

## Suitability of hemp seed for reproduction of stored-product insects

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### Extended Abstract

#### 1. Introduction

Hemp, or industrial hemp, is a high value alternative crop that has seen major increases in acreage in Canada since commercial production was legalized in 1998. The term industrial hemp applies to non-psychoactive varieties of *Cannabis sativa* L. There have been reports of insect infestations on stored hemp seed in Manitoba. The moths *Plodia interpunctella* (Hübner) Indianmeal moth, and *Ephestia kuehniella* (Zeller) Mediterranean flour moth feed on hemp seed (Hagstrum and Subramanyam, 2009). Our objectives were to determine which stored-product beetles can reproduce on hemp and the effect of dockage and seed moisture content.

#### 2. Materials and Methods

Twenty adult insects were placed on 15 g of hemp seed at two different moisture contents (~8% or ~15%) and two different dockage levels (~0% or ~15%) and held at 30°C and 60-70% relative humidity. The number of live and dead insects were counted at 3, 5, 7 and 9 weeks. Only live adults were returned to vials.

#### 3. Results and Discussion

These insect populations increased over the 9 weeks; red flour beetle [*Tribolium castaneum* (Herbst)], drugstore beetle [*Lasioderma serricorne* (F.) ] saw-toothed grain beetle [*Oryzaephilus surinamensis* (L.) ], warehouse beetle (*Trogoderma variabile* Ballion). These insect populations did not increase: rusty grain beetle [*Cryptolestes ferrugineus* (Stephens) ], lesser grain borer [*Rhyzopertha dominica* (F.) ], rice weevil [*Sitophilus oryzae* (L.) ], flour mill beetle (*Cryptolestes turcicus* (Grouvelle), confused flour beetle (*Tribolium confusum* Jacquelin du Val), cigarette beetle [*Stegobium paniceum* (L.) ]. In general, higher dockage led to higher populations. The effect of moisture content was variable.

**Keywords:** *Cannabis sativa*, reproduction, dockage, moisture

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## The use of long-lasting insecticide netting to prevent dispersal of stored product insects

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## Abstract

The lesser grain borer, *Rhyzopertha dominica*, and red flour beetle, *Tribolium castaneum*, are two notorious primary and secondary pests of stored products. Extensive research has been done to prevent the establishment and subsequent infestation of the insects in stored product facilities. Long-lasting insecticide netting (LLIN) on mosquitoes has proved effective in controlling the spread of malaria, but little research has been conducted on the LLIN's behavioral effects of stored product insects. In this study, a movement and dispersal assay were performed. In the movement assay, the video-tracking software, Ethovision, recorded the movement of *R. dominica* and *T. castaneum* after 1-10 min exposures to LLIN or control netting and a waiting period of 1 min, 24 hr, 72 hr, or 7 days after netting exposure. In the dispersal assay, *R. dominica* and *T. castaneum* were observed after 5 minutes of exposure to LLIN or control netting to measure the insects' ability to reach new food patches at three different distances. The results from the movement assay showed a significant reduction in horizontal movement and significant increase in angular velocity for beetles exposed to LLINs, indicating that movements were more erratic and less directed. The dispersal assay revealed that exposure to LLIN had a significant effect on the dispersal ability of both *R. dominica* and *T. castaneum* with averages of 0-3 from a group of 20 beetles reaching the new food patch. These results indicate that LLINs can be an effective tool for the prevention of stored product insect establishment and colonization.

**Keywords:** polyethylene netting, integrated pest management, behavior, sublethal effects, bed nets

## 1. Introduction

Together, the major three stored grains in the US (corn, soybean, and wheat) alone represent a value of \$85.9 billion (NASS, 2018), much of it exported to help feed the world's growing population. The world's population is estimated to reach 9 billion people by 2050 (Godfray et al. 2010), and agricultural output will have to more than double by that point (Ray et al. 2013). Insects are our main competitors for food on the planet, resulting in 10-50% yield loss of products after they have been harvested from the field. The key to many integrated pest management programs (IPM) for stored products is sanitation to prevent infestation by insects (Phillips and Throne, 2010). However, this is often difficult because of the success with which stored product insects can immigrate to new facilities (McKay et al. 2017; Campbell and Arbogast 2004).

