PACKAGING TECHNOLOGIES FOR THE CONTROL OF STORED-PRODUCT INSECTS

by

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B.S., University of Wisconsin – Eau Claire, 2010
M.S., University of Wisconsin – Stout, 2013

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
College of Agriculture

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Abstract

Larvae of the Indian meal moth, *Plodia interpunctella* (Hübner), have the ability to invade or penetrate packaging materials and infest the food within. Energy bars with three polypropylene packaging types were challenged with eggs (for first instars), third, and fifth instars of *P. interpunctella* to determine package resistance to larvae at 28°C and 65% r.h. to determine which provided the greatest protection against *P. interpunctella* larval penetration. Third and fifth instars showed a higher propensity to infest all packaging variations. First instars showed a reduction in package pentration abiltiy compared to third and fifth instars.

Methoprene is an insect growth regulator labeled in the USA for use as an aerosol spray, a residual surface treatment, and as a grain protectant, and recently has been impregnated into a polymer-based packing film to prevent insect infestations of packaged products. The objectives of these studies were 1) determine the effect of short term exposure time and temperature on four week old larvae, continual exposure on egg-to-adult emergence of beetles, and sub-lethal effect on adults of the red flour beetle, *Tribolium castaneum* (Herbst) and warehouse beetle, *Trogoderma variabile* Ballion, on the inside and outside surfaces of methoprene-treated woven packaging material at 27 and 32°C at 60% r.h.; 2) evaluate fecundity, egg hatch, and egg-to-adult emergence of *T. castaneum* and *T. variabile*, when exposed to two methoprene-impregnated polymer packaging on the inside and outside surfaces at 27 and 32°C at 60% r.h.; and 3) determine the effect of methoprene-treated foil packaging on larval emergence, penetration, and invasion ability of *T. variabile* and *P. interpunctella* at 27°C and 60% r.h.

Short term exposure results indicated that adult emergence from larvae of *T. castaneum* and *T. variabile* decreased with increasing exposure time. The number of eggs laid per female of *T. castaneum* and *T. variabile* did not vary from their controls. Continual exposure demonstrated

100% supression of *T. castaneum* adult emergence, irrespective of exposure to outside or inside surfaces. *T. variabile* exposed to inside surfaces were unaffected and normal adult emergence was reduced in those exposed to outside surfaces..

The number of *T. variabile* eggs laid per female was not significantly different among polymer packaging types. The methoprene-treated polyethylene terephthalate to polyethylene packaging, PET-PE reduced the number of *T. castaneum* eggs laid per female. Both polymer packaging reduced the percent hatch of both species. No *T. castaneum* adults emerged on the inside surface of PET-PE and both sides of the polyethlyene to polyethylene (PE-PE). Egg-to-adult emergence of *T. variabile* was arrested at the pupal stage on the outside surface of PE-PE packaging. The PET-PE packaging greatly reduced the number of normal adults by 87 to 97% when exposed to inside surfaces at both temperatures.

The foil packaging had no significant effect on hatch of either species. *T. variabile* were unable to penetrate/invade any foil packages. *P. interpunctella* invaded all packaging containing pinholes. Therefore, continual exposure of *T. castaneum* and *T. variabile* to methoprene impregnated packaging could be a vaiable tool to protect food packages.

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Table of Contents

List of Tables

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Dedication

I would like to dedicate my dissertation to my loving family. Thank you to my parents, Gary and Susan Scheff, and my brother Daniel Scheff, for all you love and support throughout my studies.

Chapter 1 - Literature Review

1.1. Biology of Common Stored-Product Insects

1.1.1. *Tribolium castaneum*

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is found throughout the world, readily distributed under natural conditions, and is considered one of the most abundant stored-product insects found in flour mills (Hinton, 1948; USDA-ARS, 1986; Hagstrum et al., 2013). The *T. castaneum* subgroup originates from the Indo-Australian region and includes seven species (Hilton, 1948). The *T. castaneum* subgroup includes: *T. castaneum, T. madens, T. freemani, T. cylindricum,* and *T. politum* from the oriental region and *T. waterhousei* and *T. antennatum* from the Australian Region (Hilton, 1948).

T castaneum has been associated with 233 stored commodities (Hagstrum et al., 2013). In raw stored grain, *T. castaneum* is considered a secondary pest because it feeds on broken kernels, flour, or grain dust and feeds on the germ of intact kernels. However, *T. castaneum* is a primary pest of flour mills. *T. castaneum* is capable of causing serious damage to infested food because the insect imparts a nauseous odor and taste to the infested food products (USDA-ARS, 1986). The change in taste and odor imparted by *T. castaneum* is caused by benzoquinones excreted by adults (Reichmuth et al., 2007). These benzoquinones also have insecticidal properties. The developmental time of this insect varies with temperature and relative humidity, but optimum conditions are 32-35°C and 60% r.h (Howe, 1956).

1.1.1.1. Description and Life Cycle

Female *T. castaneum* lays and average of 450 eggs per female (USDA-ARS, 1986). *T. castaneum* eggs are white in appearance, 0.4-0.6 mm in diameter, and are covered with a sticky secretion (Reichmuth et al., 2007). The secretion will make flour or other food particles stick to

the egg or allow for the egg to adhere to the sides of boxes or packages. *T. castaneum* eggs generally hatch within 5-12 days of oviposition.

Howe (1956) noted that *T. castaneum* eggs do not hatch at any relative humidity at temperatures of 15 or 17.5°C, but any temperature and humidity combination ranging from 20-40°C results in a mean egg hatch of 75-92%. As the temperature decreases the egg period for egg hatch increases from 2.7 days at 40°C to 13.9 days at 20°C and that egg development is fastest at 37.5°C (Howe, 1956).

Newly-hatched larvae appear small, slender, and cylindrical in appearance (USDA-ARS, 1986). Neonate larvae are approximately 1 mm in length and fully grown larvae are roughly 8 mm in length (Reichmuth et al., 2007). Fully grown larvae have a dark brown colored head capsule, three pairs of legs, and two prolegs at the ninth segment of their abdomen (Reichmuth et al., 2007). Late instars transition into naked pupae.

T. castaneum larvae are less tolerant to extreme conditions compared to eggs (Howe, 1956). The optimum temperature for larval development to pupae is 35°C, and increasing relative humidity decreases development time to the pupal stage (Howe, 1956). Similar to egg development, decreasing the rearing temperature increase the average larval to pupal transition (Howe, 1956).

In the initial stage of *T. castaneum* pupation, the pupae appear whitish in color. As the pupae ages, it changes from white to yellow and finally brownish in color, 3-4 mm in length, before adult emergence (USDA-ARS, 1986; Reichmuth et al., 2007). Contrary to larval developmental time, the length of the pupal period is not affect by humidity (Howe, 1956). However, temperature significantly affects developmental time. The pupal period at 40°C is 4.4

days, while at 20°C the pupal period is 24.4 days (Howe, 1956). The most rapid developmental time occurs at 37.5°C, 3.9 days, which is the same as the egg developmental stage (Howe, 1956).

The adult *T. castaneum* is reddish-brown in color with a flattened and oval shaped appearance (USDA-ARS, 1986). The upper thorax and head display minute punctures when observed under a microscope. *T. castaneum* is nearly identical to *T. confusum* except for the antennae, in which *T. confusum* gradually increase in size and has a distinct 3-segmented antennal club and *T. castaneum* abruptly increases in size (Hilton, 1948; USDA-ARS, 1986). Adult beetles range in size, 3-4 mm (Reichmuth et al., 2007). Adult females lay 2-18 eggs per day. Adult males can be distinguished from females by the setiferous patch on the posterior side of the fore femur (Bousquet, 1990).

The life cycle of *T. castaneum* ranges from 40-90 days depending on season, temperature, and relative humidity. The adult and larval stages of *T. castaneum* are known to be cannibalistic and will eat their own eggs and pupae (Howe, 1956; Ryan et al., 1970). Both *T. castaneum* and *T. confusum* are cannibals, yet *T. castaneum* is more predaceous compared to *T. confusum* (Ryan et al., 1970). When given a choice each species prefers to eat the other, but if given the right circumstances they will cannibalize their own species.

1.1.2. *Trogoderma variabile*

The warehouse beetle, *Trogoderma variabile* Ballion (Coleoptera: Dermestidae) was formerly known as *T. parabile* (Beal). *T. variabile* was first described by Beal in 1954 and was reported in 1956 as a common pest of granaries in California (Loschiavo, 1960). *T. variabile* is distributed throughout the world on six contents and associated with 119 commodities such as dried milk, rolled oats, cat food, ground dog food, and barley (Hagstrum et al., 2013). After *Trogoderma granarium* (Everts), the khapra beetle, T. *variabile* the most serious dermestid pest

(Loschiavo, 1967; Okumura, 1972). *T. variabile* is also known to feed on other dead insects (Okumura, 1972).

1.1.2.1. Description and Life Cycle

Newly laid eggs are pearly-white in color, translucent, covered in a sticky secretion and are cylindrically elongated (Loschiavo, 1960). *T. variabile* eggs are extremely fragile, but after 3 days they become more durable and can be handled with a soft-haired brush or an aspirator (Loschiavo, 1960). A day prior to hatch, neonate larvae can be seen through the transparent egg. The egg has a reddish-brown appearance (Loschiavo, 1960). The posterior end appears dark brown and the anterior end will have five distinct brown spots or ocelli (Loschiavo, 1960). The setae and larvae segments are also visible prior to hatching.

Egg hatch is generally 95%, and the duration of the egg stage is 6-8 days at 32°C and 70% r.h. (Loschiavo, 1960; Burges, 1961). Partida and Strong (1975) found that decreasing temperatures increased the number of days required for eggs to hatch. At 37.8°C and 70% r.h. eggs required 5-8 days to hatch and at 30% r.h. 7-8 days were required (Partida and Strong, 1975). However, at temperatures between 32.2 - 21.1°C, decreasing the humidity resulted in a decrease in the average number of days for eggs to hatch and an increase in percent egg hatch (Partida and Strong, 1975). At 26.7°C and 70% r.h., eggs took 9-10 days to hatch with an egg hatch of 82%, while at 30 % r.h. eggs took 8 days to hatch and had a 90% egg hatch (Partida and Strong, 1975). Partida and Strong (1975) observed at a temperature of 32.2°C oviposition lasted 4 days, while at 15.6°C oviposition lasted 12 days at constant humidity. Similar results were observed by Loschiavo (1967). Female *T. variabile* laid the highest percentage of eggs during the first day of oviposition (Partida and Strong, 1975). Loschiavo (1967) observed highest mean

egg production per female was highest at 27.5 and 30.0°C, and the upper temperature limit for egg production was between 37.5-40.0°C and the lower limit was below 17.5°C.

Newly hatched larvae will disperse in search of food upon hatching. The larvae of *T. variabile* varies in color from yellowish-white to brown as the larvae ages and is approximately 6 mm long when fully grown (Okumura, 1972). Larvae will generally under 6 molts, instars, before pupating but larvae are capable of undergoing diapause and can molt 28 times in 11 months (Loschiavo, 1960; Okumura, 1972). The first instar's head capsule width in mm is 0.19 ± 0.01, second instar 0.26 ±0.001, third instar 0.37 ± 0.1, forth instar 0.55± 0.003, fifth instar 0.75±0.01, and sixth instar 0.86 ± 0.05 (Rai, 2014).

T. variabile larvae are unique because they have two types of setae, bristles or hair; hastisetae and spicisetae (Okumura, 1972). Hastisetae are spear-headed shafts containing barbs, and spicisetae are elongated structures (Okumura, 1972). On average, *T. variabile* has 1,706 hastisetae and 2,196 spicisetae (Okumura, 1972). *T. variabile* also has hairs covering the entire surface of the larval body (Loschiavo, 1960). As the larvae ages, the hairs or tail, increases in length. The mean length of a larvae's tail is 1.6, 0.9, 0.6, 0.5, and 0.4 times the mean body length of the first, second, third, fourth, fifth, and sixth instar, respectively (Loschiavo, 1960). During the molting process, the larval skin splits along the mid-dorsal line from the head to the sixth abdominal segment, and the cast skin is left behind (Loschiavo, 1960). These cast skins, along with the setae, can be problematic to sensitive individuals (Okumura, 1972).

During the last molt of the larval skin, the pupa splits the larval skin but is not cast off (Loschiavo, 1960; Burges, 1961). The dorsal side of the pupae is visible through the split skin. Loschiavo (1960) observed that greater than 90% of pupae are found at or near the surface of a food source. The mean lengths and widths of male pupae, 4.42 mm and 1.71 mm, are smaller

than females, 6.43 mm and 2.54 mm (Loschiavo, 1960). The mean development time for pupae is 2-6 d at 32°C and 70% r.h. (Loschiavo, 1960). Partida and Strong (1975) observed that the pupal stage of *T. variabile* increased as temperatures decreased from 37.8-21.1°C at constant 50% r.h.

Recently emerged adult *T. variabile* will spend 1-7 d within the larval skin before leaving (Loschiavo, 1960). Adult *T. variabile* are approximately 2.0-4.6 mm in length and the female is larger in size than their male counterpart (Loschiavo, 1960; Reichmuth et al., 2007; Hagstrum et al., 2013). *T. variabile* adults are a black and oval-shaped, and are covered with a fine pubescence (Loschiavo, 1960). The elytra have reddish-brown maculae, but are varied throughout the species (Loschiavo, 1960). Adult males are distinguished from females by the antennae. Males have a 6-7 segmented antennal club, while females only have 4 (Bousquet, 1990).

Larvae of *T. variabile* are capable of undergoing diapause, which is an arrested development which occurs in either favorable or unfavorable conditions for an extended period of time (Loschiavo, 1960). An arbitrary threshold for larval development at 7 weeks and 30°C and 60-70 r.h., has been established as the dividing point between normal and delayed pupation in *T. variabile* (Burges, 1961). Larvae can be arrested in diapause for up to two years. *T. variabile* larvae will stop at the full grown larval stage, 6^{th} instar, and will continue to molt at irregular periods. As the larvae continue to age, the length between molts increases. Loschiavo (1960) observed that during an 11 month period, diapausing larvae molted 15-28 times. Additionally, the longest time observed between molts was 63 days (Loschiavo, 1960). Many factors play apart in inducing larval diapause. Loschiavo's study (1960) used daily disturbance and handling to induce diapause. Overcrowding and limited availability of food are other factors

which may prompt diapause in *T. variabile*. Burges (1961) noted an inverse proportional relationship between the amount of food and delayed pupation. Delayed pupation increased with decreasing amounts of food, but was not significantly related to the amount of food per larvae (Burges, 1961).

The mean adult life span ranges from 8-20 days (Loschiavo, 1960). Partida and Strong (1975) observed at an average temperatures of 32.2, 26.7, 21.1°C, the egg-to-adult development for males was 40-67, 38-57, and 62-94, respectively. For females at the same conditions developmental time was 47-70, 43-60, 64-113, respectively (Partida and Strong, 1975). In general, egg-to-adult developmental time was faster for males than females (Burges, 1961). Additionally, the average lifespan of adult males is inversely related to temperature, and females follow the same trend at temperatures under 35°C (Loschiavo, 1967). Females may lay up to 94 eggs in a single day (Loschiavo, 1960; Okumura, 1972). Loschiavo (1960) observed that the maximum oviposition occurred among females that were 3-5 d old. Additionally, females older than 7 d laid fewer eggs, and virgin females do not lay eggs (Loschiavo, 1960). Non-ovipositing females generally live 2-3.5 times longer than ovipositing females at temperatures between 22.5-35.0°C (Loschiavo, 1967).

1.1.3. *Plodia interpunctella*

The Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) was first described by Jacob Hübner in 1827 (Hamlin and Reed, 1931). Asa Fitch was the first to refer to this species by the name Indian meal moth, because he observed the larvae feeding on corn meal (Hamlin and Reed, 1931). Common names of *P. interpunctella* include cloaked-not-horn moth, dried fruit moths, horn compressed vegetable moth, mealworm moth, coppery dry fruit moth, and south storage moth (Hagstrum et al., 2013). *P. interpunctella* has been reported in every

continent except Antarctica and in nearly 50 different countries (Jonson et al., 1992). *P. interpunctella* is a cosmopolitan storage pest and can be associated with 179 commodities such as chocolate, wheat flour, pecans, and pet food (Hagstrum et al., 2013). It is one of the most general feeders among stored-product pests (Hamlin and Reed, 1931). *P. interpunctella* can be found in retail stores, warehouses, processing facilities, flourmills, and consumer homes. *P. interpunctella* infestations results in food safety and quality issues, product adulteration, and economic losses. Loses are not directly related to the amount of product consumed by the moth, but from the quality issues resulting from infestations. Larvae will leave behind excrement, cast skins, webbing, dead individuals, and pupal casings on food surfaces (Hamlin and Reed, 1931).

1.1.3.1. Description and Life Cycle:

Female moths lays between 100-300 eggs in a lifetime and the number of eggs directly depends on temperature, humidity, and availability of food (USDA-ARS, 1986). The egg sizes range from 0.3-0.5 mm in length, are grayish-white in color, and are nearly perfectly ovate (Hamlin and Reed, 1931; Subramanyam, 2011). When observed under a stereomicroscope, the eggs appear to have a grainy texture. Females will lay eggs singly or in groups depending on the availability of a food source. In general, females will lay more eggs on areas with food than those lacking food. Food odor emanating from packaged products or processing facilities will significantly affect the oviposition behavior of *P. interpunctella* (Philips and Strand, 1994). Adult moths will orient themselves towards food odors and lay more eggs on substrates containing food versus those without food (Philips and Strand, 1994). Female oviposition is also influenced by the presence of larval secretions on a surface (Philips and Strand, 1994). Larval secretions may signal to female moths that a given oviposition site can support the growth of newly-hatched eggs (Philips and Strand, 1994). In instances where direct access to food sources

are unavailable, females will lay their eggs near a food source in which young larvae may travel to upon hatching.

The larvae of *P. interpunctella* will undergo five to six instars before pupation and instars can be distinguished by their head capsule size. The mean ± SE first instar's head capsule size in mm 0.25 ± 0.001, second instar 0.43 ± 0.001, third instar 0.66 ± 0.003, forth instar 0.71 ± 0.003, and fifth instar is 0.96 ± 0.003 (Allotey and Goswami, 1990). When the larvae are fully-grown, their size ranges from 9-19 mm but average is 13 mm in length (Hamlin and Reed, 1931). Larvae begin to grow as soon as they hatch and have a higher growth ratio between the first and second instars (1.72) compared to any other stages indicting a faster growth rate at younger instars than older (Allotey and Goswami, 1990). *P. interpunctella* larvae are whitish in color but can vary to a pinkish-brown color and variation in color is also seen in larvae excrement (Hamlin and Reed, 1931). The variation in color can be a result in age and diet of the larvae. *P. interpunctella* larvae contain 10 abdominal segments, which are covered with long, fine hairs (Hamlin and Reed, 1931). The 3-6th and 10th segments each contain a pair of short prolegs (Hamlin and Reed, 1931). Spiracles are present on the prothorax and each segment minus the 9th and 10^{th} (Hamlin and Reed, 1931). Similar to eggs, the larval surface is appears granular under stereomicroscope.

Newly hatched larvae will disperse to find a food source upon hatching. Larvae are known to invade packages through existing openings or penetrate packaging materials by chewing through them. First instar larvae can invade packages containing a food source through holes less than 0.39 mm (Tsuji, 1998). The young instars are typically packaging invaders while older larvae are considered packaging penetrators. Older larvae are capable of chewing through packaging materials due to their highly developed mandibles, whereas younger larvae have

poorly developed mandibles. Research has shown that the *P. interpunctella* has the ability to chew through paper, polyethylene (25.4µm thick), cellophane (25.4µm thick), and aluminum foil (16.5µm thick) (Cline, 1978). Damage to commodities occurs only in the larvae stage because adult *P. interpunctella* do not feed. The larvae are capable of feeding on 179 commodities such as dried fruit, granola bars, nuts, and seeds (USDA-ARS, 1986). During feeding, larvae will leave behind a silken thread or webbing on the surface of wherever it crawls. This webbing material will capture food particles, cast skins, and fecal material from the larvae (Mohandass et al., 2007).

Similar to *T. variabile*, *P. interpunctella* may undergo diapause. The duration of diapause will vary by stored-product species. Generally *P. interpunctella* are reared at 25-28°C and 14:10h light: dark (L:D) photoperiod. Changing the rearing conditions of the *P. interpunctella* increases the frequencies of diapause within the culture. A maximum diapause frequency has been observed at 20°C and 8:16h L:D in populations of *P. interpunctella* from the United States and Canada (Wijayaratne and Fields, 2011). Changing temperature or photoperiod alone, has no significant effect on diapause frequencies, but it is the combination of a decrease in temperature and increase in the length of darkness triggers diapause in the *P. interpunctella* (Wijayaratne and Fields, 2011). The location of an *P. interpunctella* population will also affect diapause frequencies. Research has shown that populations at higher latitudes have a greater diapause frequency that those at lower latitudes (Wijayaratne and Fields, 2011).

Diapause is an important characteristic of *P. interpunctella*. *P. interpunctella* adults can be significantly selected for diapause induction and doing so provides the opportunity to pass along this trait to following generations. Selection of diapause induction of adult *P. interpunctella* increases diapause over the next two generation (Wijayaratne and Fields, 2011).

Since diapause has been shown to increase cold tolerances of *P. interpunctella*, this will make *P. interpunctella* harder to control by low temperatures (Wijayaratne and Fields, 2011).

The relative humidity of rearing conditions for the *P. interpunctella* will have a significant impact on growth and survival, especially in combination with temperature. At 30°C, the average development time at 25% relative humidity ranged from 35-46 days while at 70% relative humidity the interval decreases to 24-32 days (Bell, 1975). Increasing the relative humidity decreased development time. When comparing field versus lab strains of the *P. interpunctella*, field strains generally tolerated lower relative humilities than lab strains (Bell, 1975). It has been shown that development from 1st instars to adults is fastest at 30°C and 80% r.h., average 30.4 days, and slowest at 25°C and 60% r.h., average 44.3 days (Mbata and Osuji, 1983). Thus, the optimal conditions for development of the Indian meal moth are 30-35°C and 70-80% r.h. (Mbata and Osuji, 1983).

When fully grown, larvae will spin a cocoon and transform into a pupae (USDA-ARS, 1986). The 5th instar of the *P. interpunctella* will choose a pupation sites that offer protection such as a crack, corrugated cardboard, or wooden pallets. *P. interpunctella* pupae are encased in a silken cocoon and range in size from 6-11 mm (Hamlin and Reed, 1931). Early in the pupation period, the pupae are light brown or straw colored, and towards the end of pupation, there is darkening of the pupal casing especially near the wing regions and becomes black just before adult emergence (Hamlin and Reed, 1931).

Following the pupal stage, adult moths will emerge. Adult moths have an approximate wingspan of 14-22 cm and averages 16 mm (Hamlin and Reed, 1931; Hagstrum et al., 2013). *P. interpunctella* are distinguished from other moths by the reddish brown coloring on their forewings (USDA-ARS, 1986). The body is whitish-gray and the hind legs are silver in color

with long silky fringe (Hamlin and Reed, 1931). Adult moths avoid light. Moths are most active at night, early in the morning, dusk, and are strong fliers (Subramanyam, 2011). During daylight hours moths will rest on walls, ceilings, or other poor lighted areas (Hamlin and Reed, 1931). After emergence, females begin releasing a sex pheromone (*cis*-9, *trans*-12-tetradecadienyl acetate, ZETA) to attract male moths and mating can commence (Subramanyam, 2011). Commercial traps are made from this female pheromone and can be used to trap males to prevent mating or measure distances traveled by male moths (Subramanyam, 2011). The number of male moths captured in pheromone based traps decrease with increasing distance from pupation sites (Subramanyam, 2011). The number of moths captured vs. distance traveled from pupation site can be modeled by an exponential decay equation (Subramanyam, 2011)

$$y = 13.0exp^{-0.23x}$$

Therefore, more moths will be captured close to pupation sites and location of pupation sites is of upmost importance.

