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Susceptibility of *Tribolium castaneum* and *Trogoderma variabile* larvae and adults exposed to methoprene-treated woven packaging material

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ABSTRACT

Methoprene is an insect growth regulator (IGR) registered in the United States for direct application to stored raw grains, as an aerosol or residual contact insecticide, and for use as a coating for protection of packaged products. A series of tests was conducted to determine the adverse effects of short term exposure, sub-lethal exposure, and continual exposure of the red flour beetle, *Tribolium castaneum* (Herbst) and warehouse beetle, *Trogoderma variabile* Ballion, on methoprene-treated woven packaging material at 27 and 32 °C and 60% r.h. In the first test, larvae of both species were added to individual arenas and exposed for different time intervals, removed and resulting adult emergence was assessed. In the second test, eggs of both species were exposed on the packaging surfaces to determine percent egg hatchability. In the third test, adults of *T. castaneum* and *T. variabile* were added to arenas and held for 7 and 3 d, respectively, to determine number of eggs laid per female and subsequent egg hatchability. The eggs were held in arenas to determine the effect of continual exposure on egg-to-adult emergence. Results showed normal adult emergence decreased with increasing exposure time and temperature. Exposure to methoprene-treated packaging did not adversely affect fecundity of *T. variabile* adults, but did affect fecundity of *T. castaneum*. Continual exposure gave 100% suppression of *T. castaneum* adult emergence and a reduction of *T. variabile* emergence. This study indicated that methoprene-treated packaging could be a valuable addition to an existing integrated pest management program to increase protection of packaged products.

1. Introduction

Stored-product insects are serious pests in raw and processed grains, pet foods, and birdseed (Arbogast et al., 2000). Food and feed manufacturing companies take all possible measures to produce an insect free product, but they have little to no control of their product once it leaves their manufacturing facility (Platt et al., 1998). Retail stores present a serious threat for infestation of packaged products by stored-product insects (Roesli et al., 2003). Potential sources of infestation include incoming commodities that are already infested, insects entering through open doors or windows, and insect populations already present within the store (Arbogast et al., 2000; Subramanyam et al., 2001). The red flour beetle, *Tribolium castaneum* (Herbst), is a stored-product insect commonly found in retail stores (Arbogast et al., 2000; Roesli et al., 2003). A survey of eight retail pet stores in Kansas found *T. castaneum* and the warehouse beetle, *Trogoderma variabile* Ballion, to be present in seven out of eight stores surveyed (Roesli et al., 2003). *T. castaneum* and *T. variabile* have been identified as two species of primary economic importance that infest food products (Boyer et al., 2012). In retail stores, stored-product insects can be found above or underneath shelves, behind kick-plates, near the pet food department, and in accumulations of spilled food (Arbogast et al., 2000 Roesli et al., 2003).

Packaged food products are also susceptible to infestation by insects during transportation and storage in retail stores (Highland, 1991). Food packaging is designed to protect food products from the point of manufacture to consumption (Campbell et al., 2004). Insects infest packaged products by penetration or invasion. Package penetrators such as the larvae of *T. variabile* can chew through packaging material and infest food products (Highland, 1991). Package invaders such as *T. castaneum* larvae enter packages through natural defects, holes, or seam failures (Highland, 1991). The larval stage of stored-product insects cause the most damage,

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because they can invade smaller holes than adults and possess powerful mouthparts which are capable of chewing through various packaging materials (Wohlgemuth, 1979).

Methoprene, an insect growth regulator (IGR), mimics insect juvenile hormone in insects which regulates the developmental process from the egg to the adult stage (Oberlander et al., 1997; Henrick, 2007). In 2003, the United States Environmental Protection Agency granted methoprene exemption from food tolerance levels (Henrick, 2007). Methoprene is very effective in controlling a variety of lepidopterous and coleopterous pests (Henrick, 2007). Recently, methoprene has been incorporated into the adhesive matrix of packaging materials used to store food products and is registered and marketed by ProvisonGard™ (Greensboro, North Carolina, USA; http://www.pvgard.com) (Arthur, 2016; Scheff et al., 2016).

There is little published data assessing efficacy of methoprene-treated packaging on *T. castaneum* and *T. variabile*. The fecundity, egg hatchability, and egg-to-adult emergence of these two species were evaluated on untreated and methoprene-treated polyethylene-to-polyethylene (PE-PE) and polyethylene terephthalate-to-polyethylene (PET-PE) packaging at 27 and 32 °C and 60% r.h. (Scheff et al., 2016). Adverse effects, especially egg hatchability and egg-to-adult emergence, upon exposure to inside and outside surfaces of methoprene-treated packaging were more pronounced in *T. castaneum* compared with *T. variabile*. In the present investigation, adverse effects of methoprene-treated woven packaging were evaluated against these two insect species. Specific research objectives were to determine adverse effects on larvae of *T castaneum* and *T. variabile* exposed to untreated and methoprene-treated woven packaging material for short time intervals, determine effects on adult fecundity and subsequent egg hatchability, and determine effects of continuous exposure of eggs laid by adults on packaging material on adult emergence of the two species.

