ATTRACT-AND-KILL METHODS FOR CONTROL OF INDIANMEAL MOTH, *Plodia interpunctella* (Hübner) (LEPIDOPTERA: PYRALIDAE), AND COMPARISONS WITH OTHER PHEROMONE-BASED CONTROL METHODS

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PREFACE

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Father, Mother and Sister…
I dedicate all this work in your memories.

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CHAPTER I

INTRODUCTION AND RESEARCH PROBLEM

Importance of the Indianmeal moth

The Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is an important pest in bulk stored grain and seeds (LeCato, 1976, Storey 1983, Vick 1986, Cuperus 1990, Doud and Phillips 2000, Nansen *et al.* 2004), dried fruits and nuts (Johnson *et al.* 1992), dried saw palmetto berries (Arbogast *et al.* 2002), seed stores (MacFarlane and Sylvester 1969), stored groundnuts (Corby 1947, Howe 1952, Hayward 1955, Hallyday 1967, 1968, Davey *et al.* 1959) cited by Mbata and Osuji (1983), military rations (Cline and Highland 1985) and garlic bulbs (Perez-Mendoza and Aguilera-Pena 2003). The infestation and damage by *P. interpunctella* and similar species of the pyralid subfamily Phycitinae are present in confectionary factories, in warehouses, retail stores, food processing facilities, stored food commodities (Hinton 1943, Sedlacek *et al.* 1996, Arbogast *et al.* 2002), flour mills (Doud and Phillips 2000), dried vegetables commodities (Hyun and Il 2000). It is also reported as a potential pest in soy bean meal commodities (Cox and Simms 1978).

Identification

Plodia interpunctella can be differentiated in the stages of larva, pupa, and adult. The main characteristics for the identification of the adult of *P. interpunctella* are wing coloration, the scales of the apical half of the fore wing are brown and the basal half are copper. Additionally, the hind wings exhibit gray scales. *P. interpunctella* adults are

about 10 mm long and have a wingspan of 1.25 cm (Mason 2003). In this stage, feeding is not common and the span of life is short (5-7 days); they usually rest on the walls or other indoor dark regions and are more active during the night (Richards and Thomson 1932). Adult males can be distinguished from females because they have thinner abdomens and are smaller in size.

The larval stage has been identified for its coloration that varies from cream to pink; this variation in color depends on the diet. Late instar males present a dark patch on the dorsal side of the body representing the testes (Bennett *et al*. 1988).

The pupa may be protected in a silken cocoon or remain loose in the food source. Pupa measure from 6-11 mm long and are pale brown coloration (Fasulo and Knox 2004).

Damage

P. interpunctella is the most common moth in stored food facilities and is the cause of most complaints from the food industry, and sellers and consumers (Phillips *et al.* 2000). Occasionally, the larvae produce silk-webbing in the grain and on the bags, causing heating and molding of the grain. High concentrations of moisture promotes the growth of *Aspergillus spp*. on seeds (Abdel-Rahman *et al*. 1969). Kane *et al*. (1977) mentioned that webbing produced by larvae of *E. kuehniella* causes clogging of the machinery in flour mills resulting in a reduction of yield. Furthermore, webbing is used to lay eggs and as diet for larvae (Abdel-Rahman *et al.* 1969) and becomes attractive to other pests including the Confused flour beetle, *Tribolium confusum* Duv., the Broad-horned flour beetle, *Gnathocerus cornotus* (F.), and the Flat grain beetle, *Cryptolestes turcicus* (Grouv.) (Kane *et al*. 1977). However, main losses are due to the presence of feces,

secretions, and pieces of insect bodies as well as whole bodies that reduce the quality of food. Additionally, quantitative losses are due to larvae directly feeding on food. The larvae tend to prefer the grain embryo and will attack this area in undamaged grains. One larva can destroy the germ in a relatively large number of grains. Mbata and Osuji (1983) determined that the material eaten by the larvae results in losing weight of groundnuts. The construction of a tunnel in garlic bulbs also reduces weight (Perez-Mendoza and Aguilera-Pena 2003).