The egg-to-adult development of the Indian meal moth can range from 35-151 days but is dependent on temperature, moisture content of food, relative humidity, and photoperiods. At 17.5, 20.0, 22.5, 25.0, 27.5, 30.0, 32.5, and 35°C the developmental times from egg-to-adults are 150.9, 9.3, 67.3, 48.1, 37.9, 34.9, 38.4, and 49.1 days, respectively (Subramanyam, 2011). Developmental times have been shown to decrease with increasing temperatures. Developmental times at high relative humidity (85-95%) are faster compared to lower relative humidity (10-60%) (Subramanyam and Hagstrum, 1993). Adults will develop 1.2 times faster at 85-95% relative humidity than at 0-10% (Subramanyam and Hagstrum, 1993). Generally, unmated males and females live 2.4-2.7 days longer than mated adults and males live 2-3 days longer than females (though not statistically significant) (Hamlin and Reed, 1931). The

temperature for maximum development is 29.45 ± 0.51°C. Among each life stage, the percentage of time spent in egg, larvae, and pupae stages are 8, 77, and 15%, respectively (Subramanyam, 2011). These percentages have been used to develop a formula to calculate the length of egg, larvae, and pupae stages for a given temperature (Subramanyam, 2011). For example (Subramanyam, 2011):

$(1)\ Total\ development\ at\ egg\ stage = Avg.egg - to - adult\ develop.time\ \times 0.08$

$(2)\ Total\ development\ at\ larvae\ stage = Avg.egg - to - adult\ develop.time\ \times 0.77$

$(3)\ Total\ development\ at\ pupae\ stage = Avg.egg - to - adult\ develop.time\ \times 0.15$

$Total\ development\ time\ = (1) + (2) + (3)$

1.2. Infestation in Retail Stores

Stored-product insect infestations in retail stores can cause significant economic losses resulting from product contamination, damaged product, and allergic reactions in susceptible individuals, as well as public relation and company image problems. It is important to note that the size of a retail store has no significant effect on the total number of stored-product insects captured (Roesli et al., 2003). Stored-product insects are known to infest dried fruits, grains, nuts, cereals, flours, and pet foods, which is why they are commonly found in retail marketplaces. Many times the central point of infestation by insects are found on areas with an accumulation of previously infested food products, spilled food between the enclosed space between the bottom shelves and the floor, shelves spaces, and/or flat surfaces (Arbogast et al., 2000; Roesli et al., 2003). Major infestations have been found in the stock rooms of retail establishments, which may be a result of infested products awaiting disposition (Arbogast et al., 2000). Other instances, suppliers' ship infested products to the retail stores and without proper inspection upon arrival will perpetuate infestations into retail establishment.

The infestation risk in a retail stored is directly related to sanitation, inspection, and pest management practices employed (Roesli et al., 2003). Effective management methods include proper sanitation, inspection of incoming products for signs of infestation, frequent stock rotation (first in first out), continual monitoring for pests, quick disposal of infested products, and application of insecticides if necessary (Arbogast et al., 2000). Sanitation methods include removal of spilled product from shelving or in-between shelving units, removing torn bags from the shelves, cleaning or replacing trapping devices, and application of approved insecticides (Roesli et al., 2003). Consumers should look for any rips, tears, or holes in a packaged product as this may indicate an infestation. In addition, consumers should look on the package for webbing, cast skins, or fecal material. This would also indicate infestation of a packaged good. If a consumer finds an infested package, it is crucial to notify store managers so they may address the problem. If a consumer should happen to have an infested product, they may place the product inside a sealed container and place in the freezer for 1 week to ensure all life stages are killed before disposal. Any additional products that are not in containers should be immediately placed in sealed containers and monitored for infestation.

1.3. Role and Function of Food Packaging

The purpose of packing is to protect food products from infestation by stored-product insects throughout the entire distribution channel (Highland, 1991). Effective food packaging delays product deterioration, extends shelf life of products, and maintains product integrity and quality (Marsh and Bugusu, 2007). Therefore, food packaging provides protection against the three major hazards: chemical, physical and biological (Marsh and Bugusu, 2007). Food packaging provides protection from factors due to environmental influences such as water, light, or oxygen or internal changes such as water activity, pH, and rancidity (Marsh and Bugusu,

2007). Packaging protects products from physical damages such as crushing, impact, expansion/shrinkage, or foreign material during transportation and storage due to air pressure, temperature changes, or handling (Marsh and Bugusu, 2007). Packaging protects food against biological hazards such as microorganisms, insects, rodents, spoilage, and prevents odor transmission (Marsh and Bugusu, 2007). There is a direct correlation between the length of storage and severity of infestation by stored-product insects (Highland, 1991). Increasing the shelf life of packaged food products, increases the chances for product infestation to occur. Food processors can take all possible precautions to make their products insect free, but they lack direct and complete control of their product once it leaves their processing and storage facility (Highland, 1978).

Packaged food can become infested during processing, transportation, storage at the retail market, and in the consumer's pantry. Food packages are more likely to become infested during extended storage periods on the retail shelf or warehouse storage facility (Mullen et al., 2012). Consumers may hold the manufacture responsible for infested products, regardless of how or where the package became infested. Packaging materials vary in composition, structure, and thicknesses, therefore the ability of stored-product insects to penetrate or invade packages depends on these characteristics along with the insect species and life stage. Stored-product insects can infest nearly all polymer films, but glass and metal cans are the only package types resistant to insect penetration or invasion (Mullen et al., 2012). Ideally all food produced should be held in glass or metal containers, but costs, feasibility, and customer convenience are important and thus the wide use of metal or glass is minimal (Mullen et al., 2012). Adult insects are capable of entering packages with openings $\geq$ 0.53mm, and neonate larvae are capable of entering packages with holes > 0.01mm (Wohlgemuth, 1979; Cline and Highland, 1981). In

general, adult insects are capable of passing through holes nearly equal to their width (Cline and Highland, 1981). Sometimes males are smaller than their female counterpart, and can invade smaller openings in packages. Similarly, larvae are capable of entering packages approximately equal to their head capsule size (Wohlgemuth, 1979). The objective of food packaging is to enclose commodities in a cost effective manner for industrial or consumer uses, maintain food integrity and safety, and minimize the environmental impact (Marsh and Bugusu, 2007). In recent years, food-manufacturing companies have begun implementing package testing programs and packaging alternatives, to prevent stored-product insect infestations of their packaged food products (Mullen et al., 2012).

1.3.1. Properties of Common Food Packaging Materials

Foods are packaged in a wide variety of materials, including Kraft paper, foil, polymer film blends, metal cans, and glass. Food packages are created based on the specific food product and its attributes, and no one package type will provide protection needed for all aspects of a food product (Campbell et al., 2004). It is important to point out that there are two major considerations to address when selecting a food packaging type (Collins, 1963). First, the method of sealing the package must be addressed. Secondly, the type of packaging material used must be considered. The later may be of most importance (Collins, 1963). Kraft paper and cellophane are the least resistant to penetration by insects (Highland, 1978, 1991). Wohlgemuth (1979) found that 0.05 mm thick cellophane, PVDV-coated cellophane, low density polyethylene, and nitrocellulose coated cellophane did not provide complete protection (>10% of samples penetrated) from adults of the lesser grain borer, *Rhyzopertha dominica* (F.); drug store beetle, drug store beetle, *Stegobium paniceum* (L.); *T. castaneum,* and *P. interpunctella* (larvae). Rigid polyvinyl chloride (PVC) at 0.06 mm thick was completely resistant to each of

these insects (Wohlgemuth, 1979). Polypropylene (0.03 mm) was resistant to *S. paniceum* and *T. castaneum*, but did not provide complete protection to *R. dominica* and *P. interpunctella* (Wohlgemuth, 1979). Thicker films of the same polymer base are more resistant to insect penetration than thinner films of the same material (Highland, 1978). Kraft paper can be made more resistant to insect infestation by incorporating insect-resistant construction, tight closures, and/or using an insect-repellent (Secreast, 1968). Coating Kraft paper with pyrethrins and piperonyl butoxide can be effective and the Food and Drug Administration (FDA) approve both treatments (Secreast, 1968).

Aluminum foil can be used as a packaging material, but it is almost never used independently (Collins, 1963). It is commonly used in conjunction with a polymer film to create a laminated or multi-layer packaging material. This process creates a material which is more resistant to penetration by stored-product insects (Collins, 1963). Cline (1978) found that when aluminum foil (0.0165 mm) was used independently to create a food package, the cadelle beetle, *Tenebroides mauritanicus* (L.), was able to penetrate 100% of packages containing food as second or fifth instars. Similarly, the rice moth, *Corcyra cephalonica* (Stainton), also penetration 100% of foil packages as second instars (Cline, 1978). *T. variabile* penetrated 88% and 40% of foil pouches as fifth and second instars, respectively (Cline, 1978). Gerhardt and Lindgren (1954) found that laminated films consisting of pliofilm, aluminum foil, and acetate (0.254 mm total thickness), formed into a sealed package, and was resistant to penetration by ten different stored-product insects. Another laminate film package, consisting of polyethylene and aluminum foil (0.05 mm total thickness), had 8.1% of packages penetrated by the same 10 stored-product insects (Gerhardt and Lindgren, 1954). Laminated films do not have to contain aluminum foil but consists of two or more packaging materials co-extruded to form one

continuous film. Gerhardt and Lindgren (1955) studied the resistance of Mylar (polyester film) laminated with Saran film at 0.05 and 0.08 mm thick against ten insect species. They concluded that none of the laminate films were penetrated by any species of insects but 0.03 mm thick Mylar films alone were penetrated by *R. dominica, T. mauritanicus,* and *T. variabile* (Gerhardt and Lindgren, 1955).

The effectiveness of polyethylene (PE) as an insect resistant packaging material is directly proportional to the thickness of the film (Collins, 1963). PE at 0.05 mm, was capable of being penetrated by adult *R. dominica, S. paniceum, T. castaneum,* and *P. interpunctella* (larvae) (>10% of samples penetrated) (Wohlgemuth, 1979). However, a 0.02 mm thick PE film was resistant to *P. interpunctella* penetration by neonate larvae (Tsuji, 1998). The fifth and second instars of *P. interpunctella* penetrated 60 and 33% of PE packages, at 0.0254 mm thickness, respectively (Cline, 1978). Wohlgemuth (1979) did not mention the age of the *P. interpunctella* larvae used in the experiment, and the penetration found could be due to older larvae which contain stronger mandibles compared to young larvae. Bowditch (1997a) demonstrated that older larvae are able to penetrate more polyvinyl chloride packages, which indicates that penetration ability of insects varies by life stage. Linear low-density polyethylene (LLDPE), 0.04 and 0.05 mm thick, is resistant to third instar of *P. interpunctella* and adult *T. castaneum* (Chung et al., 2011). Even with 0.20 mm pinholes, *T. castaneum* adults were unable to invade the packaging material (Chung et al., 2011). Third instar *P. interpunctella* invaded less than 40% of the 0.04 mm thick LLDPE with pinholes and the 0.05 mm thick films had less than 10% invaded (Chung et al., 2011).

Polyvinyl chloride (PVC) materials can vary in their structure, such as rigid vs. flexible films. Wohlgemuth (1979) found that rigid PVC was more resistant to insect penetration

compared to flexible PVC at the same thickness. Bowditch (1997a) sealed *Cadra cautella* (Walker), tropical warehouse moth or almond moth, and *P. interpunctella* first and fifth instars inside of PVC, 0.025 mm thick, packages and monitored penetration ability of the two moths. The PVC is resistant to first instars of *C. cautella* but first and fifth instars of *P. interpunctella* are able to penetrate 25 and 63% of the time, respectively (Bowditch, 1997a). The fifth instar of *C. cautella* penetrated 69% of packages (Bowditch, 1997a). Results indicate that as the age of the larvae increase, the penetration ability of the insect also increases. Adult confused flour beetles, *Tribolium confusum* Jacquelin du Val, were tested in a similar manner and only one adult penetrated out of 15 films tested.

Polypropylene (PP) laminates offers resistance to penetration by *C. cautella, P. interpunctella,* and *T. confusum.* A PP laminate, 0.028 mm thick biaxially oriented and coated with acrylic on one side and polyvinylidene chloride on the other, was resistant to penetration by adult *T. confusum,* first and fifth instars of *C. cautella* and *P. interpunctella* (Bowditch, 1997a). Cast polypropylene (CCP) at 0.02 and 0.025 mm thickness and oriented polypropylene (OPP) at 0.02 and 0.03 mm thickness, were resistant to penetration by third instar *P. interpunctella* and adult *T. castaneum* (Chung et al., 2011). PP packages that were 0.0254 mm thick and contained food could only be penetrated by fifth instars of *T. variabile* 25% of the time, when compared to no penetration by 10 other stored-product insects (Cline, 1978). However, when food was removed from the PP packages, fifth instars of *T. mauritanicus* were able to penetration 100% of packages tested (Cline, 1978).

Polyester at a thickness of 0.05 mm was resistant to penetration by *S. paniceum, T. castaneum,* and *P. interpunctella,* but >10% of samples were penetrated by *R. dominica* (Wohlgemuth, 1979). Riudavets et al. (2007) observed that *R. dominica* and the rice weevil,

Sitophilus oryzae (L.), were able to penetrate 0.012 mm thick polyester films, but the cigarette beetle, *Lasioderma serricorne* (F.), was unable to produce holes in the film. Polyethylene terephthalate (PET) films that were 0.012 and 0.016 mm thick inhibited penetration by third instars of *P. interpunctella* and *T. castaneum* adults (Chung et al., 2011). However the introduction of a 0.20 mm pinhole, allowed larvae of *P. interpunctella* to invade packages but *T. castaneum* was unable to invade packages (Chung et al., 2011). Insect species vary in their ability to penetrate polyester. *T. variabile* second and fifth instars can easily penetration polyester at a thickness of 0.0254 mm, but other insects such as *P. interpunctella, E. cautella*, and hide beetle, *Dermestes maculates* (DeGeer), were unable to penetrate (Cline, 1978).

1.4. Physical Properties Associated with Packaging Materials

Stored-product insects have been shown to produce different types of damage to various types of packaging materials (Riudavets et al., 2007). Common physical properties of polymer films tested include elongation percentage and tensile strength. Packaging films with high elongation (%) and low tensile strength (MPa) are generally more resistant to insect penetration (Chung et al., 2011). The hole shape and size insects create in a packaging material is related to the shape of the mouthparts of a particular species, the head capsule width, and the mechanical properties of that particular film (Chung et al., 2011). Chung et al. (2011) demonstrated that *P. interpunctella* was capable of creating holes with clear cut edges in PET films, but linear low-density polyethylene (LLDPE) films contained scratches and tears around entry holes. The difference between the materials is PET films have a low elongation and high tensile strength compared to LLDPE films. High elongation in polyethylene (PE), 0.05 mm thick, produced a hole by *L. serricorne* with large amounts of filaments or fraying of the material (Riudavets et al., 2007). Harder materials such as PE and PP are less resistant to fraying and insects create holes

which are clean cut or contain scratches near the entrance (Riudavets et al., 2007). Packaging materials can be ranked in many different ways, but ultimately the resistance of a particular film is related to the films thickness, and thicker films are more resistant to insect penetration. However, among packaging types Kraft paper and cellophane are the least resistant to insect penetration among all packaging materials. Polyvinyl chloride (PVC) is less resistant to insect infestation than PP films (Bowditch, 1997a). Among packaging films tested by Chung et al. (2011), LLDPE, PET, oriented polypropylene (OPP), and cast polypropylene (CPP) were all resistant to penetration by *P. interpunctella*, which is classified as a penetrator and a serious pest of packaged products. When pinholes are introduced into the packaging material, stored-product insects are able to invade packages more readily. Between the packaging materials tested by Chung et al. (2011), 0.05 mm thick LLDPE was the most resistant to invasion by *P. interpunctella.* The resistance of polymer films to penetration or invasion by stored-product insects, varies by resins from which the films are made and their physical properties (Highland and Wilson, 1981). The multilayer or laminate films, appear to be the most resistant to insect penetration among all packaging types.

1.4.1. Methods used to Test Packaging Integrity

Several methods for testing packaging integrity against stored-product insects have be developed over the years. Generally, there are two types of testing methods employed. The first method consists of constructing "bags" containing food from the packaging materials under test, and introducing the bags into a room containing stored-product insects (Wohlgemuth, 1979). This test requires extensive room capacity and long experimental periods, and determining if the packaging material was penetrated versus invaded through packaging defects, can be difficult to

assess (Wohlgemuth, 1979). This test is suitable for quality control testing of mass produced packages (Wohlgemuth, 1979).

The second method employed, is creating a barrier from the packaging material under test and challenging the material with the test insect species. In this situation, a piece of material is placed between two metal holders, the material is held taut, and insects are placed on top of the material being tested. In this scenario, the insect can be held with or without food, and penetration of the material can be determined because once the insect penetrates the material they fall through and cannot escape. Therefore, this eliminates the possibility of insects invading the material through a defect. This test is suitable for small sample sizes, but not for large quality control type studies, which mimic retail environments (Wohlgemuth, 1979). In this testing method, the introduction of a pinhole can also be included and invasion properties of the material can be determined. The presence or absence of food, will also affect the penetration or invasion ability of stored-product insects (Newton, 1988). Insect that are starved may penetrate packages to obtain food, but in the presence of a food source they may not necessarily invade those packages. In addition, pricking packaging materials to provide access through pinholes increases the likelihood that insects will penetrate/invade the product. In instances where insects normally fail to penetrate packages, the pinhole is utilized for assessment.

1.4.2. Insect Boring Direction Determination

On a manufacturer's standpoint, it is important to determine whether insects bore through the packaging from the outside to inside and vice-versa (Wohlgemuth, 1979). This enables manufactures to determine if an infestation occurred in the product before or after final packaging. If there are no holes, tears, seam failures, or insect-bored holes in the packaging material, it is reasonable to assume that infestation originated during processing (Brickey et al.,

1973). However, if insect-bored holes are present the direction of penetration is key to determining the origin of the infestation (Brickey et al., 1973). Brickey et al. (1973) developed a methodology for determining the direction of insect penetration. One common characteristics in materials in which insects bore through is a tapered hole in which the diameter of the hole is greater on the entrance side than then exit side (Brickey et al., 1973). In foil, cellophane, and polyethylene plastics, upturned edges around the perimeter are commonly found (Brickey et al., 1973). The mandibles of insects also caused roughening to the surface or surface fraying on packaging materials formed by the pincer like action of the mandibles (Brickey et al., 1973). Though it is important to determine whether an insect bored through the packaging materials or were present inside prior to packaging, ultimately any type of flaw can easily negate the effects of a strong package design.

1.5. Biological Attributes of Insects

1.5.1. Invaders vs. penetrators

The stored-product insects that affect packaged products are found throughout the world (Highland, 1978). Athanassiou et al. (2011) proposed two scenarios that explain the presence of stored-product insects in packaged products: (1) insects are present in the product before packaging, or (2) insects invade or penetrate the product after packaging. Taking these two scenarios into consideration two classifications of stored-product pests has been identified: (1) packaging invaders, or (2) packaging penetrators (Highland, 1978). Packaging invaders include *T. castaneum, T. confusum,* the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), and *P. interpunctella* (Mowery et al., 2012). Packaging penetrators include *S. oryzae, R. dominica,* and *C. cautella*. Packaging invaders enter a package through a pre-existing opening such as a tear, seam failure, or puncture (Highland, 1984). Packaging invaders typically have weakly

developed mouthparts at the larval and adult stages (Wohlgemuth, 1979). Newly hatched larvae are of most concern because they possess the ability to invade holes as small as 0.1 mm wide (Wohlgemuth, 1979). Adults are capable of passing through hole diameters approximately equal the diameter of their body widths (Cline and Highland, 1981). Cline and Highland (1981) determined the minimum size holes in which 20 adult species of stored-product insects are capable of crawling through and size of holes ranged from 0.53-2.25 mm depending on species. The flat grain beetle, *Cryptolestes pusillus* (Schönher), is capable of crawling through the smallest opening of 0.53 mm (Cline and Highland, 1981). *Dermestes maculatus*; the yellow meal worm, *Tenebrio molitor* (L.), and *T. mauritanicus* all were unable to crawl through the largest opening of 2.25 mm (Cline and Highland, 1981). The life stage of the insect is extremely important. Larvae of *P. interpunctella* are capable of enter packages through pinholes. First instars are able to penetrate a pinhole size of 0.293 mm, but were stopped by a 0.173 mm hole (Tsuji, 1998). Information such as this is beneficial to packaging designers when considering allowable hole sizes in their packages.

Many packaged food products have mechanically produced pinholes, to avoid bulging after sealing due to air pressure or temperature changes. The pinholes created in food packaging, allow for food odors to be emitted from the packages. The food odors can be detected by insects, and orient insects towards the source (Muratore et al., 2009). Invading insects account for more than 75% of packaging infestations (Collins, 1963). However, little investigation has been conducted on the behavioral mechanism by which insects invade packages. It has been suggested that volatile compound emanating from holes in packaging materials play a role in orienting larvae and adults towards the source (Barrer and Jay, 1979; Mowery et al., 2002). Food odors originating from stored grain bins or packaged food products provide olfactory cues

towards the direction of the odor and signal to the insect a potential food source (Barrer and Jay, 1979).

Under certain circumstances, packaging invaders may become penetrators, such as *P. interpunctella* (Mullen et al., 2012). Penetrating insect can bore through flexible packaging materials due to their large and powerful mouthparts (Wohlgemuth, 1979; Highland, 1984). The ability of insects to penetrate packaging materials depends on the species, life stage, type of film used, thickness of material and the presence of creases, scratches, or tears on the material (Gerhardt et al., 1954). Insect species that are penetrators are most dangerous in the larval stages (Wohlgemuth, 1979). Both penetrating and invading insect species will exploit any packaging flaw in order to reach a food source before beginning to chew through the package (Mullen et al., 2012). *R. dominica, T. mauritanicus, T. variabile, L. serricorne,* and *P. interpunctella* are all considered as important penetrating insects (Highland, 1991). In a survey of infestation of chocolate-based products, a total of 141 bars were examined and 50 of those bars contained enough insect fragments for identification. *P. interpunctella* accounted for more than 50% of the infested bars (Bowditch and Madden, 1997b). This further emphasizes that *P. interpunctella* is a major pest of packaged products, because they possess the ability to feed on all types of products. Further research indicated that the infestation of the chocolate bars most likely occurred post packaging, because researchers failed to capture *P. interpunctella* at the processing facility (Bowditch and Madden, 1997b).

The primary method of preventing infestation is by using insect resistant packages (Mullen and Highland, 1988). An indicator of a potential infestation problem in a packaged product is the attractiveness of the food to stored-product insects (Mullen, 1993). Many stored-product insects are cosmopolitan, meaning they can feed on a variety of food products and thus

making packaged food susceptible to insect attack. Olfaction is the means by which insects can identify resources on which to feed and/or oviposit (Bell and Cardé, 1984). Insects are attracted to particular packages by odors they emit, and when an insect detects a food odor it orient to the source (Murator et al., 2009; Mowery et al., 2002). Various factors can influence an insect's response to food odors such as species, age, sex, and mating status.

1.5.2. The Effect of Metals in Insect Mandibles

Metals such as zinc and manganese, which have been found in the cutting edges of the mandibles of adult beetles, have been correlated with extreme hardness of the insect cuticle (Hillerton et al., 1984). It has been found in wasps, that those species which require the ovipositors to bore through harder substrates contain metals and those that place their eggs in soft substrates lack metals in their ovipositors (Morgan et al., 2003). Similarities can be seen between the ovipositors of wasps and the mandibles of stored-product insects. The presence or absence of metals in larval and adult mandibles may depend on the feeding habits of the insect species, external versus internal feeders. Morgan et al. (2003) found metals such as zinc and manganese, are present in the mandibles of stored-product insect larvae which are capable of boring into intact seeds, and larvae which do not have these metals present are usually unable to penetrate seeds. *R. dominica, L. serricorne, S. paniceum,* and *T. mauritanicus* all contain zinc in the mandibles of the larvae (Morgan et al., 2003). *T. variable* larval mandibles contain high levels of manganese (Morgan et al., 2003). All of these insects, are classified as packaging penetrators (Highland, 1991). Insects such as *T. castaneum* and *O. surinamensis* lack zinc and manganese in the larval mandibles and are classified as packaging invaders (Morgan et al., 2003). The metal hardening in the mandibles of stored-product insects, may enable these species to penetrate various packaging materials (Morgan et al., 2003).

1.6. Insect Growth Regulators (IGRs)

Insect growth regulators (IGRs) are formulated to mimic specific insect juvenile hormone, which regulates molting in insects (Oberlander et al., 2000; Arthur, 2006). Methoprene is an IGR and a juvenile hormone analog, which is able to disrupt molting and development in immature insects (Amos et al., 1978). Methoprene was commercially introduced by the Zoecon Company in 1973 (Oberlander and Silhacek, 2000). As of 2003, the United States Environmental Protection Agency granted methoprene exemption from food tolerance levels (Henrick, 2007). Methoprene has been approved for use as a direct application to stored grains, residual surface treatments, aerosol sprays, packaging, and have little to no mammalian toxicity (Arthur, 2006). There is little to no effect on non-target species such as birds, fish, crustaceans, or mammals when methoprene is used at appropriate application rates (Henrick, 2007).

A reduction in juvenile hormones, induces molting in insects (Mondal and Parween, 2000). Exposing young larvae to juvenile hormones will inhibit pupation and can cause supernumerary molting in young larvae, and on older larvae produces abnormal larval-pupal intermediates or pupal-adult intermediates (Mondal and Parween, 2000). Treatment of pupae result in pupal-adult intermediates or causes morphological deformities in adults such as a twisted wing (Mondal and Parween, 2000). It should be noted that the degree of morphogenetic effect on insects differs depending on mode of application, species, active dose, and age of the insects (Mondal and Parween, 2000).

Methoprene is very effective in controlling a vast variety of insect pest species, such as lepidopterous and coleopterous pests, when added to the insect diet (Henrick, 2007). Amos et al. (1978) showed that crossing deformed adult males that had been exposed to 0.1 ppm methoprene, with unexposed females produced no viable progeny. Progeny production of males

and females is adversely affected by exposure to methoprene, and males are more sensitive compared to females (Wijayaratne et al., 2012). Methoprene does not cause adult mortality or a quick knock-down effect, but methoprene works by reducing populations over extended periods of time (Mondal and Parween, 2000; Wijayaratne et al., 2012).

