evaluating food dust accumulation on the net and methods to properly clean the netting should be undertaken.

The success of LLIN as bed nets against mosquitoes and other vectors has been clear for the last several decades. Now, our data has conclusively demonstrated the promise of LLIN for application in the post-harvest environment. This is a preventative strategy that can be layered onto existing IPM programs at food facilities, while continually acting to decrease pest dispersal and pest infestation in and around the facility. The findings from our research demonstrate the beneficial impacts LLIN can have in an agricultural setting. LLIN can and should be implemented by commercial food facilities to reduce economic losses, decrease insecticide outputs, increase sustainability, and improve food security.

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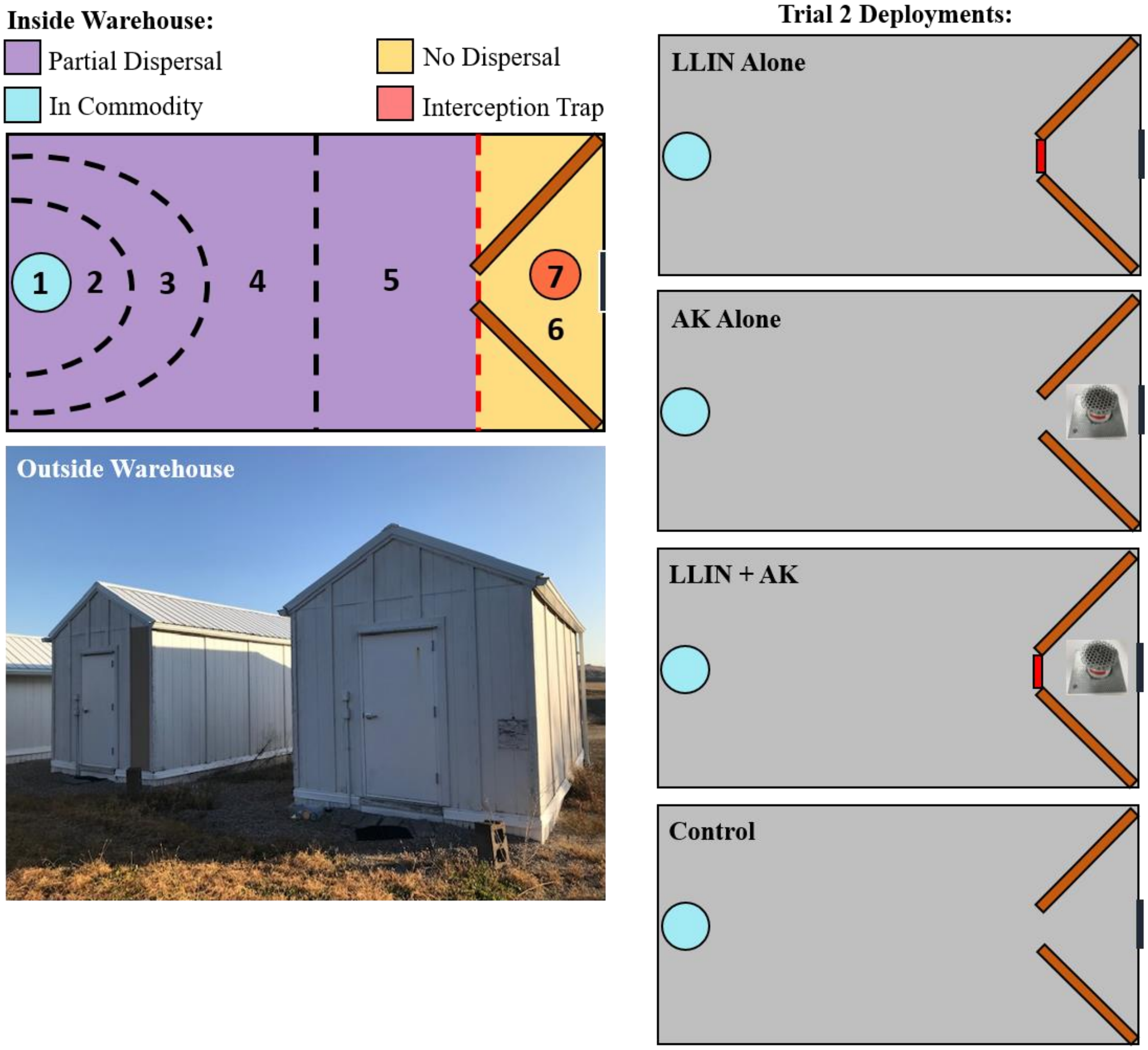
Appendix A - Supplemental Data

Chapter 4: Trial 2 zone schematic



Chapter 4: Interception trapping time axis