Development

A single female of *P. interpunctella* may lay up to 400 eggs that may be individually or in groups (Brower 1975). Once the egg is laid and depending on food source and environmental conditions, the larva takes 4-7 days to hatch (Mbata and Osuji 1983). There are critical conditions to consider like at 15 °C and 70% R.H. the larvae do not hatch (Bell 1975). Also, at conditions of 70% R.H. and temperature of 20 °C the egg hatches in 6-9 days, at 25 °C in 3-5 days, at 30 °C in 2-4 days. Once the larva hatches it immediately looks for available food and makes silky tunnels (Richards and Thomson 1932). However, when there is no food source available, the larvae may feed on conspecific egg shells, unhatched eggs, and dead adults via “cannibalism”. Larvae have five instars as determined by head capsule width (Imura and Sinha 1986, Allotey and Goswani 1990). Previous to pupation, the last instar larval “wandering stage” searches for a suitable pupation site and it takes up to 7 days to emerge as adult (Bell 1975).

Behavior in closed environments

Once the adult emerges from the pupa, it drops to the floor and walks or flies to resting areas on the wall or undersides of pallets (Silhacek *et al.* 2003). The females begin to release the sex pheromone for calling males when they reach a suitable resting area (Lum and Brady 1973). Once the male detects the pheromone plume, he immediately starts the flutter dance behavior for mating and flies upwind to seek out females (Brady and Smithwick 1968, Lum 1974). When a fluttering male makes physical contact with a calling female this is termed "mating" (Silhacek *et al.* 2003). The level of female sex pheromone decreases during the second and third day after moths are mated, and only a few unmated females continue calling (Lum and Brady 1973).

The female releases pheromone molecules that form a plume and as air currents disperse them, these plume filaments expand with distance from their source (Murlis 1986, Murlis *et al*. 2000). The plume filament is detected by a flying insect depending on the pheromone concentration and spacing (Murlis and Jones 1981). After detecting the female, the male flies upwind and the orientation is made in a zigzag pattern crosswind to increase the contact with filaments of pheromone (Baker 1990).

The wind tunnel is a helpful tool to observe and understand behavior of moths. Justus *et al*. (2002) saw that the ground speed and track angle of *C. cautella* flight were similar in plumes at 10 Hz and in continuous plumes, but when the plume is pulsed or turbulent in *C. cautella*, the ground speed and flying track is faster (Mafra-Neto and Cardé 1994). Also, Schofield *et al*. (2003) showed in wind tunnel studies that the concentration of female sex pheromone influenced the flight track, course and drift angles of *C. cautella* males, and these results helped to explain the mechanism of mating disruption. Another

application of the wind tunnel is the response of males to attract-and-kill formulations at different concentrations and active ingredients against Oriental fruit moths, *Grapholita molesta* (Evenden *et al*. 2005) and the Indianmeal moth, *P. interpunctella* (Nansen and Phillips 2004).

Sanitation

The females of storage moth species prefer to lay eggs on substrates or food sources contaminated by conspecific larvae (Corbet 1973, Phillips and Strand 1994).
The removal of larval rearing sites may result in significant eradication of *E*. *cautella* females to infest the same sites (Bowditch and Madden 1996). In order to prevent infestation by *P*. *interpunctella* in inaccessible area of warehouses it is necessary to remove debris from those areas (Arbogast *et al*. 2002). In Korea, for example, fumigants and other insecticides are prohibited on vegetable commodities because of consumer safety issues and manufacturers must rely on sanitation and physical methods for moth control (Na and Ryoo 2000).

Chemical control

There have been several methods of control used in moths but still the effectiveness is limited. Also, the continuous conventional application of insecticides in stored products has been associated with health hazards (Muller and Pierce 1992, Süss and Trematerra 1986). Fumigation is a costly method due to the difficulties of sealing, and only the milling machinery area is treated and reinfestation occurs from non-treated areas (Kane *et al*. 1977). The removal of debris from complex machinery sometimes is impractical and fumigation techniques are needed (Bowditch and Madden 1996) like fumigation with