In particular, the lesser grain borer, *Rhyzopertha dominica*, and red flour beetle, *Tribolium castaneum*, are two notorious primary and secondary pests of stored products. These represent radically different life histories among stored product insects. *Tribolium castaneum* is a secondary feeder (Hagstrum and Subramanyam 2006), feeding on already broken grain, is a relatively weaker flier, and is 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, boring into whole kernels, depositing eggs, and developing inside the grain (Hagstrum and Subramanyam 2006), while also being a strong flier (Edde et al. 2006) and long-distance disperser (Mahroof et al. 2010).

Extensive research has been done to prevent the establishment and subsequent infestation of the insects in stored product facilities. One potential alternative management tactic that has not been evaluated for control of stored product insects is long-lasting insecticide netting (LLIN). Since the 1990s, LLINs have proved effective in reducing mosquito populations to control the spread of malaria (Lengeler 2004; Kitchen et al. 2009; Alonso et al. 1991) and to kill vectors of other arthropod-borne diseases (Dutta et al. 2011). 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. This has included as a kill mechanism in traps for the brown marmorated stink bug (Kuhar et al. 2017; Morrison et al. 2017; Rice et al. 2018). Most recently, LLINs are now being considered for their ability to control post-harvest insects (Scheff et al. 2018; Rumbos et al. 2018). However, one challenge with currently available LLINs is that stored product insects are small enough to pass through the netting material, and it takes extended durations of exposure to elicit outright mortality. As a result, a natural question is whether the netting will have sufficiently pronounced effects on the behavior of stored product insects to prevent their dispersal after contact.

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 LLINs 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 LLINs against *T. castaneum* and *R. dominica*. Nonetheless, there are several other considerations that must be met for LLINs to be behaviorally compatible with the behavior of stored product pests and be potentially effective as a control tactic. Specifically, LLINs must 1) swiftly decrease the locomotion and result in the loss of coordinated movement by stored product insects, and 2) prevent dispersal to new food patches after brief contact with the material. In this study, a movement and dispersal assay were performed. We employed either a deltamethrin-incorporated polyethylene netting at 0.6% a.i. (ZeroFly, Vestergaard-Frandsen, Inc., Switzerland; LLIN hereafter) or netting with identical physical characteristics but not insecticide (control netting).

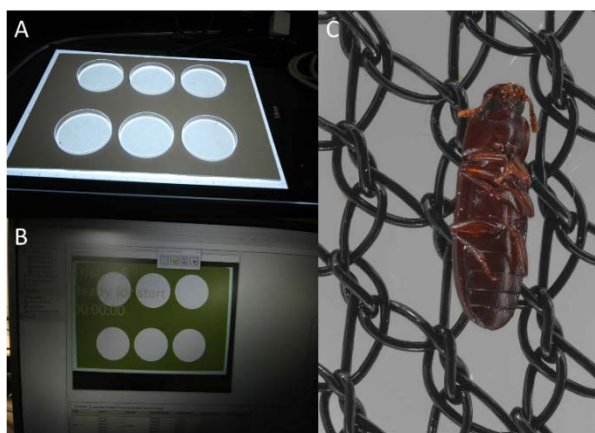
## 2. Materials and Methods

In the movement assay, the video-tracking software, Ethovision, recorded the movement of *R. dominica* and *T. castaneum* for 2 h after 1, 5, or 10 min exposures to LLIN or control netting and a waiting period of 1 min, 24 hr, 72 hr, or 7 days post-exposure (Fig. 1). The movement variables characterized were the total distance moved (cm) by adults and their mean angular velocity (deg/s). In the dispersal assay, *R. dominica* and *T. castaneum* were observed after 5 minutes of exposure to LLIN or control netting to measure the insects' ability to reach new food patches at three different distances (25, 75, or 175 cm) at the conclusion of a 48-h period. These were performed in laboratory spaces and environmental chambers under standardized abiotic conditions (30°C, 65% RH, 14:10 L:D). The data did not conform to the assumptions of normality, and thus were log-transformed prior to running the final model. Data were analyzed with an ANOVA, and upon a significant result from the model, Tukey's HSD were performed for post-hoc pairwise comparisons. All data was analyzed using the R Software (R Core Team, 2017).