Ishaaya and Yablonski (1976) found that the life span of *T. castaneum* can be up to ten-times that of untreated *T. castaneum* when diet contained IGR's. Loschiavo (1976) found diet containing 10 ppm methoprene increase larval developmental time by 35% compared to untreated diet. Additionally, larval weight of *T. castaneum* also increased nearly twice that of larvae feeding on untreated diet, 5.0 mg compared to 2.4 mg (Ishaaya and Yablonski, 1976). Loschiavo (1976) observed fully developed larvae, which failed to pupate, were larger than untreated larvae. Diet incorporating methoprene at 200 ppm increased mean larval weight of *T. castaneum* to 6.2 mg (Ishaaya and Yablonski, 1976). Ishaaya and Yablonski (1976) found the critical point at which methoprene is most effective is the period between 4th instar and pupation.

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Chapter 2 - Penetration by larvae of *Plodia interpunctella* (Hübner) into polypropylene packaging materials

2.1. Abstract

Larvae of *Podia interpunctella* (Hübner), Indian meal moth, can invade or penetrate packaging materials and infest the food within. Three different polypropylene packaging types, containing energy bars, were challenged with eggs (for first instars), third instars, fifth instars, and pupae of *P. interpunctella* to determine package resistance to larvae at 28°C and 65% r.h., and also to determine which package type provided the greatest protection against *P. interpunctella* larval penetration. Packaging samples infested with eggs, larvae, or pupae were evaluated after 21 or 42 d to count number of larvae, pupae, or adults found inside each package type. Packaging types were evaluated for number of holes, diameter of holes, and amount of damage sustained to the energy bar. Third and fifth instars showed a higher propensity to infest all packaging variations. First instars showed a reduction in package pentration abiltiy compared to third and fifth instars. Among package types, Test A was most resilient to penetration by all larval stages. In conclusion, energy bar manufacturers could improve packaging designs, utilize thicker gauge films, or use odor barrier technology to prevent penetration and infestation by *P. interpunctella* larvae.

2.2. Introduction

The presence of stored-product insects in ready-to-eat packaged products can cause product losses, decrease consumer confidence, and possibly result in allergic reactions in sensitive individuals (Subramanyam et al., 2001). In addition, economic losses faced by the food industry include fines, penalties by government agencies, possibility of litigation by consumers who may suffer from eating infested products, and insurance claims (Laschiavo et al., 1979). Infestation of packaged products can occur due to insect contamination in raw ingredients, during the manufacturing process, prior to packaging, during transportation, in retail environments, and consumer homes (Hagstrum et al., 2009). Stored-product insects are a persistent problem in retail stores, feed stores, food warehouses, and processing plants (Arbogast et al., 2000). Infestation of packaged products at the retail level could be due to incoming products being infested, insects entering the retail store through open doors or windows, or due to insect populations that are already established in the store (Roesli et al., 2003). In the retail marketplace, stored-product insects will contaminate and cause damage to food products such as candy bars, flour, and pet food (Arbogast et al., 2000). Food processors may take all possible precautions to prevent infestations in their packaged products during manufacture, but they have little or no control over their product during shipping or storage in warehouses, and retail environments (Highland, 1978; Roesli et al., 2003). Retail stores can employ effective pest management techniques to reduce or eliminate infestations. Stock rotation, use of traps, inspection of incoming product, and good sanitation are some examples of an effective pest management program (Arbogast et al., 2000; Subramanyam et al., 2001). Traditionally, retailers and pest management professionals used chemical pesticides to control insect infestations in retail environments (Arbogast et al., 2000). In recent years, there has been a trend in the industry

to find alternative measures to manage infestation problems. In order to create insect-resistant packaging, one needs to understand the mechanisms behind penetration by stored-product insects. A thorough understanding of where, how, and when the infestations occurred in the packaged product, will help to identify critical aspects of the packaging material which need to be addressed (Athanassiou et al., 2011).

The stored-product insects that affect packaged products are cosmopolitan (Highland 1978). Athanassiou et al. (2011) proposed two scenarios that explain the presence of stored-product insects in packaged products: (1) insects are present in the product before packaging, or (2) insects invaded or penetrated the product after packaging. Based on these two scenarios, stored-product insect pests have been classified as package invaders and package penetrators (Highland, 1978). Invaders enter a package through a pre-existing opening such as a tear, seam failure, or puncture (Highland, 1984). Invaders have weakly developed mouthparts in the larval and adult stages (Wohlgemuth, 1979). Newly-hatched larvae are of most concern because they can invade holes as small as 0.1 mm in diameter (Wohlgemuth, 1979). Penetrating insects can bore through flexible packaging materials due to their large and powerful mouthparts (Wohlgemuth, 1979; Highland, 1984). The ability of insects to penetrate packaging materials depends on the species, life stage, type of film used, thickness of material, and the presence of creases, scratches, or tears on the material (Gerhardt et al., 1954; Mullen et al., 2012). Insect species that are penetrators are most dangerous in the larval stages (Wohlgemuth, 1979).

The Indian meal moth, *Plodia interpunctella* (Hübner) (Leipdoptera: Pyralidae), can attack a wide range of stored products (Allotey et al., 1990). *P. interpunctella* is among the world's most economically important stored-product insect pests of raw or processed foods, and is found in food and feed processing plants, warehouses, and retail environments (Loschiavo et

al., 1979; Roesli et al., 2003). The larvae of this pest have been associated with 179 different food commodities in 48 different countries spanning six continents (Hagstrum et al., 2013). In a survey of four Hawaiian islands, 54 species of beetles and 11 species of moths were identified, of which *P. interpunctella* was the most frequently found moth (Loschiavo and Okumura, 1979). Adult moths are capable of laying 100-300 eggs per female (USDA, 1986). Newly- hatched larvae wander in search of food. While larvae are mobile, they leave behind a sticky silk and in this webbing, fecal material and cast skins of earlier instars may become entrapped (USDA, 1986). Newly-hatched larvae are able to invade films with pinholes of 0.293 mm or larger, but cannot invade pinholes of 0.173 mm or smaller (Tsuji, 1998). The size of the thorax was the primary factor limiting invasion of a pinhole in packages by *P. interpunctella* larvae, and newly-hatched larvae were unable to penetrate polyethylene films of 0.02 mm thickness (Tsuji, 1998). The second and fifth instars of *P. interpunctella* have the ability to chew through Kraft paper (114-µm thick), polyethylene (25.4 µm), and aluminum foil (16.5 µm) when held without food (Cline, 1978). In the same study, the second and fifth instars of *P. interpunctella* were unable to penetrate polyester (25.4 µm) and polypropylene pouches (25.4 µm) (Cline, 1978). Bowditch (1997) similarly found that fifth instars of *P. interpunctella* were unable to penetrate polypropylene (28 µm) pouches, but they were able to penetrate 63% of polyvinyl chloride (25 µm) pouches. In addition, 25% of polyvinyl chloride (25 µm) pouches tested were penetrated by first instars. In Cline's study (1978), second and fifth instars were unable to penetrate 25.4 µm polyvinyl chloride pouches when food was given; however, when food was removed fifth instars penetrated 33% of pouches. Bowditch (1997) and Cline (1978) demonstrated that older instars show a greater tendency to penetrate packages than younger instars, especially in the absence of food. The objective of this research was to evaluate susceptibility of three types of

polypropylene energy bar packages to penetration and infestation by *P. interpunctella* when challenged with larvae.

2.3. Materials and methods

2.3.1. Insects

Cultures of *P. interpunctella*, which have been in reared since 1999 in the Department of Grain Science and Industry at Kansas State University, Manhattan, KS, USA, were used in this experiment. Insects were reared on a diet consisting of 1000 g poultry mash, 150 ml glycerol, 150 ml honey, and 75 ml distilled water (Subramanyam and Cutkomp, 1987) at 28°C, 65% r.h., and 14:10 L:D photoperiod.

2.3.2. Properties of packaging films

Three packaging film types, each containing a single energy bar, were obtained from the manufacturer. Packaging films included Test A, package currently used in production, made of 15.24 µm oriented polypropylene/30.48 µm metalized cast polypropylene (total thickness, 48.7 µm); Test B 15.24 µm oriented polypropylene/27.90 µm metalized oriented polypropylene (total thickness, 43.14 µm), and Test C 15.24 µm oriented polypropylene/25.40µm metalized cast polypropylene (total thickness, 40.64 µml). All packaging samples were visually similar in appearance and contained the same type of macadamia nut energy bars. All packages were evaluated for pre-existing rips, tears, and seam integrity prior to use in all tests.

2.3.3. Tests with three densities of first instars

Male and female moths were collected from cultures and introduced into a 0.95-L glass jar fitted with a mesh screen. The glass jar was inverted over a 9-cm glass Petri dish, and moths were allowed to mate and oviposit. Eggs (0-24 h old) were collected and counted under a stereomicroscope and 50, 200, or 400 eggs were added to a 0.45-L glass jar containing a single

package type, Test A, B, or C, and closed with a metal lid fitted with mesh screen and filter paper. Jars were held at 28°C and 65% r.h. Observations were made after 21 d or 42 d post-infestation. Each egg density, package type, and exposure duration was replicated five times for a total of 90 glass jars. The average number of eggs that hatched was determined according to procedures described by Huang et al. (2004). Three replicates of 100 eggs each of *P. interpunctella* were collected and placed in glass Petri dishes. Dishes were placed at 28°C and 65% r.h. and examined after 7 d. The percentage of eggs that hatched out of the total (100) was calculated (Huang et al., 2004). The mean ± SE egg hatch was 79.3 ± 2.4%.

After 21 and 42 d post-infestation, packages were removed from jars and examined for the number of larvae, pupae, or adults present inside each package type. Additionally, the number and diameter of holes in each package and the amount of damage sustained to the energy bar was determined. Hole diameter was measured by stereomicroscope fitted with a calibrated ocular micrometer. The energy bar was examined and the amount of damage sustained to the bar was quantified on a 0 to 4 scale. A score of 0 represented no visible damage to the energy bar. A score of 1 indicated that the bar had 1-25% of the total surface area covered with larval webbing, cast skins, frass, or dead insects. A score of 2 indicated that the damage ranged from 26-50% of the total surface area. A score of 3 represented 51-75% damage, and a score of 4 represented 76-100% damage.

2.3.4. Tests with first, third, and fifth instars

Male and female moths, collected from cultures, were introduced into an inverted 0.95-L glass jar fitted with a mesh screen. Adult moths were allowed to mate and oviposit and eggs collected (≤ 24 h) were added to 500 g of poultry mash diet in a 0.95-L glass jar and held in a growth chamber at 28°C and 65% r.h. to facilitate larval development. Fifty eggs were counted

under a stereomicroscope and added to individual 0.45-L glass jars, as described previously, with lids fitted with mesh screens and filter papers containing a package type. Eggs were used to represent first instars, because first instars could be damaged or injured during transfer due to handling. Egg hatch was determined as described previously. The mean ± SE (n = 3) egg hatch was 87.0 ± 1.7%.

Third and fifth instars were determined by measuring the head capsule width (Allotey et al., 1990). Fifty third or 50 fifth instars, from the culture jar, were added to 0.45-L glass jars containing a package type, and held at 28°C and 65% r.h. Jars infested with eggs were examined at 21 d to count number of larvae and at 42 d to count number of pupae and adults. In the case of third instars, 21 d observations included counts of larvae and pupae and 42 d included only adult counts. In the case of fifth instars, observations were made after 21 d to count number of adults that emerged. After 21 and 42 d, packages were assessed for diameter and number of holes present, and the bars were rated for damage. Each package type, larval age, and exposure period combination, was replicated five times.

2.3.5. Tests with adults

In the 0.95-L glass jars used for rearing *P. interpunctella*, corrugated paper spools were added to the top of the diet to serve as pupation sites for wandering larvae (Huang et al., 2003). Pupae were collected from the paper spools and sexes separated using characteristics and illustrated described by Richards and Thomson (1932). Two male and two female pupae were added to 0.45-L glass jars fitted with mesh screens containing a package type, and held at 28°C and 65% r.h. for 21 and 42 d. After 21 and 42 d, packages and bars were assessed as described previously. Each test was replicated eight times.

2.3.6. Data analysis

For all experiments, a completely randomized design was used. The means and standard errors for number of larvae, number of pupae, and number of adults that emerged and for bar damage and size and number of holes were calculated and reported (SAS Institute, 2008). The number of larvae, pupae, or adults found inside energy bar packages after 21 and 42 d were transformed to $\log_{10}(x+1)$ scale for further analysis. Data obtained on the number of holes in packages, hole diameter, and damage score were not transformed. Data collected from tests at three first instar densities were subjected to two-way analysis of variance (ANOVA) by observation time to determine significant differences in each of the dependent variable measured as influenced by first instar densities and packaging type and their interaction. Data collected from first, third, and fifth instars were analyzed similarly by observation time to determine significant differences in each of the dependent variable measured among instars and packaging type and interaction of these two main effects. Means male and female adults were subjected to a one-way ANOVA, and package type was the main factor.

2.4. Results

2.4.1. Tests with three densities of first instars

After 21 and 42 d following the addition of *P. interpunctella* eggs, the amount of packages penetrated varied by packaging type (Table 2.1). At 21 d and a density of 50 eggs, at least one of each type of package was penetrated, but after 42 d there were no packages penetrated. After 21 d, no packages were penetrated by larvae in the 200 egg density treatment, but after 42 d there was penetration in Test B and Test C packages. Test B was the only package type that was not penetrated at an egg density of 400 eggs at 21 or 42 d. With the exception of the lowest egg density, *P. interpunctella* larvae penetrated more packages after 42 d exposure.

The increase in package penetration from 21 to 42 d can be associated with an increase in exposure time and instar age.

A two-way ANOVA of 21 d data show that the number of larvae found inside the packages was significantly different among egg densities (F = 4.03; df = 2, 36; P = 0.0263) but not among packaging type (F = 2.94; df = 2, 36; P = 0.0656). However, the egg density and package type interaction was significant (F = 6.85; df = 4, 36; P = 0.0003). Test C had the highest level of larvae present inside the package at an egg density of 400, but test B had the least amount of larvae present at an egg density of 50 (Table 2.2). The number of holes in the packages was not significant for egg density (F = 1.91; df = 2, 36; P = 0.1629) or package type (F = 1.91; df = 2, 36; P = 0.1629). Yet, the interaction between package and egg density was significant (F = 2.59; df = 4, 36; P = 0.0529). The size of the holes produced in the packages was significant for egg density (F = 3.32; df = 2, 36; P = 0.0474), but was not significantly different for packaging type (df = 2, 36) or package type and egg density interaction (df = 4, 36) (F_{range} = 0.21-0.50; P_{range} = 0.7393-0.8135). The damage score sustained by the energy bars was not significant for egg density (df = 2, 36) and packaging type (df = 2, 36) (F_{range} = 1.68-2.88; P_{range} = 0.0691-0.2007), but was significant among the package type and egg density interaction (F = 3.84; df = 4, 36; P = 0.0106). Test C packages subjected to 400 eggs sustained the most damage to the energy bar. This sample also had the most larvae present inside the package, which would explain the magnitude of damage observed.

Statistical analysis of 42 d exposure data showed the number of larvae present inside packages was not significant for egg density (df = 2, 36), package type (df = 2, 36), and their interaction (df = 4, 36) (F_{range} = 0.66-1.17; P_{range} = 0.3409-0.5215). However, the number of pupae found inside packages varied significantly by egg density (F = 3.79; df = 2, 36; P =

0.0322), but was not significant for packaging type (F = 1.10; df = 2, 36; P = 0.3449). The egg density and packaging type interaction was significant (F = 2.74; df = 4, 36; P = 0.0437). In packages that were penetrated after 42 d of continual exposure, test C had the highest number of adults present (Table 2). Statistical analysis showed no significant differences based on egg density (F = 2.13; df = 2, 36; P = 0.1340), but significant differences were observed among packaging types (F = 4.67; df = 2, 36; P = 0.0158). The interaction between egg density and package type was not significant (F = 1.30; df = 4, 36; P = 0.2889). The number of holes found in the packages was not significant for egg density (df = 2, 36), packaging type (df = 2, 36) or their interaction (df = 4, 36) (F_{range} = 1.00-2.00; P_{range} = 0.1501-0.4203). The size of holes in the packages was not significant based on egg density (F = 2.59; df = 2, 36; P = 0.0889), but it was significant for packaging type (F = 4.32; df = 2, 36; P = 0.0208), and Test C had the largest average hole size of 0.8 mm. The interaction between egg density and packaging type was not significant (F = 1.53; df = 4, 36; P = 0.2131). The damage score of the bars was not significant for any factor, egg density (df = 2, 36), packaging type (df = 2, 36), and their interaction (df = 4, 36) (F_{range} = 1.14-2.17; P_{range} = 0.1287-0.3531). Among the packaging types, Test C provided the least amount of resistance to penetration by *P. interpunctella* larvae.

2.4.2. Tests with first, third, and fifth instars

The third and fifth instars of *P. interpunctella* penetrated more packages that the first instars, regardless of the packaging type (Table 2.3). Additionally, the number of packages penetrated at 42 d either remained constant or showed an increase up to 40% compared to 21 d penetrations. Among the packaging types, Test A had the least number of packages penetrated by the first and third instars after 21 and 42 d exposure. In tests conducted with fifth instars, Test B package had fewest penetrated packages. The increase in the number of packages penetrate

from 21 to 42 d exposures can be associated with the increase in age of instars. As *P. interpunctella* increase in larval age, the mandibles of the larvae increase in strength, which may enable them easily chew through packaging material. Bowditch (1997) also demonstrated that fifth instars penetrated more polyvinyl chloride packages (25 μm) than first instars.

A two-way ANOVA of 21 d data, showed the number of larvae found inside packages was significant for instar ($F = 4.78$; df = 2, 36; $P = 0.0144$), but was not statistically significant among package types ($F = 2.25$; df = 2, 36; $P = 0.1196$). The package type and instar interaction was not significant ($F = 2.48$; df = 2, 36; $P = 0.0613$). Test A had the least amount of larvae present inside packages and Test C had the most, 0 and 21.6, respectively (Table 4). The number of pupae found inside the package was significantly different among instars ($F = 17.99$; df = 2, 36, $P < 0.0001$), package type ($F = 6.23$, df = 2, 36; $P = 0.0047$), and package type and instar interaction ($F = 2.75$; df = 2, 36, $P = 0.0332$). Similarly, the number of adults found inside the package was significant for instar (F = 9.50; df = 2, 36; $P = 0.0005$) and package type ($F = 2.75$; df = 2, 36; $P = 0.0332$). The package type and instar interaction was not significant ($F = 2.20$; df = 2, 36; $P = 0.088$). The increase in larval age (third and fifth instars) resulted in having more number of pupae and adults inside each package type. This was expected due to the fact these instars are further along in their life cycle, while 21 d was not adequate time for first instars to reach the pupal stage or adult stage. Additionally, the number of holes in package, size of holes, and the amount of damage to the energy bar increased with increasing larval age. Test 1 had the most holes per package, size of holes, and highest damage score among packaging types. The number of holes in packages was not significant for instar age ($F = 2.40$; df = 2, 36; $P = 0.1055$). However, the package type was significant ($F = 9.37$; df = 2, 36; $P = 0.0005$) as well as the interaction ($F = 2.88$; df = 2, 36; $P = 0.0361$). The hole size in packages was significant based

on instar age (F = 4.76; df = 2, 36; P = 0.0147), but not for packaging type (F = 2.91; df = 2, 36; P = 0.0675). The interaction between instar age and packaging type was significant (F = 5.09; df = 2, 36; P = 0.0023). The instar age did not influence damage scores (F = 2.18; df = 2, 36; P = 0.1283). Conversely, packaging type significantly influenced the amount of damage sustained to energy bars (F = 6.54; df = 2, 36; P = 0.0038). The package type and instar interaction was significant (F = 4.25; df = 2, 36; P = 0.0064).

Analysis of 42 d exposures results only include data for first and fifth instars, because after 42 d fifth instars would have completed one life cycle and therefore it would be difficult to determine which life stages found inside packages were a result of the original larvae or from F_1 adults. Furthermore, the number of larvae present inside test packages was recorded as present or absent, because after 42 d of testing there were larvae present in various life stages of development from eggs laid by F_1 adults. The first instars in Test A were the only package that did not have larvae present inside (Table 2.4). Statistical analysis showed that the number of pupae found inside packages was not significantly different among instars (df = 1, 24) package types (df = 2, 24), and their interaction (df = 2, 24) (F_{range} = 0.00-3.23; P_{range} = 0.0572-1.000). Similarly, the same trend was seen in the number of adults found inside packages (F_{range} = 0.23-3.13; P_{range} = 0.0619-0.6373). Test B had the most holes in the packaging material. The number of holes was not significant for instars (F = 2.70; df = 1, 24; P = 0.1132) but packaging type was statistically significant (F =5.30; df = 2, 24; P = 0.0124). The interaction between instar and package type was not significant (F = 3.03; df = 2, 24; P = 0.0673). The number of size of holes in the packages varied but the results were not significant among instars (df = 1, 24), package type (df = 2, 24), and their interaction (df = 2, 24) (F_{range} = 0.05-3.17; P_{range} = 0.0602-0.8290). The amount of damage sustained to energy bars was not significant based on instars (F = 0.02; df

= 1, 24; P = 0.8962). However, damage was significant for packaging type (F = 5.97; df = 2, 24; P = 0.0079), but the interaction between package type and instar was not significant (F = 1.58; df = 2, 24; P = 0.2261).

2.4.3. Tests with adults

The number of packages penetrated either increased or remained constant from 21 to 42 d exposure when subjected to male and female moth pairs (Table 5). Test A was the only package that was not penetrated after 21 d, but 75% of packages were penetrated by 42 d. Test B had the most packages penetrated at day 21, 63%, but the number of penetrated packages remained constant after 42 d of exposure. The same trend was seen with Test C packages. The number of larvae found inside packages after 21 d was significantly different among packaging types (F = 5.33; df = 2, 42; P = 0.0087) (Table 6). Test C had the most holes in packages, largest hole sizes, and greatest amount of damage to energy bars (Table 2.6). The number of holes in packages was not significant (F = 0.12; df = 2, 42; P = 0.8847). In addition, the size of holes was not significant based on packaging type (F = 0.75; df = 2, 42; P = 0.4807). The amount of damage sustained to energy bars was also not significant based on packaging type (F = 1.85; df = 2, 42; P = 0.1693). After 42 d, all samples had at least 25% of packages penetrated. Analysis of data showed that there was no statistical differences for the number of larvae (df = 2, 21), pupae (df = 2, 21), or adults (df = 2, 21) found inside packages (F_{range} = 0.50-1.00; P_{range} = 0.3847-0.6149). Additionally, the number of holes in packages (df = 2, 21), size of holes (df = 2, 21), and damage scores (df = 2, 21) were not statistically significant (F_{range} = 0.50-2.36; df = 2, 21; P = 0.1185-0.6136).

2.5. Discussion

The objective of this research was to evaluate susceptibility of three types of polypropylene energy bar packages to penetration and infestation by *P. interpunctella* larvae. This study has demonstrated that first, third, and fifth instars of *P. interpunctella* are capable of penetrating multilayer polypropylene energy bar packages with thicknesses ranging from 40.6-48.7 µm. The results of this study indicates that older instars of *P. interpunctella* are capable of causing more damage to energy bars compared to first instars, presumably because the ability of these later instars to penetrate the package is greater. Cline (1978) and Bowditch (1997) demonstrated the ability of *P. interpunctella* to penetrate packaging materials also varies among life stages, and in general, their studies found fifth instars to penetrate more packages compared to younger instars.

Shinoda et al. (1990) found that fourth and fifth instars were able to penetrate 30 µm thick polyethylene packages. Fifth instars penetrated significantly more packages than fourth instars (Shinoda et al., 1990). The results in this study are consistent to those found by Shinoda et al. (1990). Cline (1978) found that second and fifth instars were unable to penetrate 25.4 µm thick polypropylene films but were able to penetrate 25.4 µm thick polyethylene films. Bowditch (1997) found that first and fifth instars were unable to penetrate 28 µm polypropylene pouches but could penetrate 25 µm polyvinyl chloride films when pouches were exposed to larvae for five days. These two studies used films roughly half of that used in this study, and they found no penetration. A study conducted by Tsuji (1998) also demonstrated that newly hatched larvae are unable to penetrate 20 µm polyethylene film even after 2-3 weeks of exposure to larvae. In both the Bowditch (1997) and Tsuji (1998) experiments, the time larvae were exposed to packaged films was shorter compared to this study. The additional time in this study

indicates that larvae exposed to surfaces for an extended period of time may increase their ability to penetrate packages. Chung et al. (2011) found that third instars were not able to penetrate 20 µm casted polypropylene and oriented polypropylene packages within five days. Our study demonstrated increased exposure time, 21 d compared to 42 d, increased the number of packages penetrated by first, third, and fifth instars. Again the results of this study differ from previous studies, but further emphasizes that exposure time is an important factor influencing the ability of *P. interpunctella* larval penetration.