phosphine to treat stacks of dried botanicals in warehouses (Arbogast *et al.* 2002). Another fumigant regularly used is methyl bromide that is widely used to control stored insects but was banned in 2005 and alternative techniques of control are needed except for quarantine treatments (Phillips *et al*. 2000, Fields and White 2002). Alternatives such as protective sprays of synergized pyrethrins mixed with technical oil are being applied on stacks of bagged wheat against *Cadra cautella* (Wlk.), but it caused excessive staining of stored bags (Mcfarlane and Sylvester 1969). Another alternative is the fogging applications of pyrethrin in a chocolate-based consumables factory against *E. cautella* that reduced the use of pesticide as much as 80-90%, but the failure of fogging is that non-exposed insect stages are unaffected (Bowdith and Madden 1996). Another technique used to control stored product pests is the applications of Dichlorvos using slow-release strips or pellets, oil sprays, or aerosols (Green *et al*. 1966, 1968; Childs *et al.* 1966; Somme 1968; McGaughy 1973) cited by Kane *et al*. (1977). Disadvantages are deposits of oil on machinery, wastage of insecticidal vapor, and that work areas need to be vacated for 12 hr or more. However, in order to prevent infestations by *P. interpunctella*, *C. cautella* and several species of beetle pests in grains for food or seed that will be stored for a long period of time, it is necessary to protect them with applications of “grain protectants”, which involve the direct application of residual insecticides (Giga and Canhao 1991, Arthur 1994, 1997 and 1999) or insect growth regulators (Arthur 2003, Thomas W. Phillips, unpublished) to the commodity.

Temperature management

The application of high temperatures is an alternative to disinfest and protect stored commodities from *P. interpunctella* eggs. Cold disinfestations often require long exposure periods and it is more a preservation than disinfestation (Evans 1987). In contrast, heat treatments can be carried out in hours rather than days (Nakayama *et al.* 1983). The combination of heat and cold treatments decreases disinfestations more than heat or cold treatment alone (Lewthwaite *et al.* 1998).

Pheromones

Burkholder and Ma (1985) state that the use of pheromones in enclosed environments is favorable for management of insects. The species of the subfamily Phycitinae that includes *P. interpunctella* produce the female sex pheromone (*Z,E*)-9,12-tetradecadienyl acetate (*Z*9, *E*12-14:OAc) better known as "ZETA", which is attractive to males (Brady *et al.* 1971, Kuwahara *et al*. 1971, and Sower *et al.* 1974). ZETA was the first stored-product insect pheromone commercially available (Phillips 1997). A second compound, (*Z,E*)-9,12-tetradecadienol (*Z*9,*E*12-14:OH), which increases the response of male *P. interpunctella*, was found in extracts of females (Soderstrom *et al.* 1980, and Vick *et al.* 1981). Later, a third compound (*Z,E*)-9, 12-tetradecadienal (*Z*9,*E*12-14:Ald) was discovered by Teal *et al.* (1995). Finally, the most recent and fourth compound (*Z*)-9-tetradecenyl acetate (*Z*9-14:OAc) and its homologue (*Z*)-11-hexadecenyl acetate (*Z*11)-16:OAc were identified by Zhu *et al.* (1999).

Monitoring

Pheromone traps have previously been successfully employed to detect and monitor moths in food storage areas, processing factories (Hoppe and Levinson 1979, Vick *et al.* 1986), wheat storage bins (Hagstrum 2000), in and around flour Mills (Doud and Phillips 2000) and pilot feed mills (Roesli *et al.* 2003). Also the sticky trap offers advantages over visual inspections (Mullen and Dowdy 2001), is a valuable tool for spatial and temporal distribution and encourages the use of integrated pest management programs (Mueller 1998). Additionally, the longevity of lures containing the female sex pheromone can enhance trap catching (Mullen *et al.* 1991) and can suppress this pest through either mating disruption or an attracticide technique (Phillips *et al*. 2000).

Mating disruption

Large quantities of synthetic female sex pheromone emitted to the environment disrupt the response of *P. interpunctella* males (Ryne *et al.* 2001). These pheromone point sources may compete with pheromone plumes from calling females, thus creating a "false-trail following" by the males called "mating disruption" (Cardé and Minks, 1995). Mating disruption for stored-product moths has been evaluated for *Sitrotoga cerealella* (Olivier) (Vick *et al.* 1978) and *Ephestia cautella* (Walker) (Mafra-Neto and Baker 1996, Shani and Clearwater 2001) found in corn storage (Fadamiro and Baker 2002).

