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Plodia interpunctella and *Trogoderma variabile* larval penetration and invasion of untreated and methoprene-treated foil packaging

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ABSTRACT

Packaged food products inside retail stores and food warehouses are susceptible to infestation by stored-product insects. The insect growth regulator methoprene can be impregnated onto packaging materials to help limit stored-product insect population development and prevent infestations. The objectives of this study were to determine the effect of methoprene-treated foil packaging on egg hatchability and the penetration and invasion ability of the warehouse beetle, *Trogoderma variabile* Ballion, and the Indian meal moth *Plodia interpunctella* (Hübner). Twenty-five eggs of each species were added to Petri dishes containing untreated packaging or packaging containing 0.1, 0.25, and 0.5% of the active ingredient methoprene. Petri dishes were held for 7 d in an environmental chamber at 27 °C and 60% r.h. to determine percent hatchability of eggs. Additionally, 6 cm by 8 cm food packages were created from untreated and methoprene-treated packaging and placed into 0.18-L vials. First or third instars of each species were introduced into vials containing diet or no diet, to determine penetration ability after 21 and 42 d. In a separate experiment, packages were pierced with pinholes, and first instar *T. variable* or *P. interpunctella* were introduced and observed after 21 and 42 d for package invasion. The foil packaging had no significant effect on egg hatchability of either species. *T. variabile* were unable to penetrate or invade any foil packages. *P. interpunctella* invaded all packages containing pinholes. The methoprene-treated packaging reduced adult emergence of *P. interpunctella* and caused pupal and adult deformations in *T. variabile*. Methoprene-treated packaging presents a valuable option for food manufacturers to prevent insect infestations and maintain integrity of packaged food products.

1. Introduction

Stored-product insects are a common and persistent problem in food-processing facilities, warehouses, distribution centers, retail stores, and consumer pantries (Highland, 1978). The warehouse beetle, *Trogoderma variabile* Ballion, and Indian meal moth, *Plodia interpunctella* (Hübner), are cosmopolitan insect pests (Highland, 1978; Campbell et al., 2002; Arthur et al., 2014), and are associated with food package infestations in retail environments (Roesli et al., 2003). Infestations in food facilities or retail stores are a result from established insect populations within the facility, immigration of insects from the outside environment, or bringing infested products inside the facility. Infestation by stored-product insects result in food product losses, economic losses, decreased consumer confidence, and the potential risk for allergic reactions (Subramanyam et al., 2001). If a consumer finds insects in their food product, they may hold the manufacturer responsible, even though the manufacturer might have little to no direct control of their product once it leaves their facility (Mullen et al., 2012). Stored-product insects can be classified as package penetrators and/or invaders (Highland, 1978, 1991; Mullen and Mowery, 2006). *T. variabile* and *P. interpunctella* larvae are both classified as penetrators, but are capable of entering packages through existing openings such as a hole, rip, or tear and become package invaders (Highland, 1978, 1991).

Adults of *P. interpunctella* and *T. variabile* are highly mobile insects. Mark-recapture studies show *P. interpunctella* dispersal ability around food-processing facilities to range from 21 to 276 m (Campbell and Mullen, 2004). Adults of *P. interpunctella* are commonly captured around doorways to the outside, pallet wrapping equipment, and near where food products are stored (Campbell et al., 2002; Arthur et al., 2013). Adults of *T. variabile* are

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capable of moving across multiple floors and distances ranging from 7 to 216 m throughout a warehouse (Campbell et al., 2002). Arthur et al. (2014) monitored a commercial food storage facility over three years using pheromone traps, and found *T. variabile* to be among the most common insect species captured. The highest percentage of *T. variabile* and *P. interpunctella* captures were in the primary food storage area of the warehouse, but insect captures also occurred in areas which did not contain stored food and were concentrated near loading docks, demonstrating the mobility of insects throughout a facility (Arthur et al., 2013, 2014). Changes in abundance of insect captures and species of stored-product insects may vary with the type of food product stored and transportation to and from the food storage facilities (Arthur et al., 2014). Areas of high food spillage, damaged food packages, and open doors and windows are common locations where infestations can begin (Subramanyam et al., 2001). Infestations can also occur through incoming products that are previously infested either at the manufacturing step or during transportation. However, it is difficult to pinpoint a precise location of an infestation source in a warehouse or retail setting, especially if infestations are widespread throughout the facility. The constant movement and rotation of food products complicates identifying a specific location as the likely source of an infestation (Arthur et al., 2013).

Pest management techniques commonly used in warehouse and manufacturing settings are sanitation, structural fumigations, heat treatments, removal of infested packages, and spot treatments with insecticides (Campbell and Arbogast, 2004). In retail environments typical pest management techniques include sanitation, first in first out stock rotation, and monitoring with food- and pheromone-baited traps (Subramanyam et al., 2001). These pest management techniques used independently may not be enough to prevent infestations of warehouses and packaged food products, but a multi-pronged approach to pest management which incorporates sanitation, spot treatments, insecticide-treated packaging, and insect-resistant packaging would be better suited for food manufacturers to protect their packaged products in warehouses, distribution centers, and retail environments.