The results of this study demonstrated that increasing the film thickness decreased the ability of *P. interpunctella*'s ability to penetrate. Test C had the thinnest film, 40.64 µm total thickness, and was consistently penetrated by all stages of *P. interpunctella* tested. In tests with first, third, and fifth instars, Test C had the highest larvae, pupae, and adult counts found within the package. Additional, Test C had the most holes per package and the most damage to the energy bars after 21 and 42 d exposures. Both Test A and B were consistently lower on all measurements. Thus, thicker polypropylene films provide better protection against *P. interpunctella* penetration. Further studies are warranted to determine the minimum thickness that can discourage *P. interpunctella* penetration. Additional testing is underway to determine the effect of adding growth regulators to packaging materials to prevent packaging penetration of films.

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Table 2.1 Number of packages penetrated by *P. interpunctella* at three egg densities.

Trt	Egg Density	Observation Time	
		21d	42 d
Test A	50	1	0
Test B		2	0
Test C		1	0
Test A	200	0	0
Test B		0	1
Test C		0	2
Test A	400	0	1
Test B		0	0
Test C		3	3

A total of five packages were exposed at each treatment and egg density.

Table 2.2 Number of larvae, pupae, and adults of *P. interpunctella* found inside packages of energy bars after 21 and 42 d of infestation, and extent of damage to packages and energy bars.

Trt.	Egg density	Mean ± SE											
		Number of larvae		Number of pupae		Number of adults		Number of holes		Hole size (mm)		Damage score	
		21d	42 d	21d	42 d	21 d	42 d	21d	42 d	21 d	42 d	21 d	42 d
Test A	50	2.4 ± 2.4	0.0 ± 0.0	---[a]	0.0 ± 0.0	---[a]	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0
Test B		4.0 ± 3.1	0.0 ± 0.0	---	0.0 ± 0.0	---	0.0 ± 0.0	0.4 ± 0.2	0.0 ± 0.0	0.4 ± 0.3	0.0 ± 0.0	0.6 ± 0.4	0.0 ± 0.0
Test C		0.2 ± 0.2	0.0 ± 0.0	---	0.0 ± 0.0	---	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0
Test A	200	0.0 ± 0.0	0.0 ± 0.0	---	0.0 ± 0.0	---	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Test B		0.0 ± 0.0	0.4 ± 0.4	---	0.8 ± 0.8	---	1.0 ± 1.0	0.0 ± 0.0	0.4 ± 0.4	0 .0± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.8 ± 0.8
Test C		0.0 ± 0.0	0.0 ± 0.0	---	0.0 ± 0.0	---	4.4 ± 3.0	0.0 ± 0.0	0.6 ± 0.4	0.0 ± 0.0	0.6 ± 0.4	0.0 ± 0.0	1.2 ± 0.8
Test A	400	0.0 ± 0.0	3.2 ± 3.2	---	1.0 ± 1.0	---	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.8 ± 0.8
Test B		0.0 ± 0.0	0.0 ± 0.0	---	0.0 ± 0.0	---	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Test C		12.8 ± 4.6	0.0 ± 0.0	---	2.6 ± 1.1	---	2.4 ± 1.2	1.0 ± 0.5	0.6 ± 0.2	0.2 ± 0.1	0.8 ± 0.3	1.2 ± 0.4	1.8 ± 0.8

[a]No pupae or adults were found at 21 d; only larvae were found at 21 d.

Table 2.3 Number of packages penetrated by first, third, and fifth instars of *P. interpunctella.*

Treatment	Instar	Observation time	
		21 d	42 d
Test A	1	0	1
Test B		1	3
Test C		3	4
Test A	3	2	3
Test B		3	4
Test C		4	4
Test A	5	4	---[a]
Test B		0	---
Test C		5	---

A total of five packages was exposed at each treatment and instars

[a]All adults from fifth instars emerged within 21 d.

Table 2.4 Number of larvae, pupae, and adults of *P. interpunctella* found inside packages of energy bars after 21 and 42 d of infestation with first, third, and fifth instars, and extent of damage to packages and energy bars.

Trt	Instar	Mean ± SE											
		Number of larvae		Number of pupae		Number of adults		Number of holes		Hole size (mm)		Damage score	
		21d	42 d[a]	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
Test A	1	0.0 ± 0.0	N	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.2 ± 0.2
Test B		15.4 ± 7.1	Y	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	3.6 ± 2.7	0.8 ± 0.2	0.6 ± 0.4	0.7 ± 0.4	0.6 ± 0.4	1.4 ± 0.6	1.4 ± 0.9
Test C		21.6 ± 9.3	Y	0.0 ± 0.0	0.4 ± 0.2	0.0 ± 0.0	8.0 ± 3.3	1.0 ± 0.6	0.8 ± 0.2	0.3 ± 0.2	1.2 ± 0.3	1.6 ± 0.7	2.6 ± 0.7
Test A	3	1.8 ± 1.4	Y	0.6 ± 0.4	0.0 ± 0.0	2.8 ± 1.8	2.6 ± 1.5	0.4 ± 0.2	0.6 ± 0.2	0.4 ± 0.3	0.8 ± 0.3	1.0 ± 0.6	1.2 ± 0.6
Test B		0.8 ± 0.4	Y	2.6 ± 1.3	0.0 ± 0.0	3.4 ± 2.1	0.6 ± 0.6	1.2 ± 0.4	0.2 ± 0.2	1.1 ± 0.3	0.3 ± 0.3	2.2 ± 0.6	0.2 ± 0.2
Test C		0.8 ± 0.8	Y	2.4 ± 0.7	0.8 ± 0.6	6.0 ± 2.3	3.4 ± 1.9	2.0 ± 0.6	2.8 ± 1.1	1.2 ± 0.3	1.0 ± 0.3	2.2 ± 0.6	2.6 ± 0.7
Test A	5	0.2 ± 0.2	---[b]	2.6 ± 0.9	---	4.6 ± 2.1	---	1.2 ± 0.4	---	1.0 ± 0.2	---	1.8 ± 0.6	---
Test B		0.0 ± 0.0	---	0.0 ± 0.0	---	0.0 ± 0.0	---	0 .0± 0.0	---	0.0 ± 0.0	---	0.0 ± 0.0	---
Test C		1.6 ± 1.0	---	4.4 ± 0.6	---	7.4 ± 3.1	---	2.8 ± 0.7	---	1.3 ± 0.2	---	3.2 ± 0.2	---

[a]N, no larvae were present; Y, larvae were present most probably from eggs laid by F_1 adults.
[b]All adults emerged within 21 d

Table 2.5 Number of packages penetrated by *P. interpunctella* larvae emerging from eggs after the addition of two mating pairs.

Treatment	Observation time	
	21 d	42 d
Test A	0	6
Test B	2	2
Test C	5	5

A total of eight packages were exposed at each observation time

Table 2.6 Number of larvae, pupae, and adults of *P. interpunctella* found inside packages of energy bars in treatments with two mating pairs after 21 and 42 d of infestation, and extent of damage to packages and energy bars.

Trt	Mean ± SE											
	Number of larvae		Number of pupae		Number of adults		Number of holes		Hole size (mm)		Damage score	
	21d	42d	21d	42d	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
Test A	0.0 ± 0.0	2.1 ± 1.1	---[a]	0.6 ± 0.4	---	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.2	0.0 ± 0.0	0.7 ± 0.2	0.0 ± 0.0	0.9 ± 0.3
Test B	5.1 ± 4.3	1.4 ± 1.2	---	0.3 ± 0.3	---	0.0 ± 0.0	0.5 ± 0.4	0.3 ± 0.2	0.1 ± 0.1	0.4 ± 0.3	0.4 ± 0.2	0.5 ± 0.4
Test C	15.0 ± 6.7	2.1 ± 1.1	---	1.5 ± 1.0	---	0.5 ± 0.5	1.0 ± 0.4	0.8 ± 0.3	0.3 ± 0.1	1.0 ± 0.3	0.5 ± 0.2	0.9 ± 0.3

[a]No pupae or adults were found at 21 d; only larvae were found at 21.

Chapter 3 - Susceptibility of *Tribolium castaneum* and *Trogoderma variabile* exposed to methoprene impregnated birdseed packaging material

3.1. Abstract

The insect growth regulator methoprene is registered for treatment of empty storage facilities, stored grain, and packaging. Methoprene adversely affects the number of eggs laid by female beetles, egg hatch, and larval development. The objectives of this study were to determine the effect of short term exposure time and temperature on four week old larvae, continual exposure on egg-to-adult emergence of beetles, and sub-lethal effect on adults of the red flour beetle, *Tribolium castaneum* (Herbst) and warehouse beetle, *Trogoderma variabile* Ballion, on the inside and outside surfaces of methoprene-treated packaging material at 27 and 32°C and 60% r.h. Inside and outside surfaces of methoprene-treated and untreated packages were cut into discs and fitted into Petri plates. Disc edges were glued down to deter larvae from crawling underneath, and sides were coated with fluon to prevent insect escape. Approximately 1500 mg of diet was added to Petri dish arenas. Fifty third instars of *T. castaneum* or *T. variabile* were added to individual arenas and exposed to untreated and methoprene-treated surfaces for 8, 24, 48, 72, 96 h. Exposed larvae were transferred to untreated Petri dishes 500 mg of diet and observations were made weekly to determine the percentage of normal adults that emerged. Sub-leathal effect experiments used twenty-five, 3-4 d old eggs, of *T. castaneum* or *T. variabile* which were added to treatment and control arenas, respectively and held at 27 or 32°C and 60% r.h. The number of hatched larvae for each species and treatment variation was recorded and percent hatch was determined. In addition, ten mixed sex adults of either *T.*

castaneum or *T. variabile* were added to arenas and held at 27 or 32°C and 60% r.h. for 7 d or 3 d, respectively. Following exposure to each treatment combination, adults were removed and the contents of each arena were analyzed for the number of eggs laid per female and percent hatch for each species. For continual exposure 10 mixed sex adults of each species were added to individual arenas, and adult *T. castaneum* were removed after 7 d and *T. variabile* after 3 d. Observations for normal adult emergence from eggs laid were made weekly. Short term exposure results indicated that the larvae to adult emergence of *T. castaneum* and *T. variabile* generally decreased with increasing exposure time. The number of eggs laid per female of *T. castaneum* and *T. variabile* did not vary from thier controls. Continual exposure demonstrated 100% supression of *T. castaneum* adult emergence, irrespective of outside or inside surfaces of the bagging material. *T. variabile* exposed to inside surfaces were unaffected and those exposed to outside surfaces normal adult emergence was reduced compared to the untreated control. Therefore, continual exposure of *T. castaneum* and *T. variabile* to methoprene impregnated packaging could be a vaiable tool to protect food and feed products from infestation.

3.2. Introduction

Stored-product insects are serious pests in raw and processed grains, pet foods, and birdseed. Food and feed manufacturing companies can take all possible preventive controls to produce an insect free product, but they have little to no control of their product once it leaves their facility. Retail stores also present a serious threat for infestation of packaged products by stored-product insects (Roesli et al., 2003). Potential sources of infestation include incoming commodities which are already infested, open doors or windows, and insect populations already present within the store (Subramanyam et al., 2001). Beetles such as the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) and sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) can be found on or above shelves, underneath shelves, and/or below kick-plates (Subramanyam et al., 2001).

Food packaging is a consumer's last defense against infestation by stored-product insects. Insects infest packaged products by penetration or invasion. Package penetrators such as the larvae of the warehouse beetle, *Trogoderma variabile* Ballion chew through packaging material and infest food products. Package invaders such as the red flour beetle, *Tribolium castaneum* (Herbst), enter packages through natural defects, holes, or seam failures. The larval stage of stored-product insects cause the most damage, because they can invade smaller holes than adults and possess powerful mouthparts which are capable of chewing through various packaging material (Wohlgemuth, 1979).

Current methods employed by food manufacturers and warehouse facilities to control stored-product insects include fumigation, contact insecticides, aerosols, and heat treatments. In recent years, manufacturers have been looking towards alternative and safer control methods to accommodate consumer demands (Wijayaratne et al., 2012). Insect growth regulators, insect

resistant packaging, or odor barriers have all been documented as plausible alternatives to prevent infestation of packaged food products.

Insect growth regulators, such as methoprene, are formulated to mimic specific insect juvenile hormone which regulates the developmental process from the egg to the adult stage (Oberlander et al., 2000; Arthur, 2006). Methoprene is a juvenile hormone analog, which is capable of causing morphogenetic and gonadotropic effects (Amos et al., 1978). As of 2003, the United States Environmental Protection Agency granted methoprene exemption from food tolerance levels (Henrick, 2007). Methoprene, an insecticide of very low mammalian toxicity, is approved for treatment of empty storage facilities, direct applications to stored grains, and as aerosol sprays, and for use in packaging (Arthur, 2006). There is little to no effect on non-target species such as birds, fish, or mammals when methoprene is used at appropriate application rates (Henrick, 2007).

Juvenile hormones control physiological and behavioral processes in insects, along with ecdysones and molting hormones (Mondal and Parween, 2000). They also regulate the morphogenetic changes during metamorphosis. A reduction in juvenile hormones and ecdysones induces molting in insects (Mondal and Parween, 2000). Exposing young larvae to juvenile hormones such as methoprene will inhibit pupation and cause supernumerary molting to occur because the reduction in juvenile hormones is inhibited (Mondal and Parween, 2000). Juvenile hormone treatment of young larvae often produces supernumerary larvae and on older larvae, produces abnormal larval-pupal intermediates, or pupal-adult intermediates (Mondal and Parween, 2000). Treatment of pupae results in pupal-adult intermediates or causes morphological deformities in adults such as a twisted wing (Mondal and Parween, 2000). It

should be noted that the degree of morphogenetic effect on insects differs depending on mode of application, species, active does, and age of the insects (Mondal and Parween, 2000).

Methoprene is very effective in controlling a vast variety of lepidopterous and coleopterous pests (Henrick, 2007). Amos et al. (1978) showed that crossing deformed adults exposed to 0.1 ppm methoprene did not result in progeny production. Progeny production of males and females is adversely affected by exposure to methoprene, and males are more sensitive compared to females (Wijayaratne et al., 2012). Methoprene does not cause adult mortality or a quick knock-down effect, but methoprene works by reducing populations over long periods of time by slowly reducing progeny production (Mondal and Parween, 2000; Wijayaratne et al., 2012). The objectives of this study were designed to determine adverse effects of exposure to untreated and methoprene impregnated woven packaging material on development to adulthood of four week old larvae of *T. castaneum* and *T. variabile* at two temperatures, (2) effect of exposure of adults to untreated and methoprene-impregnated packaging on egg laying and egg hatch of the two species, and (3) effect of exposure of adults to untreated and methoprene-treated packaging on egg-to-adult emergence of the two species.

3.3. Materials and Methods

3.3.1. 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 have been in rearing since 1958. *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 (Arizona Nutritional Supplements, Chandler, Arizona, USA), and the top of the culture was sprinkled with 100% whole grain rolled oats (Kroger Co., Cincinnati, Ohio, USA) and maintained at 30°C and 60% r.h. and 16:8 L:D photoperiod.

Eggs were obtained by using, approximately 100 g of flour sifted through a 149 µm opening sieve (Newark Wire Cloth Company, Clifton, New Jersey., USA), placed into a 0.18-L jelly jars (Ball, Muncie, Indiana, USA), and 60 unsexed *T. castaneum* or *T. variabile* adults of mixed ages were introduced. The containers were incubated at 30°C and 65% r.h. and 16:8 L:D photoperiod to allow for mating and oviposition. After three days, the adults were removed from jars using a 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 packages the adults were removed, frozen, and separated as male and female. Male *T. variabile* were distinguished by the 6-7 segmented antennal club, whereby 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 such a setiferous patch (Bousquet, 1990).

Methoprene impregnated and untreated woven packaging materials were obtained from a commercial manufacturer of birdseed packaging. The outer layer consisted of biaxial 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 weights where the

methoprene active material was impregnated into a polymer pellet matrix and extruded. This layer is 8 μm think and is loaded with a methoprene application rate of 0.1% or 1000 ppm per area.

3.3.2. Effect of short term larval exposure on adult emergence

Eighty individual treatment arenas were constructed by cutting a 150 by 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 impregnation in the packaging material, 20 discs with the inside surface and 20 discs with the outside surface were placed individually into 150 x 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 crawling on the sides of the Petri dishes and escape. The inside and outside surfaces of packaging not treated with methoprene were placed in Petri dishes to serve as the control treatment. Treatment combinations included, packages untreated or impregnated with methoprene, 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 species had 8 treatment combinations, and 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 to 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 at 60% r.h. Larvae were exposed to methoprene-treated or untreated arenas and inside and outside surfaces for 8, 24, 48, 72, and 96 h. At each exposure

time, 10 larvae were selected and removed from the arena and transferred to new untreated Petri dishes, 100 by 15 mm for *T. castaneum* or 100 by 20 mm for *T. variabile,* along with 500 mg of the respective 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 the untreated Petri dishes. The number of normal adults that emerged did not have any visible morphological deformities were recorded and expressed as a percentage. Any beetles that remained in the larval or pupal stages or adults with morphological deformities were also recorded. Morphologically deformed adults included those with missing or deformed body parts, unsclerotized patches on the exoskeleton, or wing deformations.

3.3.3. Effect of methoprene on egg hatch of *T. castaneum* and *T. variabile*

Forty eight individual arenas were prepared as described previously, except disc sizes were 9 cm in diameter (62 cm^2) and fitted into 100 by 15 mm (*T. castaneum)* or 100 by 20 mm (*T. variabile*) Petri dishes. Twenty four arenas did not contain methoprene (control) and twenty four were methoprene-treated. Both the inside (12 arenas per treatment) and outside surfaces (12 arenas per treatment) of each material were evaluated. Disc edges were secured with adhesive caulking (DAP Products Inc.) and the inner sides were coated with polytetrafluoroethylene (Fluon®) to prevent insect escape. Each treatment combination was replicated six times.

To determine the effect of methoprene-treated packaging on *T. castaneum* and *T. variabile* egg hatch, 25, 3-4 day old eggs were added to sample arenas and held at 27 or 32°C and 60% r.h. The number of larvae that hatched for each species and treatment combination after 7 d was recorded and the percent egg hatch was determined from the number of eggs that hatched out of the total exposed.

3.3.4. Effect of methoprene on adult fecundity and subsequent egg hatch

Forty eight individual treatment arenas, six replicates per treatment combination, were constructed as described in 2.3. Approximately 500 mg of flour pre-sifted through a 150 µm opening sieve (Newark Wire Cloth Company, Clifton, New Jersey, USA) was added to each treatment arena. Ten unsexed adults of mixed ages of *T. castaneum* or *T. variabile* were added to arenas and held at 27 or 32°C and 60% r.h. for 7 or 3 days, respectively. Following exposure to each treatment combination, adults were removed, frozen, and separated into male and females using characteristics described by Bousquet (1990). The contents of each arena were sifted using a 250 µm opening sieve to remove flour but retain the eggs. The number of eggs laid female per was counted, and the unhatched eggs were transferred to new untreated Petri dishes to determine percent hatch after 7 d for each species in each treatment combination. Percentage egg hatch was based number of eggs that hatched out of the total that were laid.

3.3.5. Effect of methoprene on egg-to-adult emergence of T. castaneum and T. variabile

Individual treatment arenas were constructed as previously described. A total of 24 discs of untreated and 24 of methoprene-treated materials were cut manually. For each species, the untreated and methoprene treated discs, inside and outside surfaces, and two temperatures were replicated six times. Approximately 500 mg of insect diet was placed in each arena, flour for *T. castaneum* and vanilla shake mix for *T. variabile*. Ten unsexed adults of mixed ages of a species were placed in individual arenas and incubated in a growth chamber at 27 and 32°C at 60% r.h. Adult *T. castaneum* were held in the chambers for 7 d and adult *T. variabile* for 3 d to facilitate egg laying. After 7 or 3 d, adults were gently removed from arenas using forceps, and placed in a freezer and then separated into males and females using characteristics described by Bosquet

(1990). The arenas with eggs were placed back into the environmental growth chambers and examined every 2-4 d and food (500 mg) was added as needed. The eggs were reared to adulthood, and arenas were held at the two temperatures until F_1 larvae were present in the control arenas. The total time for this to occur spanned approximately 8-11 weeks. Adults were assessed as either morphologically normal or deformed. Deformed adults primarily included pupal-adult intermediates and adults with twisted wings. The deformed category also included supernumerary larvae that failed to pupate after 11 weeks.

3.3.6. Data analysis

Data were analyzed by species. Data on the percentage of normal adults that emerged from 4-week-old larvae at the five exposure intervals were transformed to angular values for statistical analysis (Zar, 1984). Since 10 larvae were sampled at each of the five exposure intervals, data on percentage of normal adults that emerged by exposure interval among treatment combinations were subjected to one-way analysis of variance (ANOVA). If ANOVA was significant ($P < 0.05$), differences among treatment combinations were determined by Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test (SAS Institute, 2008). Data on percent egg hatch by species were transformed to angular values and differences ($P < 0.05$) in hatch among treatment combinations were determined by one-way ANOVA and REGWQ multiple range test. In tests were adults were exposed for 7 or 3 d to determine number of eggs laid and percent egg hatch, differences in number of males and females of a species among replicates in each treatment were determined by a paired *t*-test to test for deviation from a 1:1 sex ratio. Data on the mean number of females among treatment combinations and number of eggs laid per female were transformed to $\log_{10}$ (x) scale and data on percent egg hatch were transformed to angular values. These data were subjected to one-way ANOVA and REGWQ multiple range test to find

differences among the treatment combinations. In egg-to-adult emergence tests, the number of males and females of a species among replications in each of the treatments were determined by paired *t*-tests. Data on the number of normal and deformed adults that emerged from eggs were transformed to $\log_{10}(x + 1)$, and subjected to one-way ANOVA and REGWQ multiple range test to detect difference among treatment combinations.

3.4. Results

3.4.1. Effect of exposure interval and temperature on adult emergence

The mean percent normal adult emergence of *T. castaneum* varied overtime (Table 3.1). Significant differences in normal adult emergence were noted only at 96 h among treatment combinations ($F = 4.76$; df = 7, 32, P 0.0009). The lowest percent normal adult emergence occurred on the methoprene-treated inside surface at 96 h exposure and 32°C (24.0%), and the emergence in this treatment was significant different from emergence on all untreated surfaces irrespective of the temperature. Across all methoprene-treated surfaces at 96 h of exposure, adult emergence ranged from 24.0-42.0% and the percent reduction in emergence compared to emergence of untreated surfaces ranged from 48.8-68.4%. Treatment comparisons across each of the exposure time between 8 and 48 h were not significant (F, range among exposure times = 0.82-1.65; df = 7, 32; P, range = 01566-0.5806). Lack of differences were also found among treatment combinations at the 72 h exposure time ($F = 2.25$; df = 7, 30; $P = 0.0578$). Our results show longer exposure times are needed to significantly reduce the number of normal adults which emerge from four-week *T. castaneum* old larvae exposed to methoprene-treated packaging materials.

The lowest mean percent normal adult emergence for *T. variabile* was 50% on the methoprene-treat outside surface at 32°C at 48 and 72 h exposure periods (Table 3.2). Among

all treatment combinations *T. variabile* normal adult emergence was significantly affected after 48 and 72 h of exposure to methoprene-treated packaging materials (*F*, range among treatment combinations = 3.82-4.52; df = 7, 32; *P*, range = 0.0015-0.0044). After 48 h of exposure the methoprene-treated surfaces at both temperatures was significantly lower than the untreated and methoprene-treated outside surfaces at 27°C. At 72 h, the outside methoprene-treated surface at 32°C was significantly different only from the outside untreated surface at 27°C. *T. variabile* larvae exposure for 8 h produced a significant effect on the number of normal adults which emerged ($F = 2.45$; df = 7, 29; $P = 0.0419$), but further analysis using REGWQ multiple range test failed to show significant differences among treatment combinations. The same results were also seen in exposure at 24 h ($F = 2.67$; df = 7, 32; $P = 0.0270$) but not for 96 h exposure ($F = 1.90$; df = 7, 32, $P = 0.1026$). The lack of significant differences between treatments following the REGWQ multiple range test could be due to the large standard error spreads for several observations. The REGWQ test did not apparently control the type I experiment wise error rate. The use of linear contrasts may show which of the pairwise treatment combinations is different.

3.4.2. Effect of methoprene on egg hatch of *T. castaneum* and *T. variabile*

The mean ± SE percentage of hatch of *T. castaneum* eggs added to arenas ranged from 86.2 ± 3.2 to 96.7 ± 1.1% (Table 3.3). There was a significant difference based on a global ANOVA ($F = 2.92$; df = 7, 40; $P = 0.0145$) but the REGWQ test found no significant differences among treatment combinations. These differences may reflect biological variation in egg hatch.

The mean ± SE percentage of hatch of *T. variable* eggs added to treatment arenas ranged from 59.2 ± 4.5 to 87.0 ±2.9% (Table 3.3). The was a significant difference between the treatments ($F = 8.77$, df = 7, 40; $P < 0.001$). The outside surface of the control packaging at 27°C had the highest percentage of egg hatch, and the inside surface of the treatment package at 32°C

had the lowest percent hatch. At 27°C, both the outside and inside methoprene-treated surfaces showed a lower percent hatch compared to the untreated surface. However, at 32°C the untreated surfaces had a lower percent hatch compared to the methoprene-treated surfaces.