Attracticide

The "attracticide" is the combination of an attractant such as a sex pheromone and a killing agent (pathogen or insecticide) (Lanier 1990), also known as "lure and kill", "attract-and kill" and "attraction-annihilation." This technique may lead to the

annihilation of males, females or both. Its advantage over chemical applications are 1) insecticides are not broadcast over large areas; 2) insecticides are not applied directly onto food materials; 3) non-target insects (beneficials) are unlikely to be affected; and 4) only small amounts of pesticide are needed to treat a facility (Nansen and Phillips 2004). In Lepidoptera, efficiency of control has been shown by attracticides on forest trees and Western spruce budworm, *Choristoneura occidentalis* Freeman (Sower and Shorb 1985); European pine shoot moth *Rhyacionia buoliana* (Denis and Schiffermuller) (Sukovata *et al.*, 2004); for field crops with the Egyptian cotton leaf worm, *Spodoptera litoralis* Boisduval (De Souza *et al.* 1992, Downham *et al.* 1995); Pink bollworm, *Pectinophora gossypiella* (Saunders) (Haynes *et al.* 1986, Miller *et al.* 1990); for fruit orchards with the Codling moth *Cydia pomonella* (L.) (Charmillot and Hofer 1997, Charmillot *et al.* 2000, Losel *et al*. 2000, Krupke *et al.* 2002); Oriental fruit moth *Grapholita molesta* (Evenden and McLaughlin 2004); light brown apple moth *Epiphyas postvittana* (Walker) (Brockerhoff and Suckling 1999, Suckling and Brockerhoff 1999); and in stored-products with navel orange worm *Amyelois transitella* (Phelan and Baker 1987); Mediterranean flour moth *Ephestia kuehniella* Zeller (Trematerra and Capizzi 1991), and Indianmeal moth *Plodia interpunctella* (Hübner) (Nansen and Phillips 2004).

RESEARCH PROBLEM

The Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) is a very important pest in bulk stored grain and seeds, dried fruits, nuts, dried saw palmetto berries, seed stores, stored groundnuts, military rations and garlic bulbs. Infestations by *P. interpunctella* and similar species of the subfamily Phycitinae are present in confectionary factories, warehouses, retail stores, food processing facilities, stored food commodities, flourmills, dried vegetable commodities, and potential pest in stored soy bean meal (Cox and Simms 1978). Damage caused by *P. interpunctella* begins during the larval stage by feeding directly on the food and they are so aggressive that they might penetrate packing materials. At the time of feeding, the larvae produce webbing that raises the temperature and humidity which allows the growth of fungus (Abdel-Rahman *et al*. 1969), and produce optimal conditions for other related pests that become serious economic problems. Methods used to control *P. interpunctella* infestations are fumigation with methyl bromide, fogging treatments, and spray applications (protectants). Although these methods are effective in controlling this pest, they produce secondary effects. Methyl bromide was banned in stored products starting in 2005 (Phillips *et al*. 2000, Fields and White 2002). Fogging treatments are costly because they require continuous application. Finally, spray applications (protectants) retard the consumption of the grain and some formulations might leave residues on the grain which is not well accepted by consumers. Therefore, using insecticides becomes subject of public health concern.

The detection and monitoring of *P. interpunctella* is done by using slow-release devices, “lures” containing a specific female sex pheromone (*Z,E)*-9,12-tetradecadienyl

acetate (ZETA) on sticky traps to attract males. The pheromone by itself has been used at high doses to disrupt mating; this means that males keep flying toward the pheromone source and cannot find the female. Another use of the pheromone (ZETA) consists in placing it on sticky traps to trap a high density of insects (mass-trapping). Recently, synthetic sex pheromone is being used in combination with a small amount of insecticide; once the insect is attracted to the pheromone, the insect touches the killing agent, and it is effectively eliminated. This technique has been known as “lure and kill”, “attraction-annihilation”, “attract-and-kill” or “attracticide”.