2. Materials and methods

2.1. Insects

Cultures of *T. castaneum* and *T. variabile* used in this study were obtained from the United States Department of Agriculture's Center for Grain and Animal Health Research (USDA-CGAHR) in Manhattan, Kansas. *T. castaneum* cultures were reared on 95% unbleached whole-wheat flour (Hudson Cream Flour, Stafford Country Flour Mills Co., Hudson, Kansas, USA) with 5% by wt. of brewer's yeast (MP Biomedicals LLC, Solon, Ohio, USA) and maintained at 27 °C and 60% r.h. in constant darkness. *T. variabile* cultures were reared on 50% Purina One lamb and rice formula (Nestlé Purina PetCare Company, St. Louis, Missouri, 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 are considered proprietary information and due to confidentiality agreements, cannot be disclosed. Cultures were maintained at 30 °C and 60% r.h., and 16:8 L:D photoperiod.

Eggs of both species were obtained by using approximately 100 g of flour sifted through a 150 μm opening sieve (Newark Wire Cloth Company, Clifton, New Jersey., USA), placed into a 0.18-L jelly jar (Ball, Muncie, Indiana, USA), and 60 unsexed *T. castaneum* or *T. variabile* adults of mixed ages were introduced. The jars were incubated at 30 °C and 65% r.h., and 16:8 L:D photoperiod to allow for mating and oviposition. After 3 d, the adults were removed from jars using an 850 μm opening sieve on the top to retain the adults. The flour passed through the bottom 250 μm opening sieve and was collected in a pan. Eggs were retained on top of the 250 μm sieve. Eggs were collected and counted using an aspirator.

In tests with adults, unsexed adults of mixed ages were used. Adults were directly aspirated from culture jars. After exposure to treatment arenas the adults were removed, frozen, and separated as male and female. Adult *T. castaneum* were held for 7 d on arenas, and *T. variabile* adults were held for 3 d. These exposure periods were determined based on preliminary experiments examining progeny production suitable for experimentation without overcrowding the arenas. Male *T. variabile* were distinguished by the 6–7 segmented antennal club, whereas females only have 4 segmented antennal club (Bousquet, 1990). Male *T. castaneum* possess a setiferous patch on the posterior side of the fore femur, while the female lacks the patch (Bousquet, 1990).

2.2. Methoprene-impregnated packaging material

Methoprene-treated and untreated woven packaging materials were obtained from a commercial manufacturer (ProvisonGard™, Greensboro, North Carolina, http://www.pvgard.com). The specific extrusion process is proprietary information, but the process of impregnating the methoprene is as follows: the outer layer of the packaging consisted of biaxially oriented polypropylene that was 18 μm thick (18 g/m^2 weight), and the inner woven layer consisted of a 60 g/m^2 of fabric weight. The middle adhesive resin layer of 20 g/m^2 weight contained the active methoprene layer which was mixed into the polymer pellet matrix and extruded. This layer is 8 μm thick and is loaded with a methoprene application rate of 0.1% or 1000 ppm per area.

2.3. Effect of short term larval exposure on adult emergence

Eighty individual treatment arenas were constructed by cutting 150 × 25 mm (137 cm^2) circular discs from packaging material that either contained methoprene (40 discs) or did not contain methoprene (40 discs). Out of the 40 discs containing methoprene, 20 discs with the inside surface and 20 discs with the outside surface were placed individually into separate 150 × 25 mm Petri dishes. The dish edges were secured down by using adhesive caulking (DAP Products Inc., Baltimore, Maryland, USA) and the inner sides were coated with polytetrafluoroethylene (Fluon®) (Sigma-Aldrich Co., St. Louis, Missouri, USA) to prevent insects from crawling on the sides or under the material. The inside and outside surfaces of the untreated packaging were placed in Petri dishes, in the same manner. Treatment combinations included, untreated or methoprene-treated material, two surfaces (inside vs. outside), two temperatures 27 and 32 °C at 60% r.h, and two insect species, *T. castaneum* and *T. variabile*. Each treatment combination was replicated five times.

Testing methodology was modified from that described by Arthur and Fontenot (2012). Fifty 4-week-old larvae of each species were exposed on each of the 40 methoprene-treated and untreated (control) arenas. Each arena was supplemented with 1500 mg of flour with yeast (*T. castaneum*) or vanilla shake mix (*T. variabile*). Treatment and control arenas were placed in an environmental chamber at 27 and 32 °C, both at 60% r.h. Larvae were exposed to methoprene-treated or untreated arenas and inside or outside surfaces for 8, 24, 48, 72, and 96 h. At each exposure time, 10 larvae were selected at random and removed from the arena and transferred to new untreated Petri dishes, 100 × 15 mm for *T. castaneum* or 100 × 20 mm for *T. variabile*, along with 500 mg of the respective untreated insect diet and held in the environmental chambers at the two temperatures for 3–4 weeks until the adults emerged. Diet was added as needed to sustain growth. The number of normal adults that emerged, and did not have any visible morphological deformities, were recorded and expressed as a percentage of the original number exposed. Any beetles that remained in the larval or pupal stages or adults with morphological deformities were also