3.4.3. Effect of methoprene on fecundity and egg hatch of *T. castaneum* and *T. variabile*

The mean ± SE number of female *T. castaneum* adults ranged from 4.2 ± 0.7 to 6.2 ± 0.6. A paired *t*-test between females and male *T. castaneum* for each treatment showed no significant differences (*t*, range among treatments = -1.27 - 1.91; df = 5; *P*, range = 0.1099-1.000). The mean ± SE number of *T. variabile* females per arena ranges from 3.8 ± 0.7 to 5.0 ± 0.6. A paired *t*-test between females and males, showed no significant differences for each of the treatments (*t*, range among treatments = -1.81-0.42; df = 5; *P*, range = 0.1303-1.0000)

The methoprene treated woven bags did not have a significant effect on the number of eggs laid per female or the subsequent egg hatch of *T. variabile* (Table 3.4) (*F*, range among treatments combinations for eggs laid and egg hatch = 1.17-1.37; df = 7, 40; *P*, range = 0.2440-.3397). The mean number of eggs laid per female *T. variabile* ranged from 42.8-67.9. Adult *T. variabile* exposed to the inside and outside methoprene-treated surfaces had fewer eggs laid than the untreated surfaces at the same temperature. The percent egg hatch for *T. variabile* ranged from 80.32-95.82%. In all comparison except the inside surfaces at 27°C, the percent hatch of *T. variabile* eggs was lower on the methoprene-treated surface.

The mean number of eggs laid per female was significant for *T. castaneum* (*F* = 2.56; df = 7, 40; *P* = 0.0281). The outside surface of methoprene treated packaging held at 32°C had significantly the most eggs laid per *T. castaneum* female (mean ± SE) 17.1 ± 3.1, while the methoprene-treated inside surfaces at 27°C had significantly the fewest eggs laid, 7.6 ± 1.8 and

7.4 ± 2.0. All other treatments did not differ significantly from these. There were significant differences among the mean percent hatch of *T. castaneum* eggs (F = 2.35; df = 7, 40; P = 0.0415). The inside surface of the methoprene-treated material 27°C had the highest percent egg hatch, 81.4 ±5.4%. The outside surface of the methoprene-treated material at 32°C had the lowest percent egg hatch 36.31±3.66%. The other treatments did not differ significantly from these two.

3.4.4. Effect of continual exposure of methoprene-treated packaging on egg-to-adult development of *T. castaneum* and *T. variabile*

The mean ± SE number of *T. castaneum* females ranged from 4.7 ± 0.4 to 5.8±0.7. A paired *t*-test indicated that there was no significant differences between male and female *T. castaneum* in each of the treatments (F, range among treatments = -0.79 - 1.58; df = 5; P, range = 0.1747 - 1.0000). The mean ± SE number of female *T. variabile* ranged from 3.8 ± 0.6 to 4.8 ± 0.6. A paired *t*-test found a significant difference between the number of female and males on the outside surface of the untreated material at 32°C (t = -2.80; df = 5; P = 0.0379).

The methoprene packaging had a significant effect on the number of normal *T. castaneum* adults (F = 95.70; df = 7, 40; P < 0.0001) and deformed adults (F = 38.70; df = 7, 40; P < 0.0001). The methoprene packaging had a 100% reduction of normal adult emergence among all treatment combinations (Table 3.5). Additionally, the number of deformities was significantly higher for methoprene surfaces. The deformities of *T. castaneum* consisted of supernumerary larvae and pupal-adult intermediates.

Results of exposing *T. variabile* to methoprene treated packaging showed significant differences in normal adult emergence (F = 23.34; df = 7, 40; P < 0.0001). The outside surface of the methoprene packages held at 32°C had significantly the least amount of

normal adults, and a 96.4% reduction in normal adult emergence compared to the control surface (Table 5). All other methoprene packages were not significantly different from the control, and the percent reduction ranged from 33.9-40.9% compared to the control packaging. The number of deformities was significant among treatments ($F = 21.44$; df = 7, 40; $P < 0.001$). Since the outside surface of the methoprene packaging held at 32°C had the fewest number of normal adults emerge, it therefore has significantly the highest deformities among all treatments. Compared to the respective control, the methoprene had a greater number of deformities seen. Both methoprene-treated surfaces at 27 and 32°C, varied significantly from their respective untreated surfaces.

3.5. Discussion

Comparisons of the effect of methoprene-treated materials for *T. castaneum* and *T. variabile* indicated significant reduction in normal adult emergence for *T. castaneum* after 96 h of exposure and 48 and 72 h exposure for *T. variabile*. The lowest mean adult emergence for both species occurred at the 32°C temperature. Our study indicated that 96 h or greater are needed to show a significant effect on normal larval development in *T. castaneum* and *T. variabile*. The lack of significance in shorter time exposure periods indicates that the larvae of either species lacks the required time to absorb the methoprene and thus making it ineffective at short term exposures. Arthur (2006) demonstrated that increasing the exposure time of *Plodia interpunctella* to hydroprene on concrete dishes, increases the time required for 5th instar larvae to emerge as adults. Hydroprene, also and IGR, works in a similar manner as methoprene. The results from the short-term exposure of four-week old larvae are comparable to those seen by Arthur (2006). Arthur (2006) found that within a specific exposure time period the developmental time decreased as holding temperatures increased, indicating that at higher

temperatures immature stages develop faster. Arthur (2006) found a relationship between time of exposure and temperature in relation to inhibition of adult emergence.

Wijayaratne et al. (2012) found that only some of the larvae exposed to methoprene treated wheat were susceptible and displayed adverse effects. This is similar to the current short term exposure study. Since the methoprene was incorporated into an adhesive matrix of the birdseed packaging, there may be an inherent variation of methoprene distribution throughout the packaging material which could account for the variation in normal adult emergence between replicates. In addition, there could be a defined window of sensitivity at which methoprene is most effective during *T. castaneum* and/or *T. variabile* development (Wijayaratne et al., 2012). Since the current study used late instars, there is an inherent variation in the rate of development especially at the two experimental temperatures. *T. castaneum* and *T. variabile* develop faster at 32°C compared to 27°C. At the higher temperature, both insects are ready to pupate after 96 h of exposure to methoprene and at 27°C the pupae stage is slightly delayed. The slight delay in development may provide adequate time for methoprene uptake.

Wijayaratne et al. (2012) found that methoprene had no effect on progeny production of 2-4 d old adult *T. castaneum*, indicating that the juvenile hormone, such as that in methoprene, may not be involved in the final stages of the reproductive system development once the adult beetle has emerged. Conversely, the levels of juvenile hormone may be too high rendering the application of methoprene ineffective in disrupting the reproductive system development (Wijayaratne et al., 2012).

3.6. References

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Table 3.1 Percentage of normal adult emergence of *T. castaneum* over short time periods.

Surface	Temp. (°C)	Treatment	Mean ± SE[a]				
			Exposure time (h)				
			8[b]	24[c]	48[d]	72[e]	96
Outside	27	Control	68.0±3.7	66.0±6.8	58.0±9.7	78.0±7.3	78.0±7.3a
		Methoprene	80.0±7.1	76.0±8.1	78.0±7.3	70.0±13.5	34.0±5.1ab
	32	Control	68.0±8.6	66.0±8.1	64.0±7.5	82.0±7.3	78.0±10.2a
		Methoprene	60.0±4.5	82.0±8.6	74.0±18.9	68.0±17.1	38.0±15.0ab
Inside	27	Control	72.0±8.6	88.0±5.8	70.0±4.5	78.0±3.7	82.0±6.6a
		Methoprene	80.0±3.2	68.0±3.7	54.0±12.1	92.0±4.8	42.0±9.2ab
	32	Control	76.0±10.3	76.0±6.8	82.0±5.8	78.0±9.7	76.0±11.2a
		Methoprene	72.0±5.8	62.0±8.6	74.0±9.3	38.0±16.9	24.0±15.0b

[a]Mean for each exposure time followed by different letters are significantly different ($P < 0.05$; by Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test).

[b]There were no significant differences among the treatment combinations ($F = 0.94$; df = 7, 32; $P = 0.4894$; by one-way ANOVA).

[c]There were no significant differences among the treatment combinations ($F = 1.65$; df = 7, 32; $P = 0.1566$; by one-way ANOVA).

[d]There were no significant differences among the treatment combinations ($F = 0.82$; df = 7, 32; $P = 0.5806$; by one-way ANOVA).

[e]There were no significant differences among the treatment combinations ($F = 2.25$; df = 7, 30; $P = 0.0578$; by one-way ANOVA).

Table 3.2 Percentage of normal adult emergence of *T. variabile* over short time periods.

Surface	Temp. (°C)	Treatment	Mean ± SE[a]				
			Exposure time (h)				
			8[b]	24[c]	48	72	96[d]
Outside	27	Control	84.0±5.1	88.0±5.8	84.0±9.3a	90.0±5.5a	86.0±5.1
		Methoprene	78.0±2.0	86.0±2.4	92.0±3.7a	77.5±10.3ab	84.0±9.3
	32	Control	60.0±10.8	60.0±0.0	70.0±12.2ab	58.0±14.6ab	70.0±6.3
		Methoprene	70.0±3.2	70.0±8.4	50.0±8.4b	50.0±4.5b	68.0±8.6
Inside	27	Control	82.0±5.8	84.0±5.1	86.0±4.0ab	88.0±3.7ab	86.0±7.5
		Methoprene	88.0±4.9	86.0±5.1	84.0±5.1ab	87.5±4.8ab	80.0±5.5
	32	Control	60.0±14.7	66.0±12.1	75.0±2.9ab	66.0±5.1ab	78.0±3.7
		Methoprene	62.5±4.8	76.0±5.1	52.0±8.0b	64.0±6.8ab	62.0±9.2

[a]Mean for each exposure time followed by different letters are significantly different ($P < 0.05$; by REGWQ multiple range test).

[b]There were significant differences among the treatment combinations ($F = 2.45$; df = 7, 29; $P = 0.0419$; by one-way ANOVA), but the REGWQ multiple range test failed to show which treatment combinations were different.

[c]There were significant differences among the treatments ($F = 2.67$; df = 7, 32; $P = 0.0270$; by one-way ANOVA), but the REGWQ multiple range test failed to show which treatment combinations were different.

[d]There were no significant differences among the treatment combinations ($F = 1.90$; df = 7, 32; $P = 0.1026$; by one-way ANOVA).

Table 3.3 Percent hatch of *T. variabile* and *T. castaneum* eggs exposed to treatment combinations.

Surface	Temperature (°C)	Treatment	Mean ± SE[a]	
			T. castaneum[b]	*T. variabile*
Outside	27	Control	89.1±1.2	90.9±2.6a
		Methoprene	96.7±1.1	87.8±2.0ab
	32	Control	95.5±2.4	67.9±3.9dc
		Methoprene	95.0±1.4	82.5±3.0abc
Inside	27	Control	86.2±3.2	87.0±3.0ab
		Methoprene	89.4±3.0	81.7±2.0abc
	32	Control	94.4±1.8	59.3±4.5d
		Methoprene	96.5±1.3	74.0±5.8bcd

[a]Means for each species and variable studied followed by different letters are significantly different ($P < 0.05$; by REGWQ multiple range test).

[b]There were significant differences among the treatment combinations ($F = 2.92$; df = 7, 40; $P = 0.0145$; by one-way ANOVA), but the REGWQ multiple range test failed to show which treatment combinations were different.

Table 3.4 Effects of exposure of *T. castaneum* and *T. variabile* adults to methoprene-treated package surfaces on number of eggs laid by females and percent egg hatch.

Surface	Temp. (°C)	Treatment	Mean±SE[a]					
			T. castaneum			*T. variabile*		
			Number of females[b]	Number of eggs/female	% Egg hatch	Number of females[b]	Number of eggs/female[d]	% Egg hatch[e]
Outside	27	Control	5.2±0.3	10.5±1.5ab	69.0±8.3ab	4.7±0.6	51.9±6.3	88.9±3.7
		Methoprene	5.2±0.8	8.9±1.4ab	63.2±7.7ab	4.7±0.5	47.0±7.0	86.2±3.8
	32	Control	4.7±0.3	13.5±2.3ab	56.5±2.9ab	5.0±0.6	67.0±11.5	95.0±1.1
		Methoprene	4.2±0.7	17.1±3.1a	36.3±3.7b	3.8±0.7	42.8±7.0	87.0±7.1
Inside	27	Control	6.2±0.6	7.6±1.8ab	63.3±6.5ab	4.8±0.4	67.9±12.2	80.3±7.5
		Methoprene	5.0±0.8	7.4±2.0b	81.4±5.4a	4.5±0.3	44.7±11.7	90.4±3.9
	32	Control	5.5±0.3	11.4±2.4ab	61.0±13.7ab	4.3±0.5	66.6±9.7	95.8±2.1
		Methoprene	4.2±0.7	11.2±1.8ab	56.7±4.0ab	4.5±0.5	46.1±8.7	92.0±0.8

[a]Mean for each species and variable followed by different letters are significantly different ($P < 0.05$; by RGEWQ multiple range test).

[b]There were no significant differences among the treatment combinations (F, range among treatment combinations and species = 0.66-1.16; df = 7, 40; P, range = 0.3493-0.7046).

[c]There were no significant differences among the treatment combinations ($F = 1.17$; df = 7, 40; $P = 0.3397$).

[d]There were no significant differences among the treatment combinations ($F = 1.37$; df = 7, 40; $P = 0.2440$).

Table 3.5 Effect of continuous exposure to methoprene treated surfaces on *T. castaneum* and *T. variabile* egg-to-adult development..

Surface	Temp. (°C)	Treatment	Mean±SE[a]			
			T. castaneum adults		*T. variabile adult*	
			Normal	Deformed	Normal	Deformed
Outside	27	Control	16.5±2.2a	0.8±0.3b	164.7±14.6a	12.8±4.9bc
		Methoprene	0.0±0.0b	11.0±2.0a	106.5±28.6a	31.0±4.4b
	32	Control	12.3±4.0a	1.2±0.4b	149.2±15.6a	4.0±1.1cd
		Methoprene	0.0±0.0b	14.8±1.5a	5.3±2.9b	122.3±22.8a
Inside	27	Control	20.3±4.2a	0.5±0.3b	131.2±10.0a	1.8±0.7c
		Methoprene	0.0±0.0b	15.3±2.9a	77.5±28.5a	7.7±3.0cd
	32	Control	12.7±2.2a	0.2±0.2b	175.0±21.0a	9.0±2.7d
		Methoprene	0.0±0.0b	12.5±3.2a	115.7±14.7a	14.0±3.7bc

[a]Means among treatment combinations for each species and variable studied followed by different letters are significantly different ($P < 0.05$; by RGEWQ multiple range test).

Chapter 4 - Effect of methoprene impregnated polymer packaging on fecundity, egg hatch, and egg-to-adult emergence of *Tribolium castaneum* and *Trogoderma variabile*

4.1. Abstract

Methoprene is an insect growth regulator used in aerosol sprays, for residual surface treatments, and as a grain protectant. Methoprene was impregnated into a polymer-based packing film to prevent insect infestations of packaged products. The study objectives were to determine fecundity, egg hatch, and egg-to-adult emergence of, *Tribolium castaneum* (Herbst), red flour beetle, and *Trogoderma variabile* Ballion, warehouse beetle, when exposed to methoprene-impregnated packaging. The number of eggs laid by beetles, egg hatch, and egg-to-adult development on the inside and outside surfaces of methoprene-impregnated polyethylene-to-polyethylene (PE-PE) and polyethylene terephthalate-to-polyethylene (PET-PE) packaging were studied at 27 and 32°C and 60% r.h. Inside and outside surfaces of the packaging were cut into discs and fitted into 62 cm^2 Petri plates, and 500 mg of flour (*T. castaneum*) or vanilla shake mix (*T. variabile*) were added to these arenas. The number *T. variabile* eggs laid per female was not significantly different between the packaging types. Methoprene-treated PET-PE packaging reduced the number of *T. castaneum* eggs laid per female. The polymer packaging reduced the percent egg hatch of both species. None of the *T.castaneum* adults emerged on the inside and outside surfaces of the PE-PE treated packages at both temperatures. Only the inside surface of PET-PE packaging resulted in 100% suppression of *T. castaneum* adult emergence. Egg-to-adult emergence of *T. variabile* was arrested at the pupal to adult stage transformation at both temperatures on the outside surface of PE-PE packaging, whereas on the inside surface *T.*

variabile were able to complete development to adulthood. The PET-PE packaging greatly reduced normal adult emergence by 87-97% when exposed to inside surfaces at both temperatures. Our results show that exposure of eggs to methoprene imprenated packaging reduced egg hatch of both species and adult emergence of *T. castaneum*, and to a lesser extent, emergence of *T. variable*.

4.2. Introduction

Stored-product insects are a reoccurring and persistent problem in retail and pet food stores where they cause damage to packaged food and animal products (Arbogast et al., 2000). Stored-product insects may be present in the store or are introduced during product deliveries from infested trailers or warehouses (Arbogast et al., 2000). Infestations of human food or pet food products results in product losses, loss of consumer confidence, and possible allergic reactions by susceptible individuals. Research studies have shown heaviest infestations are located in or near the pet food departments (Arbogast et al., 2000; Roesli et al., 2003). Arbogast et al. (2000) found that the most intense infestation can be associated with bagged birdseed in seven out of eight stores surveyed. Specifically in pet food stores, stored-product insects can be found in bagged birdseed and bulk stored pet food products (Roesli et al., 2003). Roseli et al. (2003) found at least 30 species from 20 different families among eight pet food stores in Kansas sampled over a seven month period. Among the 30 species, there were 27 different stored-product insect species captured in food-baited and pheromone traps (Roesli et al., 2003). *Tribolium castaneum* (Herbst), red flour beetle, and *Trogoderma variabile* (Ballion), warehouse beetle, were found in seven out of eight pet food stores, which make both species a concern when dealing with infestations in retail environments (Roesli et al., 2003). The types and numbers of insects found in retail stores may be related to the level of sanitation and pest management practices, type of food products stored, and inspection and management of incoming products (Roesli et al., 2003).

Stored-product insects can be found in flour and feed mills, livestock ranches, food warehouses, containers, and commercial processing facilities (Loschiavo and Okumura, 1979; Campbell et al., 2002; Semeao et al., 2013; Arthur et al., 2014). Common sources of stored-

product insects can be caused by dispersal from the field, infested machinery, other structures which contain stored grain products, and food material which has accumulated outside the building (Semeao et al., 2013). Loschiavo and Okumura (1979) studied several location types throughout four Hawaiian Islands and found *T. castaneum* in every type of premise studied and they considered it to be the most common and widespread stored-product insect (Loschiavo and Okumura, 1979). Semeao et al. (2013) studied a commercial processing facility, feed mill, and a flour mill in the central United States, and likewise found *T. castaneum* in every facility studies. Loschiavo and Okumura (1979) found *T. variabile* to be a serious pest in containers. Similar to *T. castaneum*, Semeao et al. (2013) found *T. variabile* in every facility studied. Arthur et al. (2014) found *T. castaneum* and *T. variabile* inside a food facility in the Midwest, but differences in species abundance were often widely distributed throughout the facility and time of year. Arthur et al. (2014) observed very little trap catches within the food facility from November-May. During this time period in the Midwest, often temperatures drop below optimum conditions for insect growth and reproduction. This effect could influence the trap catches of these insects, however insects inside a structure could maintain a population if the building is heated. Campbell et al. (2002) observed the spatial distribution of insects throughout a facility varies. Campbell et al. (2002) found two specific hotspots within a facility, but observed that insects are highly mobile can the "hot spots" can vary location from time to time. Historically retailers, mill managers, and consumers depended on chemical insecticides and facility fumigations to control stored-product insects, but with an increasing awareness by consumers on the environmental and human health impacts, retailers are looking for a more targeted approach to reduce the total amount of area treated and the reduce the amount of pesticide usage (Arbogast et al., 2000).

Methoprene is an insect growth regulator and a juvenile hormone analog which acts on stored-product insects by mimicking the hormone that regulates the developmental process of molting (Mondal and Parween, 2000). The presence of the juvenile hormone in the insect allows for molting to occur but inhibits adult differentiation (Henrick, 2007). As the juvenile hormone decreases in the last instar, the insect undergoes the larval-pupal molt which leads to the metamorphosis to the adult stage (Henrick, 2007). The goal of juvenile hormone analogs is to maintaining the levels of this hormone over time in order to disrupt the insect from undergoing the larval-pupal and pupal-adult molting. In addition, juvenile hormone is also needed in the adult stages of some insects for reproductive functions (Henrick, 2007).

Methoprene has low mammalian toxicity and is exempt from the Food and Drug Administration tolerance levels when used to control insect larvae. Methoprene can be sprayed on stored grains, treat surfaces as an aerosol application, as a liquid spray applied directly to the surface, and impregnated into packaging materials. As of 2003, the United States Environmental Protection Agency granted methoprene exemption from a food tolerance (Henrick, 2007). Using methoprene at the appropriate application rates, there is little to no effect on non-target species such as birds, fish, or mammals (Henrick, 2007). Additionally, there are no harmful effects to humans when products containing methoprene are used (EPA, 2015). Methoprene has high activity across many different insect species belonging to orders Lepidoptera, Coleoptera, Homoptera, Siphonaptera, Hymenoptera, Blattodea, and Diptera (Henrick, 2007). Methoprene has been shown to be effective over several years against lepidopterous and coleopterous pests (Henrick, 2007). Manzelli (1982) demonstrated that 10 ppm of methoprene applied to tobacco and stored for four years still gave protection against the *Lasioderma serricone* (L.), cigarette beetle, and *Ephestia elutella* (Hübner), tobacco or warehouse moth. Loschiavo (1976) found that

increasing concentrations of methoprene on *T. castaneum* diet from 1 ppm to 20 ppm reduced larval survival by 89%. At 20 ppm of methoprene, larvae of *T. castaneum* failed to pupate (Loschiavo, 1976). However, larvae which failed to develop into pupae weighed more and were longer in length than larvae reared on control diets. In the case of larvae of *T. castaneum* that pupated, none emerged as normal adults on diets with 10 or 20 ppm of methoprene (Loschiavo, 1976).

Larvae of *T. variabile* were thought to be extremely susceptible to juvenile hormone analogues, because the number of instars is indeterminate due to their ability to undergo diapause (Klein and Burkholder, 1984). Klein and Burkholder (1984) found that a direct application of 10 ppm methoprene to rearing medium caused a delay in the development of *Trogoderma glabrum* (Herbst), glaborous cabinet beetle. However, virgin males exposed to surfaces treated with 100 $\mu g/cm^2$ for one to four days, and mated with an untreated female, showed no effect on the number of progeny produced (Klein and Burkholder, 1984). Exposing virgin females to 100 $\mu g/cm^2$ treated surfaces for four days and then mating them with untreated males significantly reduced the average number of progeny by 97% compared to insects exposed to the control surface (Klein and Burkholder, 1984).

The use of methoprene-impregnated packages for birdseed and other food products is a potential control strategy for stored-product insects. One company that manufactures methoprene-impregnated packages is ProvisionGard LLC (Wayzata, Minnesota, USA). The manufacturer of ProvisionGard provided packages that were impregnated with methoprene and those that were not impregnated with methoprene for evaluations with insects. The objectives of the present study were to determine the effect of two methoprene impregnated polymer

packaging materials on number of eggs laid, egg hatch, and egg-to-adult emergence of *T. castaneum* and *T. variabile* held at 27 or 32°C and 60% r.h..

4.3. Materials and methods

4.3.1. Insects

T. castaneum 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 (USDA-CGAHR) in Manhattan, Kansas. *T. castaneum* cultures have been in rearing since 1958. Cultures were reared in total darkness at 27°C and 60% r.h. on 95% unbleached whole-wheat flour (Hudson Cream Flour, Stafford Country Flour Mills Co., Hudson, Kansas, USA) with 5% (w/w/) brewer's yeast diet (MP Biomedicals LLC, Solon, Ohio, USA) in 0.95-L mason jars fitted with wire-mesh and filter paper lids. Cultures of *T. variabile* were reared on 50% Purina One lamb and rice formula (Nestlé Purina PetCare Company, St. Louis, Missouri, USA) and 50% Pharmanex vanilla shake mix (Arizona Nutritional Supplements, Chandler, Arizona, USA). The top of the diet in 0.95-L jars was sprinkled with 100% whole grain rolled oats (Kroger Co., Cincinnati, Ohio, USA), and culture jars were maintained at 30°C, 60% r.h., and 16:8 L:D photoperiod.

To obtain eggs, approximately 100 g of flour, sifted through a 150 µm opening sieve (Newark Wire Cloth Company, Clifton, New Jersey, USA) was placed into a 118 ml jelly jars (Ball, Muncie, Indiana, USA), and 60 unsexed *T. castaneum* or *T. variabile* adults of mixed ages were introduced. The containers were incubated at 30°C, 60% r.h., and 16:8 L:D photoperiod. After three days, adults were removed from jars using an 841 µm opening sieve on top and a 250 µm opening sieve on the bottom with a bottom pan. The adults were retained on the top sieve

and eggs on the bottom sieve with the flour ending up in the bottom pan. Eggs were gently collected from the bottom sieve.