Methoprene is an insect growth regulator (IGR), that has previously shown to be effective against lepidopterous and colepterous pests (Henrick, 2007). Additionally, the United States Environmental Protection Agency granted methoprene exemption from food tolerance levels in 2003 (Henrick, 2007). Methoprene impregnated woven and polymer packaging have previously shown to produce adverse effects against the red flour beetle, *Tribolium castaneum* (Herbst), and *T. variabile* larvae (Scheff et al., 2016, 2017). Continual exposure of *T. castaneum* larvae on methoprene-treated woven or polyethylene to polyethylene (PE-PE) layered packaging gave 100% suppression of normal adult emergence. The outside surface of the PE-PE packaging gave >99% reduction in normal adult emergence of exposed *T. variabile* larvae (Scheff et al., 2016, 2017). *P. interpunctella* has shown reduced adult emergence from eggs exposed to topical application of methoprene to a variety of surfaces and packaging materials (Jenson et al., 2009). However, the effect of methoprene-treated foil packaging has not been tested on stored-product insects nor the effectiveness of methoprene-treated packaging on preventing package penetration or invasion by *P. interpunctella* or *T. variabile*. This is the first study to describe the effects of methoprene-treated foil based packaging against *P. interpunctella* and *T. variabile* and its effectiveness on prevent package penetration or invasion by larvae.

Trogoderma variabile and *P. interpunctella* are among the most important penetrating stored-product insects, but are also capable of invading packages through openings when presented with the opportunity (Highland, 1991). These species also represent the most-common insects associated with packaged foods (Mullen and Mowery, 2006). It is beneficial to insect pest management programs and food manufacturers to know if the methoprene-treated packaging can protect food products from infestation, and could thus be incorporated into a packaging technology program or into a food safety program. Therefore the objectives of this study were to determine the effect of methoprene-treated foil packaging on egg hatchability, and penetration and invasion ability of *T. variabile* and *P. interpunctella* larvae into food packages.

2. Materials and methods

2.1. Insects

Plodia interpunctella and *T. variabile* used in this study were obtained from laboratory cultures at the United States Department of Agriculture's Center for Grain and Animal Health Research, in Manhattan, Kansas, USA. *P. interpunctella* was reared on a diet consisting of cracked wheat and wheat shorts (4.4 kg), brewer's years (22 g), sorbic and benzoic acids (9.5 g each), honey (240 ml), glycerin (240 ml), and water (120 ml) (Jenson et al., 2009) at 27 °C and 60% r. h and 16:8 L:D photoperiod. *T. variabile* cultures were reared on a 50% Purina One lamb and rice formula (Nestlé Purina PetCare Company, St. Louis, MO, USA), 50% Pharmanex vanilla shake mix, and the top of the culture was sprinkled with 100% whole grain rolled oats (Kroger Co., Cincinnati, Ohio, USA). The contents of the vanilla shake mix cannot be disclosed due to confidentiality agreements, and therefor considered as proprietary information. *T. variabile* cultures were maintained at 30 °C and 60% r. h. and 16:8 L:D photoperiod.

2.2. Effect of methoprene-treated packaging on egg hatchability of *P. interpunctella* and *T. variabile*

Plodia interpunctella eggs were obtained by collecting pupae from rearing jars. Corrugated paper spools were added on top of *P. interpunctella* diet in jars to provide pupation sites for wandering larvae (Huang and Subramanyam, 2003). Pupae were collected from the paper spools and transferred into 1.9-L glass jars fitted with a mesh screens and filter papers. Glass jars were inverted and moths were allowed to mate and oviposit. Eggs (< 24 h) were collected and counted using an aspirator with a small glass collection vial.

To obtain *T. variabile* eggs, approximately 100 g of 95% unbleached whole-wheat flour (Hudson Cream Flour, Stafford Country Flour Mills Co., Hudson, Kansas, USA) was sifted through a 150 μm opening sieve (Newark Wire Cloth Company, Clifton, New Jersey, USA) and placed into a 0.18-L glass jelly jar (Ball, Muncie, Indiana, USA). A total of 50 unsexed *T. variabile* adults of mixed ages were introduced into the jar. The jars were held in an environmental growth chamber at 30 °C and 65% r. h. and 16:8 L:D photoperiod. After 3–4 d, adults were removed from jars using a sieve with 850 μm opening. The flour was sifted through a 250 μm opening and the eggs were retained on top of the sieve. Eggs were counted and collected using an aspirator with a small glass collection vial.

Methoprene-treated and untreated foil packaging materials were obtained from a commercial manufacturer (ProvisonGard™, Greensboro, North Carolina, USA, http://www.pvgard.com). The specific manufacturing process is proprietary information, but the methoprene-treated foil packaging contained the active ingredient methoprene at loading rates of 0.1, 0.25, and 0.5% incorporated into the adhesive matrix of the material. Eighty individual arenas were prepared for this study. From the foil packaging, 9 cm diameter circles were cut by hand and further cut to form a semicircle from the methoprene-treated and untreated materials. Out of the 80