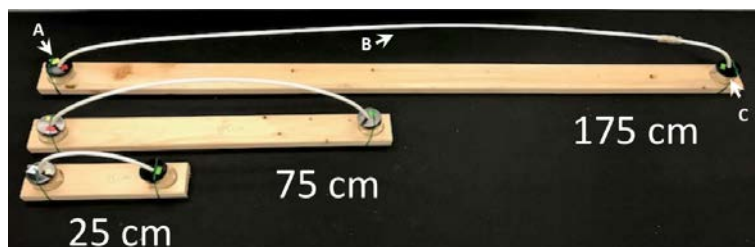
## 3. Results

In the movement assay (Fig. 1), brief bouts of 1-min exposure to LLINs resulted in the same numbers of affected and dead as longer 10-min exposure to the same nets for both species (ANOVA: *T. castaneum*,  $F = 0.073$ ;  $df = 2, 404$ ;  $P < 0.93$ ); *R. dominica*,  $F = 1.38$ ;  $df = 2, 407$ ;  $P = 0.25$ ). Importantly, movement was decreased by 3-fold for both species after exposure to LLINs (*T. castaneum*:  $F = 102$ ;  $df = 1, 404$ ;  $P < 0.0001$ ; *R. dominica*:  $F = 28.2$ ;  $df = 1, 407$ ;  $P < 0.0001$ ). There was some recovery of *T. castaneum* at 72 h and 7 d, but not for *R. dominica*. Movement was immediately reduced by half after exposure, and by 24-72 h later, movement was reduced by 4 to 9-fold compared with adults exposed to control netting. The behavioral effects of exposure extended out to 7 d later for both species where movement was still reduced by half or more compared to control netting-exposed adults. Angular velocity was elevated for LLIN-exposed adults compared to those exposed to control netting (*T. castaneum*:  $F = 289$ ;  $df = 1, 404$ ;  $P < 0.0001$ ; *R. dominica*:  $F = 38.1$ ;  $df = 1, 407$ ;  $P < 0.0001$ ), though this effect attenuated by 7 d after exposure for *R. dominica*, but not *T. castaneum*.

The dispersal assay (Fig. 2) revealed that exposure to LLIN had a significant effect on the dispersal ability of *T. castaneum* ( $F = 2151$ ;  $df = 1, 89$ ;  $P < 0.0001$ ) with averages of ~1 from a group of 20 beetles reaching the new food patch after exposure to LLINs, whereas almost the full set of 20 control netting-exposed adults reached the novel food patch. Out of over 1,400 *R. dominica* tested, not a single LLIN-exposed adult reached the novel food resource ( $F = 701$ ;  $df = 1, 54$ ;  $P < 0.0001$ ). The distance that adults had to disperse did not impact their ability to disperse; the primary determinant was whether they were exposed to netting with insecticide. The dispersal distance did not affect the dispersal capacity of either species for the range of distances tested (*T. castaneum*:  $F = 1.59$ ;  $df = 2, 89$ ;  $P = 0.21$ ; *R. dominica*:  $F = 2.31$ ;  $df = 2, 54$ ;  $P = 0.11$ ).



**Fig. 1** The movement assay used with A) individual adult *Tribolium castaneum* or *Rhyzopertha dominica* placed in 100 × 15 mm petri dishes, 2) their movement tracked with a video camera and sent to software on a computer, and C) the effect of netting on the mobility of *T. castaneum*. Please note that figures (photographs or graphs) shall be provided in the best possible resolution without frames.



**Fig. 2** The dispersal assay that tested the ability of *Tribolium castaneum* and *Rhyzopertha dominica* to move to a novel food resource after a 48 h period, with A) 20 adults introduced in the introduction chamber, B) a single line of twine threaded through the system from the bottom of the introduction chamber to halfway down the jar with the novel food resource so movement was only unidirectional, and C) a dispersal chamber with 15 g of organic, unbleached flour.

#### 4. Discussion

This is the first study to examine, in-depth, the sublethal effects of exposure to LLINs on any stored product insects. We have shown here that even brief exposure times of 1-min are sufficient to induce the same dramatic decreases in movement and increase in disorientation as longer 10-min exposures compared to controls. Exposure to LLINs reduced adult movement of both species by 3 to 4-fold. 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. Radically diminished dispersal capacity held steady even after a 2-3 d period during which adult *T. castaneum* or *R. dominica* could have recovered, but did not. As a result, this suggests that while mortality may be initially incomplete after exposure, brief bouts of contact with LLIN are adequate in preventing adults from reaching novel food patches. Overall, these results indicate that LLINs are a promising tool for the prevention of stored product insect establishment and colonization.

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