In tests with adults, unsexed adults of mixed ages were used, and these were directly collected from the culture jars. After exposure to packages, the adults were frozen and separated as male and female. Male *T. variabile* have a 6-7 segmented antennal club, and females only have 4 segmented one (Bousquet, 1990). Male *T. castaneum* have a setiferous patch on the posterior side of the fore femur, while the females lack the patch (Bousquet, 1990).

4.3.2. Effect of methoprene on egg hatch

Two types of polymer packaging were used in these experiments: polyethylene-to-polyethylene (PE-PE) and polyethylene terephthalate-to-polyethylene (PET-PE). The two layers of polymers were extruded by using a solventless two part adhesive securing the two polymers structures together to form the final packaging material. Out of the two parts, one part has just the adhesive whereas the other has adhesive with methoprene. For each packaging type, methoprene purity was at was used at 1% active, such that that when the two adhesive parts were combined to form the packaging and extruded, the methoprene was diluted to 0.5% active throughout. The adhesion layer was approximately 1 to 2 μm thick. The untreated control packaging used the same solventless two part adhesive, but without the addition of methoprene.

Forty eight individual discs of 9 cm diameter with a surface area of 62 cm^2 were cut by hand from the PE-PE packaging containing methoprene or PET-PE materials. A similar number of discs were cut from each of the two materials that did not contain methoprene (control). Out of the 48 discs, 24 discs with the inside surface of the each packaging material treated with methoprene or untreated with methoprene were placed individually into 100 mm by 15 mm (*T. castaneum)* or 100 mm by 20 mm (*T. variabile*) Petri dishes (arenas). Similarly, 24 discs with the

outside surface of each packaging material were placed into Petri dishes. The disc edges were secured with adhesive caulking (DAP Products Inc, Baltimore, Maryland, USA). The inner sides of Petri dishes were coated with polytetrafluoroethylene (Fluon®) to create the final sample arena (Sigma-Aldrich Co., St. Louis, Missouri, USA). The treatment combinations included, two package types, packages untreated and treated with methoprene, two package surfaces (inside vs. outside), two temperatures (27 and 32°C), and two insect species. Each of the 16 treatment combinations was replicated six times.

To determine the effect of packaging types at the treatment combinations on egg hatch, 25, 3-4 d-old eggs of *T. castaneum* or *T. variabile* were added to the sample arenas and held in growth chambers at 27 and 32°C and 60% r.h. The number of larvae that hatched for each species and treatment combination was recorded and percent hatch was determined from number of eggs that hatched out of the total.

4.3.3. Effect of methoprene on adult fecundity and egg hatch

To determine the effect of *T. castaneum* and *T. variabile* adult exposure to treatment combinations on adult fecundity and subsequent egg hatch, 96 discs of each package type were prepared as described previously. Approximately 500 mg of flour presifted through a 150 μm opening sieve (Newark Wire Cloth Company, Clifton, N.J., USA) was added to each treatment arena. Ten unsexed adults of mixed ages of *T. castaneum* and *T. variabile* were added to separate arenas and held in growth chambers at 27 and 32°C and 60% r.h. for 7 and 3 d, respectively. Adults were removed, frozen, and separated into male and female using characteristics described by Bousquet (1990). The eggs and flour from each arena were sifted using a 250 μm opening sieve to collect the eggs. The number of eggs laid per female was counted. These eggs were then transferred to new untreated Petri plates and percent egg hatch for

each species was determined after incubation in a growth chamber after 7 d at the two temperatures.

4.3.4. Effect of methoprene on egg-to-adult emergence

Individual treatment arenas were constructed as described above using 96 discs for each package type. Each surface, temperature, and species combination was replicated six times. Approximately 500 mg of flour (*T. castaneum*) or vanilla shake mix (*T. variabile*) were added to the discs. Ten mixed sex and aged adults were placed onto individual arenas and placed into growth chambers at 27 and 32°C and 60% r.h. Adult *T. castaneum* were held in the chambers for 7 d and adult *T. variabile* were held for 3 d. The adults of both species were removed from arenas and placed in a freezer and sexed following characteristics described by Bosquet (1990).

After adult removal, the arenas with eggs were placed in environmental growth chambers at the two temperatures. The arenas were examined every 2 to 4 d and food was added as needed. The eggs were reared through adulthood, and the number of normal or deformed adults that emerged was counted. Deformed adults primarily included supernumerary larvae, pupal-adult intermediates, and adults with separated or twisted wings.

4.3.5. Data analysis

All experiments were run as a completely random design. Data were analyzed by package type and species. The percent egg hatch data were transformed to angular values (Zar, 1984). The number of males and females by replicate and treatment were compared using a paired *t*-test (SAS Institute, 2008) to determine if the sex ratio was 1:1 among the replications. The number of females found per arena among treatment combinations in different experiments were also compared using a one-way analysis of variance (ANOVA) after transformation of data to $\log_{10}(x)$ scale (SAS Institute, 2008). Data on the number of eggs laid by female were

transformed to $\log_{10}(x)$ scale for analysis, and egg hatch data were transformed to angular values. Data on normal and deformed was adults were transformed to $\log_{10}(x+1)$ scale and subjected to a one-way ANOVA. If the ANOVA was significant, differences among treatment combinations for the variables studied were separated using the Ryan-Einot-Gabriel-Welsch multiple range tests at $\alpha = 0.05$ (SAS Institute, 2008).

4.4. Results

4.4.1. Effect of methoprene on egg hatch

The mean ± SE egg hatch of *T. castaneum* eggs on PE-PE methoprene-treated arenas ranged from 49.6 ± 6.8 to 66.2 ± 3.7% and that on untreated arenas ranged from 80.7 ± 11.1 to 87.4 ± 3.3% (Table 4.1). Egg hatch of added *T. castaneum* eggs showed significant differences among treatment combinations ($F = 5.10$; df = 7, 40; $P = 0.0003$). Generally, egg hatch was consistently lower on the methoprene-treated arenas compared with untreated arenas. On PE-PE arenas, the mean ± SE egg hatch of *T. variabile* eggs on methoprene-treated arenas ranged from 53.9 ± 5.4 to 77.4 ± 4.4% and on untreated arenas egg hatch ranged from 61.5 ± 7.1 to 83.9 ± 3.8%, and differences were observed among the treatment combinations ($F = 4.74$; df = 7, 40; $P = 0.0006$). The egg hatch of *T. variabile* eggs exposed to the inside and outside surfaces in methoprene treatment at 32°C was significantly different from the egg hatch of eggs exposed to the inside surface in the control treatment at 27°C. The egg hatch of *T. variabile* on both methoprene-treated surfaces at 32°C was 34-36% lower compared with the inside untreated surface at 27°C.

On PET-PE packaging, mean ± SE egg hatch of *T. castaneum* eggs ranged from 56.8 ± 3.2 to 98.0 ± 0.9 (Table 4.1), and differences were observed among treatment combinations ($F = 15.83$; df = 7, 40; $P < 0.0001$). The inside surface of PET-PE packaging material at 32°C had the

lowest percent egg hatch (57%), and the inside untreated surface at 27°C had the highest percent egg hatch (98.0%). Egg hatch in methoprene treatments when eggs were exposed to the inside surfaces at 27 and 32°C was significantly lower than corresponding egg hatch in the control treatments.

The mean ± SE egg hatch of *T. variabile* eggs ranged from 66.3 ± 3.1 to 92.3 ± 3.4%, and although differences in treatment combinations were detected ($F = 4.05$; df = 7, 40; $P = 0.0019$), consistently more eggs hatched in methoprene than control treatments. Thus, the significant differences observed among the treatment combinations were due to increased egg hatch in methoprene treatments relative to the control treatments, and not due to any adverse effects of methoprene on *T. variabile* egg hatch.

4.4.2. Effect of methoprene on adult fecundity and egg hatch

Paired *t*-tests showed no difference in the number of males and females added to the different treatments using PE-PE packaging material for *T. castaneum* (range in *t* values among treatments was -1.57 to 1.73; df = 5; range in *P* values was 0.1438 to 0.8969) or *T. variabile* (*t* value range, -2.30 to 1.94; df = 5; *P* value range, 0.0699 to 0.6109). The mean ± SE number of female *T. castaneum* on PE-PE packaging in our tests ranged from 4.2 ± 0.5 to 6.0 ± 0.6 and for *T. variabile* females ranged from 3.7 ± 0.6 to 6.0 ± 0.5 (Table 4.2). The mean number of females of *T. castaneum* on discs was not significant among treatment combinations ($F = 0.93$; df = 7, 40; $P = 0.4959$). However, numbers of female *T. variabile* varied significantly among treatment combinations ($F = 3.21$; df = 7, 40, $P = 0.0086$). The outside surface of the untreated disc at 32°C had the fewest mean ± SE number of females per arena (3.7 ± 0.6) and was significantly different than the inside of methoprene-treated surface (6.0 ± 0.5). All other comparisons were not significantly different from one another.

The mean ± SE number of eggs laid ranged from 7.1 ± 0.3 to 12.0 ± 2.1 for *T. castaneum* and 29.1 ± 6.9 to 54.1 ± 20.5 for *T. variabile* (Table 4.2). On PE-PE packaging, one-way ANOVA showed no significant differences on the number of eggs laid per female for *T. castaneum* ($F = 1.13$; df = 7, 40; $P = 0.3663$) on untreated and methoprene-treated packaging. The number of eggs laid per female *T. variabile* was not significantly different between untreated and methoprene-treated packaging ($F = 0.67$; df = 7, 39; $P = 0.6926$). The percent egg hatch of *T. variabile* was not significantly different among treatment combinations ($F = 0.52$; df = 7, 39; $P = 0.8132$). However, the percent egg hatch of *T. castaneum* eggs was significantly different among treatment combinations ($F = 2.67$; df = 7, 40; $P = 0.0231$). The percent egg hatch for the outside surfaces of methoprene packaging at both temperatures differed significantly from the inside methoprene-treated surfaces at 27°C. All other comparisons were not significantly different from one another.

Paired *t*-tests of PET-PE data, showed no significant differences in the number of males and females of *T. castaneum* in each of the treatments (range in *t* values among treatments -1.94 to 1.69; df = 5; range in *P* values was 0.1099 to 0.9049). Only in one case, *T. variabile* at 27°C on PET-PE inside surface, there were more males per arena than females ($t = -3.80$; df = 5; $P = 0.0127$). In each of the other treatments there were no differences in number of females and males (range in *t* values among treatments was -2.08 to 1.57; df = 5; range in *P* values was 0.0925 to 0.4341). The mean ± SE number of *T. castaneum* females on PET-PE packaging ranged from 3.8 ± 0.6 to 6.5 ± 0.9; female *T. variabile* ranged from 3.8 ± 0.3 to 5.7 ± 0.5 (Table 4.3). The number of female *T. castaneum* was not significantly different among treatment combinations ($F = 1.39$; df = 7, 40; $P = 0.2374$). There was a significant difference in the number of *T. variabile* females among treatments ($F = 2.95$; df = 7, 40; $P = 0.0137$). In order to

find which treatment combinations were different linear contrasts were used to compare treatment combinations pairwise (SAS Institute, 2008). The treatment combinations that showed significant differences in number of females per disc are shown in Table 4.4. At 27°C, the number of females on untreated outside surface was not significantly different among all pairwise comparisons (*F*, range among pairwise comparisons = 0.02 to 2.80; df = 1, 40; range in *P* values was 0.1022 to 0.8888). The number of females on the outside methoprene treated surface at 27°C was not significantly different from those found among all pairwise comparisons (*F*, range among pairwise comparisons = 0.02 to 2.58; df = 1, 40; range in *P* values was 0.1158 to 0.8888). The number of females found on the inside and outside methoprene surfaces were not significantly different from those found at 32° (*F* = 4.03; df = 1, 40; *P* = 0.0514). At 27°C, the number of female on the inside surface of packaging that was untreated was not significantly different from females on both the methoprene-treated surface at 27°C and the outside surface at 32°C (*F*, range between pairwise comparisons = 0.11 to 1.02; df = 1, 40; range in *P* values was 0.3178 to 0.7434). The number of females on the outside untreated surface at 32°C was not significantly different from those on the untreated inside surface at 32°C and the inside treated surface at 32°C (*F*, range between pairwise comparisons = 0.00 to 0.04; df = 1, 40; range in *P* values was 0.8380 to 0.9702). The number of females on the inside untreated surface at 32°C was not significantly different from those found on methoprene-treated surface at 32°C (*F* = 0.03, df = 1, 40; *P* = 0.8673). Additionally, numbers of females on the methoprene-treated inside surface at 27 and 32°C were not significantly different from one another (*F* = 0.86; df = 1, 40; *P* = 0.3603).

The mean ± SE number of eggs laid per female for *T. castaneum* ranged from 3.1 ± 0.8 to 6.8 ± 1.0, and for *T. variabile* range from 27.1 ± 4.5 to 44.8 ± 6.3 (Table 4.3). Although one-

way ANOVA showed differences among treatment combinations in the number of eggs laid per *T. castaneum* female on PET-PE (F = 2.55; df = 7, 40; P = 0.0286), only the inside surface of methoprene treatment at 32°C was significantly different ($P < 0.05$) from the outside surface control treatment at 32°C. There were no differences in the number of eggs laid by *T. castaneum* female among the other treatment combinations. On methoprene-treated surface at 32°C females laid the fewest number of eggs compared with the other treatment combinations. There was no significant differences in the number of eggs laid by *T. variabile* females among the treatment combinations (F = 1.30; df = 7, 40; P = 0.2734). The percent egg hatch of eggs among treatment combinations were not significant for both *T. castaneum* (F = 0.76; df = 7, 40; P = 0.0667) and *T. variabile* (F = 2.09; df = 7, 40; P = 0.6256).

4.4.3. Effect of methoprene on development from egg to adult

Paired *t*-tests on the initial adults added to lay eggs, showed no differences in number of females and males on untreated and methoprene-treated PE-PE packaging for *T. castaneum* (range in t values among treatments was = -1.27 to 0.81; df = 5; range in P values was 0.2586 to 1.000). The number of initial adults added to facilitate egg laying for *T. variabile* was not significantly different between untreated and methoprene-treated packaging (range in t values among treatments was = -1.00 to 1.17; df = 5; P, range = 0.2956 to 0.9083). The mean ± SE number of *T. castaneum* females on PE-PE packaging ranged from 4.5 ± 0.6 to 5.3 ± 0.3, and that of female *T. variabile* ranged from 4.7 ± 0.4 to 5.5 ± 0.5 (Table 4.5). The number of females for both species were not significantly different among all treatment combinations (F, range between species = 0.35-0.36; df = 7, 40; P, range = 0.9204-0.9259).

The continual exposure to the PE-PE methoprene packaging from egg to adult on had a significant effect on normal adult *T. castaneum* emergence (F = 332.4; df = 7, 40; $P < 0.0001$).

No normal adults emerged in methoprene treatments, irrespective of the temperature and surfaces tested (Table 4.5). These results indicate a 100% reduction in adult emergence when eggs laid by adults are exposed to the PE-PE methoprene packaging. Consequentially, the number of deformities was significantly different between methoprene and control treatments (F =25.76; df = 7, 40; P <0.0001), and the number of deformed adults was essentially similar in methoprene treatments at the two temperatures and on the inside and outside surfaces. The deformities seen on the methoprene surfaces included supernumerary larvae and pupae-adult intermediates.

The number of normal adults of *T. variabile* that emerged was significantly different among the treatment combinations (F = 75.26; df = 7, 40; P < 0.0001). The number of normal adults on the outside surfaces of the PE-PE methoprene packages at both temperatures was significantly lower than all other treatments (Table 4.5). Compared to the control these treatment combinations had a 99.9% reduction in adult emergence. There was a 47.3% reduction in adult emergence between the inside surface of the methoprene-treated packaging at 32°C compared with the untreated inside surface of the packaging. This treatment also was significantly different when compared outside surface of methoprene treatments at 27 and 32°C. Only the inside surface in methoprene treatments at 27 and 32°C had significantly greater deformed adult emergence compared with other treatment combinations. The deformities seen with *T. variabile* on methoprene packaging consisted of pupal-adult intermediates, and such individuals failed to emerge as viable adults.

On PET-PE packaging tests, a paired *t*-test indicated a significant difference between the number of female and male *T. castaneum* initially placed on the packaging materials to facilitate egg laying for the inside methoprene-treated surface treatment at 27°C (t = 5.65; df = 5; P =

0.0024). Difference in the number of *T. castaneum* females and males for each of the other treatments was not significant (range in *t* values among treatments was = -2.50to 1.20; df = 5; range in *P* values was 0.0545 to 1.0000). A paired *t*-test between *T. variabile* females and males initially placed on the untreated and methoprene-treated packaging was not significantly different (range in *t* values among treatments was = -2.42 to 1.17; df = 5; range in *P* values was 0.0599 to 1.0000). Female counts on PET-PE packaging for *T. castaneum* ranged from 3.3 ± 0.7 to 6.8 ± 0.5, and female *T. variabile* ranged from 4.2 ± 0.3 to 5.8 ± 0.6 (Table 4.6). One-way ANOVA showed no significant differences in number of females among all treatment combinations for *T. castaneum* ($F = 1.90$; df = 7, 40; $P = 0.0951$) and *T. variabile* ($F = 0.83$; df = 7, 40; $P = 0.5651$).

The emergence of normal adults of *T. castaneum* on the PET-PE packaging varied significantly among treatment combinations ($F = 128.00$; df = 7, 40; $P < 0.0001$). The inside surface of the methoprene treatment had no normal adult emergence at both temperatures. This was the only treatment which had a 100% reduction in emergence of normal adults, and pupal-adult intermediates were found in these treatments. On the outside surface of the PET-PE methoprene packaging at both temperatures, the number of normal adults that emerged was 22 and 50% less than the corresponding control treatments. The number of deformed adults of *T. castaneum* that emerged was significantly different among treatments ($F = 15.9$; df = 7, 40; $P <0.001$). The inside surface of the methoprene packaging had significantly more deformities compared to all other treatments.

The inside surface of the PET-PE methoprene packaging had a significant effect on the number of normal adults of *T. variabile* that emerged ($F = 29.21$; df = 7, 40; $P < 0.0001$). The deformities seen on the methoprene packages predominantly consisted of pupae-adult

intermediates which failed to emerge as an adult. In methoprene treatments where the inside surface was exposed to eggs at 27 and 32°C, there was a 87.1 and 96.8% reduction in adult emergence, respectively, when compared with emergence in the corresponding control treatments. These two treatments were significantly different from all other treatments. The number of deformed adults varied among the treatment combinations ($F = 5.81$; df = 7, 40; $P < 0.0001$). The inside surface of the methoprene packaging at 32°C and the outside methoprene packaging at 27°C had the most adult deformities.

4.5. Discussion

Klein and Burkholder (1984) found a relationship between egg mortality and egg age when *T. glabrum* eggs were exposed to cardboard treated with methoprene at 100 µg/cm^2. They found that the viability of *T. glabrum* eggs was reduced by 83% when eggs were exposed during the first day after oviposition, but when 5-6 day old eggs were exposed there was no effect on hatch (Klein and Burkholder, 1984). In our study, exposure of *T. variabile* eggs to methoprene did not adversely affect egg hatch. Consistently, low egg hatch was observed when T. castaneum eggs were exposed to methoprene-treated packaging. The eggs of *T. castaneum* in this study were 3-4 days old, which is consistent with Klein and Burkholder (1984) study, and this may explain lack of adverse effects on hatch in methoprene treatments. In another test, *T. variabile* and *T. castaneum* adults were exposed to arenas for 3 and 7 d, respectively, before they were removed and eggs transferred to untreated arenas. This test was done to determine if short term exposure to methoprene treatments would result in reduced egg laying by *T. castaneum* and *T. variabile*. The short term exposure to methoprene did not adversely affect the number of eggs laid by females of both species and the egg hatch. Additionally, hatch of the eggs among treatment combinations in tests with PE-PE and PET-PE packaging was essentially similar.

Although the eggs laid were exposed for the first developmental days before transfer to untreated arenas, the duration of exposure may not have been long enough to have a significant effect on percent hatch. In addition, insect diet was provided for adults and studies have shown that a decrease in effectiveness of treated medium may be due to the interaction of methoprene and the diet (Klein and Burkholder, 1984). The observed changes in percent hatch also could be due to physical handling of the eggs, during sifting, counting, and transferring to untreated dishes, and not due to methoprene or differences in number of females on surfaces and number of eggs laid by females. The last two variables were generally not different among the treatment combinations. Observations on egg hatch untreated and methoprene treated surfaces, as opposed to adults moving to untreated surfaces before laying eggs, may have shown adverse effects of methoprene on egg hatch.

Loschiavo (1976) found that *T. castaneum* larvae failed to complete development and emerge as normal adults on methoprene-treated diet, and also reported that *T. castaneum* failed to produce eggs when exposed to diet treated with 5, 10, and 20 ppm of methoprene. Additionally, Loschiavo (1976) found that the duration of larval development to pupae was delayed in diet treated with 10 ppm methoprene by 5 days when compared to larval development in the control treatment. In male and female *T. castaneum* pairs that emerged in methoprene-treated diet, no eggs were produced at concentrations of 5, 10, and 20 ppm indicating gonadotropic effects of methoprene (Loschiavo, 1976). However, *T. castaneum* produced 15 eggs/female/day in untreated food and 8 eggs/female/day in 1 ppm treated food which indicated higher levels of methoprene are needed to adversely affect egg production and/or egg laying (Loschiavo, 1976). In our study we observed no significant differences in the number of eggs laid by *T. castaneum* or *T. variabile* females exposed to PE-PE methoprene surfaces when

compared with untreated surfaces. Adults of *T. variabile* exposed to PET-PE packaging also did not show decreased egg laying. The number of *T. castaneum* eggs laid on PET-PE packaging was only significantly different between the outside control surface and the inside treated surface at 32°C, but all other treatments were not significantly different from one another. The results of our study differ from those presented by Loschiavo (1976). In our study, the main mode of action is the diffusion of the methoprene through the polymer layers, whereas Loschiavo (1976) directly incorporated methoprene into a food source.

In general, juvenile hormones analog structures consist of an unsaturated lipophilic backbone with polar substituents at both ends (Mondal and Parween, 2000). The chain length of the lipophilic backbone can vary and the optimum chain length varies between species (Mondal and Parween, 2000). The chemical structure of methoprene, and other juvenile hormone analogs, can vary. Some insects can respond to a wide variety of structures, while others will only respond to a specific analog (Henrick, 2007). *T. castaneum* is more susceptible to methoprene-impregnated packaging compared to *T. variabile.* The structure of the methoprene used in the packaging may be a factor as to why *T. castaneum* is more susceptible. The methoprene structure contains a chiral center at the C-7 carbon atom, which leads to r and s-enantiomers. The r-enantiomer is an inert diluent and the s-enantiomer is the active isomer, and is why s-methoprene is used in insecticide formulations (Henrick, 2007). However, the natural form of methoprene does not contain a chiral center (Henrick, 2007). This could explain the lack of effect on *T. variabile. T. variabile* may be an insect that responds to a specific structure, compared to *T. castaneum* which will respond to a variety of structures. Manzelli (1982) used a racemic mixture of methoprene, and applied it to long term storage of tobacco. This racemic

mixture was effective on both *L. serricone* and *E. elutella*. It is possible that *T. variabile* could have a stronger response to a racemic mixture of methoprene instead of the s-methoprene only.

On PE-PE packaging containing methoprene, irrespective of surfaces and temperatures tested, no *T. castaneum* adults emerged from the eggs laid by females. However, such an adverse effect with *T. variabile* was observed only on inside surfaces of methoprene treated PE-PE packaging only at 27°C. There may be a difference in the thickness between the inner and outside layer in the PE-PE packaging, and this may be a plausible explanation for lack of adverse effects on *T. variabile*. On PET-PE packaging, 100% reduction in normal adult emergence was seen on inside surface in methoprene treatments at both temperatures with *T. castaneum* and <87.1 reduction was seen with *T. variabile*. The results with *T. variabile* normal adult emergence on the inside surfaces PET-PE packaging in methoprene treatments are at variance from PE-PE packaging inside surface. In the PET-PE packaging, the PET layer is on the outside. The PET layer is four times as thick (50 μm) compared with the inside PE layer (12.5 μm). Methoprene offers some volatility which is why it can be incorporated into packaging materials (Henrick, 2007). Comparing the two polymers, PET polymer has a lower oxygen and carbon dioxide transmission rates compared with PE polymers. Though the methoprene is not an emitter of these two gases, it can be implied that the transmission rate of the volatile methoprene will also be inhibited strongly by the PET layer versus the PE layer. This may explain the significant difference observed in normal adult emergence between the inside and outside surfaces of the methoprene treated packaging. Comparing the structures of PET and PE, the PET polymer contains a cyclic ring capped with esters while the PE polymer is a repeating unit of ethylene. The methoprene in this packaging was impregnated within the adhesive layer between the two polymer layers. Therefore the release of the methoprene onto the packaging surfaces occurs due

to diffusion through the polymer layers. We hypothesize that the two esters groups and cyclic ring in the PET polymer structure provides static hindrance to the methoprene from diffusing through. In contrast, the simplicity of the repeating ethylene structure provides the smallest amount of static hindrance to the methoprene molecule, thus allowing the molecule to more easily diffuse through the PE layer. This may be a reason why the inside surface more effective against *T. variabile* and *T. castaneum.*

Methoprene does not have an acute effect on insects, but rather its use is designed for long term control of stored-product insects. In essence, the stored-product population will decrease over generations. Research has shown that long exposure to juvenile hormones, such as methoprene, on young larvae results in supernumerary larvae (Mondal and Parween, 2000). This effect would explain the supernumerary larvae seen on the PE-PE and PET-PE packaging on *T. castaneum*. This affect can be highly useful in the warehouse and retail setting, when products sit on the shelves for extended periods of time. This experiment has shown the effectiveness of methoprene impregnated polymer packaging against *T. castaneum* and *T. variable.* This type of packaging can be used as a preventive method to control for stored-product insects on a wide variety of products such as birdseed, cereal, granola bars, and flour.

4.6. References

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Table 4.1 Egg hatch of *T. castaneum* and *T. variabile* eggs exposed to methoprene-treated or untreated PE-PE and PET-PET packaging.

Surface	Temp. (°C)	Treatment	% Mean ± SE egg hatch[a]			
			PE-PE		PET-PE	
			T. castaneum	*T. variabile*	*T. castaneum*	*T. variabile*
Outside	27	Control	87.4±3.3a	61.5±7.1ab	93.2±2.1ab	80.4±3.4ab
		Methoprene	62.5±5.5ab	64.4±4.7ab	83.4±4.0bc	92.0±4.7a
	32	Control	81.1±2.9a	72.6±4.6ab	83.6±2.1bc	82.3±2.9ab
		Methoprene	49.6±6.8b	53.9±6.0b	82.3±1.5bc	92.3±3.4a
Inside	27	Control	84.5±3.8a	83.9±3.8a	98.0±0.9a	81.6±3.8ab
		Methoprene	65.2±3.6ab	77.4±4.4ab	71.1±3.4cd	88.4±5.9a
	32	Control	80.7±11.1a	75.1±4.0ab	82.3±3.3bc	66.3±3.1b
		Methoprene	66.2±3.7ab	55.3±5.4b	56.8±3.2d	81.7±5.0ab

[a]Means for each packaging type and species followed by different letters are significantly different ($P < 0.05$; by Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test).

Table 4.2 Effect of PE-PE methoprene packaging on fecundity and egg hatch of *T. castaneum* and *T. variabile.*

Surface	Temp. (°C)	Treatment	Mean ± SE[a]					
			T. castaneum			*T. variabile*		
			Number of females[b]	Number of eggs/female[c]	% Egg hatch	Number of females	Number of eggs/female[d]	% Egg hatch[e]
Outside	27	Control	5.2±0.7	8.5±0.4	62.7±4.3ab	5.3±0.2ab	39.8±3.4	86.3±2.1
		Methoprene	5.5±0.8	12.0±2.1	53.6±3.8b	5.5±0.6ab	39.5±7.7	82.7±1.7
	32	Control	4.8±0.5	9.5±1.2	63.7±4.1ab	3.7±0.6b	29.1±6.9	84.7±4.6
		Methoprene	4.2±0.5	8.7±1.9	53.4±4.5b	4.5±0.4ab	41.9±9.0	84.6±1.2
Inside	27	Control	5.2±0.9	7.1±0.3	64.5±2.9ab	5.2±0.3ab	37.2±2.1	80.6±3.1
		Methoprene	5.3±0.5	10.3±0.7	69.9±3.9a	4.5±0.3ab	46.5±6.1	84.3±2.9
	32	Control	6.0±0.6	8.4±1.3	56.5±1.7ab	4.3±0.4ab	54.1±20.5	84.4±2.6
		Methoprene	4.2±0.6	10.9±1.4	58.1±2.5ab	6.0±0.5a	30.7±6.9	80.1±3.9

[a]Means for each species and variable studied followed by different letters are significantly different ($P < 0.05$; by REGWQ multiple range test).

[b]There were no significant differences among treatment combinations ($F = 0.93$; df = 7, 40; $P = 0.4959$; by one-way ANOVA).

[c]There were no significant differences among treatment combinations ($F = 1.13$; df = 7, 40; $P = 0.3663$; by one-way ANOVA).

[d]There were no significant differences among treatment combinations ($F = 0.67$; df = 7, 40; $P = 0.6926$; by one-way ANOVA).

[e]There were no significant differences among the treatments ($F = 0.52$; df = 7, 39; $P = 0.8132$; by one-way ANOVA).

Table 4.3 Effect of PET-PE methoprene packaging on fecundity and egg hatch of *T. castaneum* and *T. variabile*

Surface	Temp. (°C)	Treatment	Mean ± SE[a]					
			T. castaneum			*T. variabile*		
			Number of females[b]	Number of eggs/female	% Egg hatch[c]	Number of females[d]	Number of eggs/female[e]	% Egg hatch[f]
Outside	27	Control	5.2±0.7	6.3±0.9ab	76.6±6.5	4.7±0.5	30.1±5.5	84.2±2.3
		Methoprene	4.5±0.8	6.8±1.0ab	76.4±3.2	4.7±0.3	31.2±3.7	85.1±4.0
	32	Control	4.8±0.8	7.7±1.5a	78.7±5.3	5.7±0.4	31.3±8.6	86.2±2.4
		Methoprene	5.5±0.5	5.1±0.7ab	78.8±9.1	4.3±0.4	44.8±6.3	93.6±1.4
Inside	27	Control	4.5±0.6	5.9±1.0ab	77.9±2.7	4.2±0.4	33.5±4.9	86.4±2.8
		Methoprene	3.8±0.6	4.7±0.8ab	67.5±8.3	3.8±0.3	27.1±4.5	87.0±0.9
	32	Control	6.5±0.9	4.9±0.7ab	71.7±7.5	5.7±0.5	30.9±6.6	89.7±4.0
		Methoprene	6.2±0.7	3.1±0.8b	59.7±8.3	5.5±0.3	44.5±4.6	93.1±1.4

[a]Means for each species and variable studies followed by different letters are significantly different ($P < 0.05$; by REGWQ multiple range test.
[b]There were no significant differences among the treatments ($F = 1.39$; df = 7, 40; $P = 0.2374$; by one-way ANOVA).
[c]There were no significant differences among the treatments ($F = 0.76$; df = 7, 40; $P = 0.6256$; by one-way ANOVA).
[d]There were significant differences among the treatments ($F = 2.95$; df = 7, 40; $P = 0.0137$; by one-way ANOVA), but the REGWQ multiple range test failed to show significant differences among treatment combinations. Significant differences were separated using linear contrasts (see Table 4).
[e]There were no significant differences among the treatments ($F = 1.30$; df = 7, 40; $P = 0.2734$; by one-way ANOVA).
[f]There were no significant differences among the treatments ($F = 2.09$; df = 7, 40; $P = 0.0667$; by one-way ANOVA).

Table 4.4 Linear contrasts showing significant pairwise comparisons of *T. variabile* female numbers on PET-PE packaging.

Treatments compared[a]	Mean Square	*F*-value[a]	*P*-value
Untreated inside surface at 27°C vs Untreated outside surface at 32°C	0.057	6.47	0.0149
Untreated inside surface at 27°C vs Untreated inside surface at 32°C	0.055	6.28	0.0164
Untreated inside surface at 27°C vs Methoprene-treated inside surface at 27°C	0.048	5.46	0.0246
Untreated outside surface at 32°C vs Methoprene-treated inside surface at 27°C	0.087	9.85	0.0032
Untreated outside surface at 32°C vs Methoprene-treated outside surface at 32°C	0.043	4.90	0.0326
Untreated inside surface at 32°C vs Methoprene-treated inside surface at 27°C	0.085	9.62	0.0035
Untreated inside surface at 32°C vs Methoprene-treated outside surface at 32°C	0.042	4.74	0.0354
Methoprene-treated inside surface at 27°C vs Methoprene-treated inside surface at 32°C	0.076	8.60	0.0055
Error	0.009		

[a]The degrees of freedom (df) for each pairwise comparison is 1, 40.

Table 4.5 Effect of PE-PE packaging on emergence of normal and deformed adults of *T. castaneum* and *T. variabile*

Surface	Temp. (°C)	Treatment	Mean ± SE[a]					
			T. castaneum			*T. variabile*		
			Number of females[b]	Normal adults	Deformed adults	Number of females[c]	Normal adults	Deformed adults
Outside	27	Control	5.3±0.3	23.5±2.7ab	1.8±0.7b	5.0±0.7	156.8±26.4ab	23.5±8.0c
		Methoprene	5.0±0.3	0.0±0.0c	19.8±4.6a	4.7±0.5	0.2±0.2c	116.3±23.3ab
	32	Control	4.5±0.6	33.5±5.5ab	3.3±2.4b	5.5±0.5	278.8±28.6a	11.0±3.4cd
		Methoprene	4.7±0.7	0.0±0.0c	27.2±3.8a	4.8±1.0	0.2±0.12c	207.8±29.3a
Inside	27	Control	4.7±0.8	27.0±4.2b	1.8±0.5b	5.2±0.3	145.8±18.1ab	19.7±10.1cd
		Methoprene	4.7±0.7	0.0±0.0c	19.5±4.0a	5.2±0.2	209.0±8.6a	29.8±6.3bc
	32	Control	4.5±0.6	37.2±5.0a	0.3±0.2b	4.8±0.6	202.8±38.3a	4.5±2.7d
		Methoprene	4.7±0.6	0.0±0.0c	16.3±3.5a	4.7±0.4	106.8±37.7b	30.7±9.7bc

[a]Means for each packaging type, species, and variable studied followed by different letters are significantly different ($P < 0.05$; by REGWQ multiple range test).

[b]There were no significant differences among treatment combinations ($F = 0.35$; df = 7, 40; $P = 0.9259$; by one-way ANOVA).

[c]There were no significant differences among treatment combinations ($F = 0.36$; df = 7, 40; $P = 0.9204$; by one-way ANOVA).

Table 4.6 Effect of PET-PE packaging emergence of normal and deformed adults of *T. castaneum* and *T. variabile.*

Surface	Temp. (°C)	Treatment	Mean ± SE[a]					
			T. castaneum			*T. variabile*		
			Number of females[b]	Normal adults	Deformed adults	Number of females[c]	Normal adults	Deformed adults
Outside	27	Control	5.2±0.9ab	38.5±5.0a	2.5±1.5b	5.3±0.5	181.8±25.8a	50.0±15.4abc
		Methoprene	3.3±0.7ab	19.3±2.7cd	1.2±0.6b	4.2±0.3	115.8±16.3a	7.7±3.0c
	32	Control	5.0±0.8ab	21.3±3.5bcd	1.8±0.7b	5.2±0.7	181.8±25.8a	15.0±3.8bc
		Methoprene	4.8±0.8ab	16.7±3.1d	4.2±1.9b	5.0±0.3	192.0±12.9a	20.8±14.4c
Inside	27	Control	4.7±0.7ab	34.2±5.0ab	3.8±1.1b	5.7±0.7	172.3±28.1a	15.2±7.7b
		Methoprene	6.8±0.5a	0.0±0.0e	41.0±6.5a	5.8±0.6	22.2±21.8b	127.3±33.1ab
	32	Control	5.7±0.6ab	29.3±2.7abc	3.2±1.0b	5.5±0.4	157.7±15.7a	6.3±2.2c
		Methoprene	5.2±0.5ab	0.0±0.0e	25.0±3.6a	4.7±0.7	5.0±1.0b	148.0±28.9a

[a]Means for each packaging type, species, and variable studied followed by different letters are significantly different ($P < 0.05$; by REGWQ multiple range test).

[b]There were no significant differences among the treatments ($F = 1.90$; df = 7, 40; $P = 0.0951$; by one-way ANOVA).

[c]There were no significant differences among the treatments ($F = 0.83$; df = 7, 40; $P = 0.5651$; by one-way ANOVA).

Chapter 5 - Penetration ability of *Plodia interpunctella* and *Trogoderma variabile* on methoprene impregnated foil packages

5.1. Abstract

Retail stores and food warehouse are prime locations for stored-product insect infestation to occur. The objective of this study was to determine the effect of methoprene-treated foil packaging on egg hatch and penetration ability of first and third instars of *Tribolium variabile* Ballion, warehouse beetle, and *Plodia interpunctella* (Hübner), Indian meal moth. Untreated and methoprene-treated packaging at 0.1, 0.25, and 0.5% active were fitted into 9 cm diameter semicircles and 25 eggs of either species were added to plates, monitored for 7 d, to determine percent egg hatch. A 6 cm by 8 cm foil food packages were created using a heat sealer, and placed into 0.18-L plastic vials. First instar or third instar larvae were introduced into each vial either containing 500 mg diet or no diet in order to determine penetration ability and development of each species at 21 or 42 d exposure periods. Additionally, foil packages were pierced with pinholes, 500 mg diet added, and first instar *T. variable* or *P. interpunctella* were introduced to determine if either species could invade the food packages. The foil packaging had no significant effect on egg hatch of either species when placed on the methoprene-treated foil or on untreated Petri plates. *T. variabile* were unable to penetrate any foil packages. *P. interpunctella* penetrated all packaging containing pinholes. Methoprene-treated foil packages adversely affected *T. variabile* development, when held with diet. Deformed pupae and adults were observed at all levels of methoprene-treated packaging. The methoprene-treated packaging reduced the adult emergence of *P. interpunctella.*

5.2. Introduction

Stored-product insects are a common and persistent problem in processing facilities, warehouses, distribution centers, retail stores, and consumer pantries (Highland, 1978). *Tribolium castaneum* (Herbst), red flour beetle, *Trogoderma variabile* Ballion, warehouse beetle, and *Plodia interpunctella* (Hübner), Indian meal moth, are three serious stored-product insects found in these types of locations and throughout the world (Highland, 1978; Campbell et al., 2002; Arthur et al., 2014). Infestations in food facilities result from established insect populations within the facility, immigration of insects from the outside environment, or bringing infested products inside the facility (Campbell and Arbogast, 2004). *T. castaneum*, *T. variabile*, and *P. interpunctella* are all highly mobile insects. *P. interpunctella* are capable of traveling large distances, while Campbell et al. (2002) stated *T. variabile* was capable of moving across multiple floors and distances ranging from 7-216 m throughout a warehouse.

Arthur et al. (2014) monitored a commercial food storage facility over three years using pheromone traps, and *T. castaneum* and *T. variabile* were among the most common insect species captured, along with *Lasioderma serricorne* (F.), the cigarette beetle, and *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle. Arthur et al. (2014) also noted capture of these species in a large room where no food was stored, but was adjacent to a food storage area with access through open bay doors. *P. interpunctella* are commonly captured around doors to the outside, pallet wrapping equipment and near where food products are stored (Campbell et al., 2002; Arthur et al., 2013). Campbell et al., (2002) found an infestation of *T. variabile* near a conveyer system that carried packaged food products. Additional inspection revealed larval cast skins present in the conveyor system (Campbell et al., 2002). Insect captures tend to be temporally and spatially variabile (Campbell et al., 2002; Arthur et al., 2014). Arthur et al.

(2014) observed insect densities varied according to the calendar months, and noted very little trap cultures from November to May, which corresponds to winter months in the central United States. However, trap catches increased during the warmer months. Changes in abundance of insect captures 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, open doors and windows, and incoming products that are already infested are common locations for infestation sites (Subramanyam et al., 2001). However, it is difficult to pinpoint a specific location of an infestation source in a warehouse or retail setting, because of the constant movement of food products (Arthur et al., 2013).

Infestation by stored-product insects result in product losses, decreased consumer confidence, and potential risk for allergic reactions (Subramanyam et al., 2001). Commonly used pest management techniques in warehouse 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 traps (Subramanyam et al., 2001). However, this may not be enough for adequate insect control. The use of insect resistant packaging is becoming increasingly prevalent in stored-product packaging.

Exposure of insects to methoprene treated contact surfaces can result in insect deformities such as deformed adult genitalia, reduced egg production, incomplete scleorotization of adult legs and antennae, and inability to complete larval-pupal transformation (Klein and Burkholder, 1984). *T. variabile* is capable of undergoing multiple larval stages and molts. A potential problem with using an IGR, is the continuation of the larval stage (Klein and Burkholder, 1984). The objective of this study was to determine the effect of methoprene-treated foil packaging on

egg hatch and penetration and invasion ability of first or third instars of *T. variabile* and *P. interpunctella.*

5.3. Materials and methods

5.3.1. Insects

P. interpunctella and *T. variabile* species used in this study were obtained from laboratory cultures at the United States Department of Agriculture's Center for Grain and Animal Health Research (USDA-CGAHR) in Manhattan, Kansas. *P. interpunctella* cultures used in all experiments were derived from a laboratory strain established in 1969. *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 acid (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 50% Purina One lamb and rice formula (Nestlé Purina PetCare Company, St. Louis, MO, USA), 50% Pharmanex vanilla shake mix (Arizona Nutritional Supplements, Chandler, AZ, USA), and the top of the culture was sprinkled with 100% whole grain rolled oats

5.3.2. Properties of packaging films

Foil packages, 6 cm by 8 cm, were constructed using a 15 mm portable hand sealer (Model KF-150CST, Global Industrial Marketplace, Northridge, CA, USA). Foil packages were heat sealed on all four sides using a 1.5 cm ridged seal. Prior to sealing, respective diet was added to each packaging. *P. interpunctella* diet used consisted of poultry mash, glycerol, honey, and distilled water (Subramanyam and Cutkomp, 1987). *T. variabile* diet used ground cat food (Meow Mix, Big Heart Pet Brands, Decatur, AL, USA). Packages were filled with 3.07 ± 0.06 g of diet for *P. interpunctella* and 2.41 ± 0.05 g of diet for *T. variabile*. All packages were evaluated for seam integrity prior to use in all tests. Foil packages were added to 0.18-L plastic

vials and capped with filter paper and a sealed lid which contained a hole covered by wire mesh to allow passage of air.

5.3.3. Effect of methoprene on egg hatch of P. interpunctella and T. variabile

P. interpunctella eggs were obtained by collecting pupae from rearing jars. Corrugated paper spools were added to the *P. interpunctella* diet to provide pupation sites for wandering larvae (Huang et al., 2003). Pupae were collected from the paper spools and transferred into 1.9-L glass jars fitted with a mesh screen and filter paper. Glass jars were inverted and moths were allowed to mate to oviposit. Eggs, 0-24 h old, were collected and counted using an aspirator with a small glass collection jar.

To obtain *T. variabile* eggs, approximately 100 g of flour was sifted through a 850 µm opening sieve (Newark Wire Cloth Company, Clifton, N.J., USA), placed into a 0.18-L jelly jars (Ball, Muncie, IN, USA) and 50 unsexed *T. variabile* adults of mixed ages were introduced to the jar. The containers were held in an environmental growth chamber at 30°C and 65% r.h. and 16:8 L:D photoperiod. After three to four days, adults were removed from jars using a sieve with 250 µm opening. The flour was sifted through a 149 µm opening and the eggs were retained on top of the sieve. Eggs were then counted and collected using an aspirator with a small glass collection jar.

Forty individual arenas were prepared for this study. From the foil packaging 9 cm diameter semicircles were cut by hand from all packaging materials containing methoprene and those that did not. Out of the 40 discs, 20 were for *P. interpunctella* and 20 were for *T. variabile.* The semicircle discs were secured to the divided Petri plate with adhesive caulking (DAP Products Inc, Baltimore, MD, USA). The inner sides and the divider of Petri dishes were coated with polytetrafluoroethylene (Fluon®) (Sigma-Aldrich Co., St. Louis, MO, USA) to

create the final sample arena. The treatment combinations included untreated and methoprene-treated foil packaging at three levels, 0.1, 0.25, and 0.5% active, eggs exposed to foil surfaces and those that were not, and the two species. All experiments were conducted on the outside surface of the material at 27°C and 60% r.h. Each combination was replicated five times.

To determine the effect of methoprene on the egg hatch from eggs, 25 eggs of *P. interpunctella* (0-24 d old) or *T. variabile* (3-4 d old) were added to the sample arenas either on the foil side or the non-foil side. The number of larvae that emerged for each treatment combination and species was recorded and the percent egg hatch was determined.

Egg hatch *P. interpunctella* and *T. variabile* were determined according to modified procedures described by Huang and Subramanyam (2003). Three replicates of 50 eggs of each *P. interpunctella* and *T. variabile* were collected via aspirator and placed into glass Petri plates, as described previously. Petri plates containing eggs, were placed at 27°C and 60% r.h. and examined after 7 d. The average egg hatch out of the total number of eggs (50) was calculated (Huang and Subramanyam, 2003). The mean ± SE egg hatch was 45.3 ± 7.0% for *P. interpunctella* and 80.7 ± 1.8% for *T. variabile*.

5.3.4. Effect of methoprene on penetration ability of first instar P. interpunctella and T. variabile

Foil packages were created as described previously, 6 cm by 8 cm, and placed into 0.18-L plastic vials, and covered with filter paper and a lid which contained a hole covered with wire mesh. Twelve vials contained approximately 500 mg of diet, chicken mash for *P. interpunctella* (Subramanyam and Cutkomp, 1987) or cat food for *T. variabile*, and 12 vials did not contain any diet. Treatment combinations included two species, diet and no diet, one control and three methoprene-treatment levels (0.1, 0.25, and 0.5%), 21 and 42 d exposure intervals. All treatment

combinations contained 6 replicates. This was a total of 96 vials per treatment combination per species.

The methods used for this study were modified from those used in Chapter 2 of this dissertation. Fifty eggs of *P. interpunctella* or *T. variabile* were collected using an aspirator with a glass collection vial, and were added to individual vials containing a single foil package. Vials contained either 500 mg of diet or contained no diet. The eggs of *P. interpunctella* and *T. variabile* were used to represent first instars of each species, because first instars could be injured or damaged during transfer due to handling. Egg hatch was determined as described previously (Huang and Subramanyam, 2003). The mean ± SE (n = 3) egg hatch for eggs added to vials containing food was 50.0±2.3% for *P. interpunctella* and 88.0±1.2% for *T. variabile.* The mean ± SE (n = 3) egg hatch for eggs added to vials containing no food was 41.3±6.4% for *P. interpunctella* and 76.7±4.4% for *T. variabile.* All vials were placed into a growth chambers at 27°C and 60% r.h. for 21 or 42 d exposure periods.. Vials were examined at 21 to count the number of larvae and pupae, and at 42 d to count the number of larvae, pupae, and adults found inside and outside of the foil packaging. Foil packages were assessed for number and diameter of holes (mm) present at both observational times.

5.3.5. Effect of methoprene on invasion ability of first instar P. interpunctella and T. variabile through artificially created pinholes.

The invasion ability of first instar *P. interpunctella* and *T. variabile* were assessed by using foil packages, described previously, manually punctured with a 150 µm pinhole at one of three locations; bottom third, middle third, or top third, of the packaging surface on one side. Packages contain pinholes were placed inside of a 0.18-L plastic vial and approximately 500 mg of respective diet was added. Fifty eggs of either species was added to vials and placed inside a

growth chamber at 27°C and 60% r.h. for 21 of 42 d. Treatment combinations included two species, one control and three treatment levels (0.1, 0.25, 0.5%), 21 and 42 d exposure levels, and all treatment combinations were replicated six times.

The eggs of *P. interpunctella* and *T. variabile* were used to represent first instars of each species. Egg hatch was determined as described previously and the mean (±SE, n = 3) egg hatch for *P. interpunctella* was 44.0±4.2% and for *T. variabile* 90.0±3.5%. The vials were examined at 21 and at 42 d to count the number of larvae, pupae, and adults found inside and outside of the foil packaging. Foil packages were also overserved for number and diameter of holes (mm) present at both observational times.

5.3.6. Effect of methoprene on penetration ability of third instar P. interpunctella and T. variabile.

Third instars were used in this study base off the results seen by Chapter 2 of this dissertation, in which third instars have a higher propensity to penetrate foil based packaging compared to first instars. Third instar *P. interpunctella* were determined by measuring mean head capsule width, 0.66 mm (Allotey and Goswami, 1990). Third instar *T. variabile* was obtained by measuring head capsule width under stereomicroscope. The mean head capsule width for third instars ranged from 0.31-0.37 mm (Rai, 2014). Ten *P. interpunctella* or 20 *T. variable* larvae were added to vials containing a single packaging type, with 500mg of diet or no diet added, and held at 27°C and 60% r.h. for 21 or 42 d. Vials were examined after 21 or 42 d for larvae, pupae, or adults that emerged. Foil packages were observed for diameter (mm) and number of holes present.

5.3.7. Data analysis

All experiments were run as a completely random design. Data were analyzed by package type and species. The means and standard errors were calculated and reported (SAS Institute, 2008). The mean egg hatch between non-foil and foil exposed surfaces were compared using a paired *t*-test (SAS Institute, 2008) to determine if significant differences existed between the two surfaces. The percent egg hatch data were transformed to angular values and analyzed using a one–way analysis of variance (ANOVA) (Zar, 1984). The number of larvae, pupae, and adults found inside or outside of the foil packaging was transformed to $\log_{10}(x+1)$ scale for further analysis. Data obtained for the number and diameter of holes were not transformed. All data collected was subjected to an analysis of variance (ANOVA) by species and observation time to determine significant differences. If the ANOVA indicated significant differences, the variables among treatment combinations were separated using a Tukey's adjustment and significance was determined at $\alpha = 0.05$ (SAS Institute, 2008).

5.4. Results

5.4.1. Effect of methoprene on egg hatch of P. interpunctella and T. variabile

The packaging film had little effect on the egg hatch of *T. variabile*. The mean egg hatch on untreated surfaces exposed to foil was 85.6 ± 3.7% and exposed on the non-foil side was 93.6 ± 2.7% (Table 5.1). The mean egg hatch of *T. variabile* on exposed on the no-foil side of the methoprene-treated samples ranged from 88.0 ± 3.6 to 93.6 ± 2.4%. Egg hatch from direct exposure to the methoprene-treated foil ranged from 80.0 ± 2.9 to 84.8 ± 5.6%. In all instances the 0.5% treated foil had the lowest percent egg hatch. A paired *t*-test between foil and non-foil surfaces was not significant for any treatment (range in *t* values among treatments was 1.14 to 2.59; df = 4; range in *P* values was 0.3188 to 0.0608). Similar to *P. interpunctella*, the untreated

surface had a lower egg hatch of eggs directly on the foil side versus the non-foil side, 85.6 and 93.6%, respectively. Additionally, the egg hatch was lower on the methoprene-treated foil compared to the control. The 0.5% methoprene foil had the lowest percent egg hatch among all treatments at 80.0±2.9%, which was a 14.5% reduction from eggs exposed to the non-foil side of the untreated arena.

The mean egg hatch for *P. interpunctella on* untreated surfaces was 36% on the non-foil side and 22.4% on the foil side. The egg hatch on the non-foil side for treatment arenas ranged from 30.4 to 29.6%, with the 0.25% methoprene-treated foil having the lowest percent emergence (Table 5.1). On the foil side, the 0.5% methoprene-treated surface had the lowest egg hatch, 20.8%. A paired *t*-test between the non-foil and foil sides showed no significant differences between pairs (range in *t* values among treatments was 0.10 to 2.45; df = 4; range in *P* values was 0.9273 to 0.0705). *P. interpunctella* on the non-foil side was lower for all the methoprene-treated surfaces compared to the control. This could indicate that the volatility of the methoprene has an effect on egg hatch, even without direct contact on the methoprene-treated packaging material. However, only the 0.5% treated packaged had a lower percent egg hatch compared to the untreated packaging when eggs were exposed directly to the foil. The difference in egg hatch was not significant and the differences observed could be related to do experimenter handling and transfer of the eggs. Comparing egg hatch of *P. interpunctella* eggs exposed to the non-foil side compared to the foil side, the eggs exposed to the foil side had a lower egg hatch rate. This indicates that the direct exposure to the methoprene-treated foil material had an effect on egg hatch.

5.4.2. Effect of methoprene on penetration ability of first instars of P. interpunctella and T. variabile

T. variabile larvae were unable to penetration any packaging, untreated or methoprene-treated, after 21 or 42 exposure periods when held with or without diet. There was no significant difference in the number of larvae found in vials after 21 d (F =0.43; df = 3, 20; P = 0.7357) or 42 d exposure (F = 1.19; df = 3, 20; P = 0.1519) when held with 500 mg of diet. In 21 d vials, the number of larvae ranged from 23.5 ± 2.7 to 28.5 ± 3.8 between all treatments and in 42 d vials larvae ranged from 14.2 ± 3.0 to 22.5 ± 2.2. In 21 d vials, only larvae were found. However in 42 d vials, all four life stages were observed in the untreated packages held with diet. All packaging types contained pupae (4.7 ± 0.8 to 1.3 ± 0.4) but counts were not significantly different between treatments (F = 3.09; df = 3, 20; P = 0.0505). Deformed half pupal-adult intermediates were observed in the methoprene-treated packages but not in the untreated packages containing food. Similarly, deformed adults were also only seen in the methoprene-treated packages at all treatment rates but not in the control. Deformities observes consisted of white-translucent antennae and legs of the adult beetle and half pupae-adults, as described in Chapters 3 and 4. The untreated packaging contained a mean of 3.5±0.6 morphogenically normal adults and the methoprene-treated packages contained zero normal adults. In vials without diet containing *T. variabile,* there was not a significant difference in the mean number of larvae found per treatment vial at 21 or 42 d exposure periods (range in F values was 1.27 to 2.38; df = 3, 20; range in P values was 0.1002 to 0.3117). After 42 d, untreated or methoprene-treated packaging vials did not containing any pupae or adults. Additionally, the mean number of larvae found per vial decrease between 21 and 42 d exposure periods. The decline in mean larvae could be due to cannibalistic effects of the larvae due to the lack of diet provided.

After 21 d exposure to packages which contained 500 mg diet there was no significant difference in the mean number of larvae per treatment combination of *P. interpunctella* (F = 1.53; df = 3, 20; P = 0.2386), and mean larvae ranged from 6.0 ± 1.3 to 10.0 ± 2.3. The same result was observed for *P. interpunctella* after 42 d exposure (F = 0.51; df = 3, 20; P = 0.6786). The untreated packages at 21 and 42 d contained pupae, 2.3 ± 1.2 and 1.2 ± 0.6, respectively. *P. interpunctella* adults were observed in the untreated package vials only. At 21 d three packages of 0.1% methoprene were penetrated by larvae. The mean diameter of the holes was 5.3 ± 0.8 mm and the mean larvae inside the package were 15.2 ± 6.7. The packages were observed under stereomicroscope and each penetration point was along the bottom seam of the sealed packaged, and the chewing pattern was in a linear fashion. Additionally, the penetration points display stress lines which result from an imperfect seal and stress on the package. The penetration holes seen in Chapter 2, were circular in appearance. Therefore the penetration seen at 21 d will be regarded as a seam/seal failure. In a similar fashion, 0.1% methoprene-treated packaging at 42 had one instance of penetration. There were no larvae present inside the package and the point of penetration was linear in fashion. Packaging samples containing no diet at 21 d only produced larvae for untreated and methoprene-treated packages. The mean larval density ranged from 12.2 ± 1.5 to 15.0 ± 1.7 over all treatment combinations. There was no significant difference between treatments (F = 0.64; df = 3, 20; P = 0.5969). Likewise packages held for 42 d without food only produced larvae of *P. interpunctella* which ranged from 14.0 ± 2.3 to 17.2 ± 1.8.

5.4.3. Effect of methoprene on invasion ability of first instar P. interpunctella and T. variabile through pinholes.

When presented with pinholes in three different locations, *T. variabile* was unable to invade any package type. There were no significant differences in the number of larvae found in

untreated and methoprene-treated vials after 21 or 42 d exposures (range in *F* values was 0.16 to 2. 86; df 3, 20; range in *P* values was 0.0627 to 0.9221. Larvae were the only life stage present at 21 d (Table 5.2), as well as packages with a pinhole on the top after 42 d. Methoprene-treated foil packages at 42 d contained deformed pupae and adults. Deformed pupae were seen as half pupal-adult intermediates whereby they died. Deformed adults contain antennae and legs which appeared white and translucent in color, but remained alive. Deformed pupae and adults were only seen in the methoprene-treated packaging samples. Conversely, only normal adults were seen in untreated packaging vials.

Compared to *T. variabile, P. interpunctella* invaded all untreated and methoprene-treated packaging materials after 21 or 42 d exposure periods. There were no significant differences in the diameter of holes between untreated and methoprene-treated packaging at 21 d (range in *F* values was 0.44 to 1.42; df = 3, 20; range in *P* values was 0.2652 to 0.7286). At 42 d the untreated packages had the smallest mean diameter holes at the bottom and middle locations, 1.0 and 1.1, respectively. This correlates to instars invading the packages at a younger age compared to methoprene-treated packaging because young instars have small head capsule sizes. The number of larvae found in each vial was significantly different between untreated and methoprene-treated packaging at both exposure periods. At 21 d 0.1% methoprene-treated packaging had the highest mean larvae present at all pinhole locations. However at 42 d the 0.25% methoprene had the highest mean larvae per vial (Table 5.3). In both exposure times, the untreated packaging had the fewest larvae present. In the 42 d vials, the untreated material had the presence of young larvae (data not shown). The number of pupae present varied significantly between untreated and methoprene-treated packaging at both exposure periods and pinhole locations, except for the top pinholes at 42 d. In each occurrence, the untreated material had the

highest mean pupae found inside the vials, ranging from 6.3 to 21.5 (Table 5.3). Methoprene-treated packaging had a significant effect on the mean number of adults found inside vials. There were no adults found in methoprene-treated packaging at 21 d and only a mean of 0.2 was seen at 42 d at the top pinhole location. The methoprene-treated packaging might not have inhibited invasion into foil packages, but it was effective in preventing larval development into the pupal and adult stages.

5.4.4. Effect of methoprene on penetration ability of third instars of *P. interpunctella* and *T. variabile*

T. variabile was not able to penetrate any packages after 21 or 42 d exposures when testing was initiated with third instars. At 21 d there were no significant differences seen in the number of larvae or pupae observed in untreated and methoprene-treated vials (range in *F* values was 01.02 to 2.52; df = 3, 20; range in *P* values was 0.0873 to 0.4042). There were also no significant differences observed in the number of larvae present at 42 d ($F = 0.44$; df = 3, 20; $P = 0.7297$). When third instar larvae were exposed to methoprene-treated packaging, deformed pupae and adults were observed. Deformities were only seen in the methoprene-treated packaging samples. The methoprene-treated packaging vials contained a significantly higher amount of deformed pupae at 21 and 42 d exposure times (Table 5.4). Among the treatments, the 0.50% methoprene-treated package had the highest mean number of deformed pupae at 21 and 42 d, 7.7 and 15.8, respectively. The methoprene-treated packaging at 42 d also had significantly more normal pupae. The normal pupae observed in the methoprene-treated packaging vials could be newly developed pupae and the deformed pupae could be at the end of the pupal cycle hence the longer exposure to methoprene the increased chance of deformities.

Additionally, there were no normal adult emergence in any treatment at 0.25% or higher, and untreated samples contained 7.7 adults at 21 d and 17.7 adults at 42 d (Table 5.4).

T. variabile was not able to penetrate any packages when held without a food source. Furthermore, *T. variabile* were unable to develop past the larval stage after 21 or 42 d exposure period to both untreated and methoprene-treated packaging. The number of larvae observed after 21 d exposure was significantly higher in the untreated versus methoprene-treated packaging vials ($F = 7.24$; df = 3, 20; $P = 0.0018$). There were no significant differentness in the number of larvae after 42 d of exposure ($F = 1.12$; df = 3, 20; $P = 0.3634$) (Table 5.5).

Third instar *P. interpunctella* succeeded only one time to penetrate the foil packaging when held with and with a food source. A 0.5625 mm hole located on the bottom third of the untreated packaging was observed, but there were no larvae present inside the packaging. There were no significant differences in the number of larvae observed at 21 or 42 d exposure periods between the methoprene-treated and untreated packaging (range in F values was 0.58 to 2.46; df 3, 20; range in P values was 0.0922 to 0.6379). The number of larvae present was significantly different between untreated and methoprene-treated packaging. The 0.50% methoprene-treated packaging at 21 d exposure period had the highest mean pupae 7.5 ± 0.8 and at 42 d, the 0.25% methoprene-treated packaging had the highest mean pupae 6.7 ± 0.5 (Table 5.6). However, all methoprene-treated packaging at 42 and 21 d exposures were significantly higher than the untreated packaging material. The mean number of adults observed at 21 d was significantly different among treatments. The 0.50% methoprene-treated packaging had the lowest mean adults per vial, 2.0 ± 0.6, which was significantly different from the untreated and 0.10% methoprene treated packaging. After 42 d there were no significant differences among all treatments and the mean number of adults ranged from 2.5 to 5.0 (Table 5.3).

When third instar *P. interpunctella* were held without a food, significant differences were observed between the untreated and methoprene-treated packaging. The mean number of larvae was significantly higher for 0.25% methoprene-treated packaging at 21 d compared to the untreated packaging, 1.8 to 0.2, respectively, but did not differ significantly from all other treatment levels (Table 5.4). However, there were no significant differences observed at 42 d exposure (F = 2.29; df = 3, 20; P =0.0599). The number of pupae that developed inside the vials was not significantly different between the untreated and methoprene-treated packaging at 21 or 42 d range in F values was 0.13 to 1.01; df = 3, 20; range in P values was 0.4069 to 0.9403). At 21 d and 42 d, the 0.10% methoprene-treated packaging had the highest mean number of pupae present per vial, 5.2 and 5.0, respectively. After 21 d and 42 d exposures to methoprene-treated packaging, there were no adults observed at any level of methoprene (Table 5.5).

5.5. Discussion

Stored-product insects such as *P. interpunctella* and *T. variabile* are capable of chewing through and penetration multiple types of packaging materials such as cellophane, kraft paper, and aluminum foil (Cline, 1978b). Cline (1978b) found that when young larvae are confined with no access to food, penetrated a greater tendency to penetrate food packages compared to larvae confined in pouches with food. The results of our study are in contrast to Cline's study. *T. variabile* and *P. interpunctella* were not able to penetrate any foil packaging, except in one isolated incidence, when confined with no food. Additionally when larvae were held without access to food, the developmental rate was delayed. *T. variabile* were not able to develop past the larvae stage, but when given access to food *T. variabile* were able to complete development to the adult stage.

Klein and Burkholder (1984) applied 0.1, 1.0, and 10 ppm of methoprene to rearing diet of *Trogoderma glabrum* (Herbst), glaborous cabinet beetle, and exposed 1 d old eggs, and only the 10 ppm treatment caused a delay in the developmental rate. Jenson et al. (2009) found methoprene had little effect on adult emergence of eggs exposed to methoprene treated Kraft paper at 0.00015, 0.0003 and 0.00045 mg (AI/cm^2). Silhacek and Oberlander (1975) found that the timing of exposure of juvenile hormone to *P. interpunctella* was more critical than dosage in determining whether *P. interpunctella* will undergo metamorphosis.

Cline (1978a) studied the clinging and climbing ability of stored-product insects including *P. interpunctella* and *T. variabile* on nine different materials including aluminum foil, cellophane, and six different polymer films. *P. interpunctella* is capable of climbing all types of materials tested at any angle up to 90°C (Cline, 1978a). *T. variabile* was only able to climb Kraft paper to an angle of 90°C, but all other materials tested were at angles less than 25°C (Cline, 1978a). The foil packages used in this study were held at an angle at approximately 90°C. This study used packages with and without pinholes to measure the penetration/invasion ability of *T. variabile* and *P. interpunctella* on methoprene-treated packaging. The lack of penetration of *T. variabile* in packages containing pinholes could be due to the lack of ability of larvae to climb the foil packaging. This is consistent with the results seen by Cline (1978a). However, *P. interpunctella* penetrated all packaging materials, untreated or methoprene-treated, when pinholes were provided. Pinholes in this study were placed at three locations, bottom third, middle third, and top third. *P. interpunctella* penetrated all packages irrespective of the pinhole location. This reaffirms the ability of *P. interpunctella's* ability to climb multiple types of surfaces. The fact that *T. variabile* has a difficult time climbing slick packaging material could reiterate the need for proper storage of food packages. Tightly wrapped polymer films tend to be

more resistant to insect infestations compared to loosely wrapped packages (Cline, 1976a). Storing food packages upright, could also increase resistance to infestations.

The retail marketplace is the final point of contact between food processors and consumers (Platt et al., 1998). Research has shown stored-product insects are commonly found in retail stores and are capable of invading packaged products. Platt et al. (1998) survey 322 grocery stores in the south-central United States and found that approximately 25% of service calls to pest control companies occurred more than 45 days apart. *P. interpunctella* and *T. variabile*'s life cycle is within the 45 day treatment window, thus infestations of newly acquired packaged food products would allow for populations of these insects to reproduce and grow (Platt et al., 1998). Stored-product insects have been documented in grocery stores and pet food stores, and this study demonstrated the ability of stored-product insects to infest food packages. The use of methoprene impregnated packaging could be a valuable new tool that food product manufacturers could utilize to limit infestations of their products. Additionally, it will help retail stores prevent further infestations in their stores.

5.6. References

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Table 5.1 Egg hatch of *P. interpunctella* and *T. variabile* exposed to methoprene-treated packaging material and non-treated material on Petri plates.

Treatment	% Egg hatch (Mean ± SE)			
	P. interpunctella[a]		*T. variabile*[b]	
	Non-foil side	Foil side	Non-foil side	Foil side
0.00%	36.0±2.2	22.4±6.0	93.6±2.7	85.6±3.7
0.10%	30.4±3.5	28.0±4.2	93.6±2.4	84.8±5.6
0.25%	29.6±7.4	28.8±1.5	92.0±2.5	82.0±5.7
0.50%	35.2±3.9	20.8±5.3	88.0±3.6	80.0±2.9

[a]There were no significant differences among the treatments ($F = 1.41$; df = 7, 32; $P = 0.2360$, one-way ANOVA).

[b]There were no significant differences among the treatments ($F = 2.07$; df = 7, 32; $P = 0.0760$, one-way ANOVA).

Table 5.2 Effect of methoprene on foil packing contain pinholes against *T. variabile*

Pinhole location	Treatment	Mean ± SE[a]					
		21 d	42 d				
		Larvae	Larvae	Pupae	Deformed Pupae	Adults	Deformed Adults
Bottom	0.00%	17.5±3.3	9.7±2.5	4.3±0.8	0.0±0.0c	5.8±0.8a	0.0±0.0b
	0.10%	16.7±3.9	10.3±1.3	3.5±1.0	0.7±0.3bc	0.0±0.0b	2.8±0.7a
	0.25%	18.8±5.1	9.5±2.6	2.8±0.5	2.2±0.8ab	0.0±0.0b	0.2±0.2b
	0.50%	19.3±5.0	14.0±1.7	2.3±0.6	2.7±0.6a	0.0±0.0b	0.2±0.2b
Middle	0.00%	19.8±5.2	8.0±3.1	3.5±0.6ab	0.0±0.0b	8.5±2.0a	0.0±0.0c
	0.10%	18.2±2.7	4.7±2.7	9.3±2.2a	1.5±1.1ab	0.0±0.0b	6.2±1.6a
	0.25%	18.0±4.8	10.0±1.3	2.3±0.4ab	3.0±0.6a	0.0±0.0b	2.5±0.8ab
	0.50%	16.3±4.2	11.7±2.1	1.7±0.7b	4.0±1.3a	0.0±0.0b	0.3±0.3bc
Top	0.00%	17.7±2.2	9.3±3.5	--	--	--	--
	0.10%	17.3±1.2	11.5±3.0	--	--	--	--
	0.25%	16.2±1.7	13.2±1.3	--	--	--	--
	0.50%	12.0±2.2	15.8±3.0	--	--	--	--

[a]Means for each insect stage followed by different letters are significantly different ($P<0.05$; by Tukey's adjustment)

Table 5.3 Effect of methoprene on penetration ability of *P. interpunctella*

Pinhole location	Trt.	Mean ± SE[a]									
		21 d					42 d				
		Outside of package			Inside of package	Diameter of hole	Outside of package			Inside of package	Diameter of hole
		Larvae	Pupae	Adults	Larvae		Larvae	Pupae	Adults	Larvae	
Bottom	0.00%	0.3±0.3c	12.8±2.1a	0.5±0.5	1.2±0.5	1.1±0.1	0.0±0.0b	7.0±1.9a	6.5±1.9a	5.3±2.6	1.0±0.1b
	0.10%	17.8±3.1a	0.0±0.0b	0.0±0.0	1.5±1.1	1.3±0.1	14.5±1.3a	0.3±0.3b	0.0±0.0b	1.3±0.6	1.3±0.1a
	0.25%	11.0±1.8ab	6.5±1.6a	0.0±0.0	0.5±0.3	1.2±0.1	14.8±1.8a	0.3±0.3b	0.0±0.0b	1.5±0.8	1.3±0.1a
	0.50%	5.5±1.7b	6.7±2.3a	0.0±0.0	0.0±0.0	1.2±0.2	12.5±0.7a	0.0±0.0b	0.0±0.0b	4.7±1.0	1.5±0.1a
Middle	0.00%	0.5±0.3b	6.3±1.8a	10.2±1.5a	0.0±0.0b	1.1±0.1	0.0±0.0b	7.0±2.0a	10.0±1.1a	8.0±4.8	1.1±0.1b
	0.10%	15.0±0.5a	0.8±0.4b	0.0±0.0b	1.2±1.0ab	1.3±0.1	11.5±1.0a	0.7±0.3b	0.0±0.0b	0.5±0.2	1.2±0.1b
	0.25%	10.5±2.3a	2.5±2.5b	0.0±0.0b	4.7±1.2b	1.4±0.1	12.8±1.4a	0.0±0.0b	0.0±0.0b	3.7±0.6	1.4±0.1a
	0.50%	10.3±2.5a	0.0±0.0b	0.0±0.0b	4.0±1.3b	1.2±0.2	12.5±2.0a	1.5±1.5b	0.0±0.0b	2.2±0.6	1.3±0.1ab
Top	0.00%	0.8±0.5b	21.5±2.1a	1.7±0.6a	0.5±0.3c	1.1±0.1	0.0±0.0b	2.3±1.0	12.2±1.0a	0.8±0.4c	1.1±0.1
	0.10%	15.3±1.9a	0.0±0.0b	0.0±0.0b	4.0±0.4b	1.2±0.1	10.0±0.9a	2.0±0.5	0.2±0.2b	2.3±0.3b	1.1±0.1
	0.25%	11.0±1.0a	0.0±0.0b	0.0±0.0b	8.0±0.7a	1.1±0.1	12.5±1.0a	0.7±0.2	0.2±0.2b	5.5±0.8a	1.2±0.1
	0.50%	10.7±1.6a	0.0±0.0b	0.0±0.0b	12.0±1.8a	1.2±0.1	10.3±1.9a	0.7±0.2	0.0±0.0b	5.3±0.8ab	1.1±0.1

[a]Means for each insect stage followed by different letters are significantly different ($P<0.05$; by Tukey's adjustment)

Table 5.4 Development of third instar *T. variabile* exposed to untreated and methoprene-treated packaging containing 500 mg food

Trt.	Mean ± SE[a]									
	T. variabile									
	21 d					42 d				
	Larvae	Pupae	Deformed Pupae	Adults	Deformed Adults	Larvae	Pupae	Deformed Pupae	Adults	Deformed Adults
0.00%	11.7±5.7	3.5±1.1	0.2±0.2c	7.7±1.4a	0.0±0.0b	1.0±0.4	0.0±0.0b	0.0±0.0d	17.7±0.7a	0.0±0.0c
0.10%	9.8±0.7	1.7±0.5	3.3±0.8b	0.0±0.0b	4.0±0.9a	0.5±0.2	0.2±0.2ab	5.3±0.7c	1.8±0.9b	11.0±1.5a
0.25%	12.2±0.6	1.3±0.8	5.5±0.8ab	0.0±0.0b	1.0±0.4b	1.2±0.6	1.2±0.3a	10.8±1.4b	0.0±0.0c	5.2±1.8b
0.50%	11.0±0.5	1.0±0.6	7.7±0.4a	0.0±0.0b	0.2±0.2b	0.7±0.2	0.7±0.4ab	15.8±0.9a	0.0±0.0c	1.8±0.9b

[a]Means for each insect stage followed by different letters are significantly different ($P<0.05$; by Tukey's adjustment)

Table 5.5 Development of third instar *T. variabile* and *P. interpunctella* exposed to untreated and methoprene-treated packaging containing no food.

Treatment	Mean ± SE[a]							
	T. variabile		*P. interpunctella*					
	21 d	42 d	21 d			42 d		
	Larvae	Larvae	Larvae	Pupae	Adults	Larvae	Pupae	Adults
0.00%	7.0±1.5a	3.5±0.4	0.2±0.2b	3.3±0.7	2.7±0.8a	0.0±0.0	3.8±0.6	3.8±0.8a
0.10%	4.0±0.6b	3.8±0.5	0.7±0.2ab	5.2±1.7	0.0±0.0b	0.5±0.2	5.0±1.8	0.0±0.0b
0.25%	3.5±0.4b	2.7±0.3	1.8±0.4a	2.2±1.4	0.0±0.0b	0.8±0.3	4.0±1.8	0.0±0.0b
0.50%	2.8±0.2b	3.8±0.9	1.5±0.7ab	4.3±1.5	0.0±0.0b	1.2±0.5	5.0±1.8	0.0±0.0b

[a]Means for each insect stage followed by different letters are significantly different ($P<0.05$; by Tukey's adjustment)

Table 5.6 Development of third instar *P. interpunctella* exposed to untreated and methoprene-treated packaging containing 500 mg food.

Treatment	Mean ± SE[a]					
	P. interpunctella					
	21 d			42 d		
	Larvae	Pupae	Adults	Larvae	Pupae	Adults
0.00%	0.5±0.3	1.5±0.3b	7.7±0.6a	2.5±0.9	0.8±0.3b	5.0±1.7
0.10%	0.2±0.2	4.3±0.5a	5.5±0.6a	0.5±0.3	4.7±0.6a	4.3±0.7
0.25%	0.7±0.3	4.7±0.8a	4.5±0.8ab	0.7±0.3	6.7±0.5a	2.5±0.8
0.50%	0.5±0.2	7.5±0.8a	2.0±0.6b	0.3±0.2	6.2±0.6a	3.5±0.4

[a]Means for each insect stage followed by different letters are significantly different ($P<0.05$; by Tukey's adjustment)