This research pursues the following objectives:

1. Evaluate the toxicity of several insecticides for potential attract-and-kill formulations against *P. interpunctella* using contact toxicity tests.
2. Evaluate the toxicity of insecticides screened in Objective 1 applied to several surfaces.
3. Conduct behavioral studies in response to several attract-and-kill formulations using a wind tunnel, and determine mortality of *P. interpunctella*.
4. Evaluate effectiveness of the attracticides for suppressing *P. interpunctella* populations in simulated warehouses.
5. Evaluate suppression of *P. interpunctella* population by attract-and-kill formulations in commercial establishments, and compare this suppression with mating disruption and mass-trapping.

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CHAPTER II

CONTACT TOXICITY OF INSECTICIDES FOR ATTRACT-AND-KILL APPLICATIONS AGAINST ADULT *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

Abstract

Toxicities of thirteen commercially formulated contact insecticides that represent six insecticide groups were evaluated at registered label rates and higher rates for control of Indianmeal moth (IMM), *Plodia interpunctella* (Hübner), adults. The ultimate objective of this work is to develop attract-and-kill technologies for males. Initial bioassays were conducted by exposing two-day old adult males of IMM to surface-treated Petri dishes for two seconds, and then pairing them with non-treated virgin females for mating and oviposition over a 24-hr period. Five products belonging to the pyrethroid insecticide group (Cyfluthrin, Deltamethrin, and Permethrin), and pyrethrins (organic Pyrethrum and Pyrethrum plus a synergist) caused over 70% mortality to IMM adult males. Oviposition by females paired with treated males was significantly impacted by these five insecticides while hatching rate of larvae from the eggs was not. A second experiment tested the eight-week residual toxicity of Cyfluthrin, Permethrin, and Pyrethrum at label rates and at a higher rate of 2% active ingredient on five surfaces: a plastic-coated paper, metal, painted plastic, plastic, and wood. Permethrin at 2.0% suppressed IMM adult males at over 80% for up to 8 weeks and retained activity on surfaces made with plastic-coated paper, metal or plastic. Effective surface treatments had variable impacts on oviposition

by paired females for up to eight weeks. However, egg-hatch rate was generally unaffected by treatment. This research suggests that effective attract-and-kill surfaces can be developed for killing male IMMs, thereby lead to reduced reproduction and population suppression.

Keywords: Stored-product insects, residual insecticide, surface sprays, oviposition, pest control.

INTRODUCTION

The Indianmeal moth, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), is a common pest in confectionary factories, food warehouses, retail stores, food processing facilities, bulk-stored commodities (Hinton 1943, Sedlacek *et al*. 1996, Arbogast *et al*. 2002), flour mills (Doud and Phillips 2000), and dried vegetable commodities (Hyun and Ryoo 2000). *P. interpunctella* is also reported as a potential pest in soy bean meal commodities (Cox 1978). The infestation and damage by *P. interpunctella* and similar species of stored-product moths in the subfamily Phycitinae are the cause of most complaints from food manufacturers, retailers and consumers (Phillips 2006). There have been several methods of pest control for stored-product moths, but the effectiveness is limited and alternatives are needed. The fumigant methyl bromide was widely used to control storage insects, but its use has been banned or is currently being curtailed, except for quarantine treatments (Phillips 2006, Fields and White 2002). Protective sprays of synergized pyrethrum mixed with technical oil have been applied to stacks of bagged wheat against *Cadra cautella* (Wlk.), but it caused excessive staining of stored bags (McFarlane and Sylvester 1969). Aerosol applications using pyrethrin in a chocolate-

based consumables factory against *C. cautella* reduced the use of other pesticide by as much as 80-90%, but there were control failures due to survival of non-exposed insect life stages (Bowditch and Madden 1996).

Pheromone-based methods for controlling stored product insects have been of interest to researchers and pest managers as the use of traditional chemical controls has become limited due to regulations or low biological activity (Phillips et al. 2000). The attract-and-kill method utilizes an attractant, such as a sex pheromone, to lure insects of the target species to an insecticidal surface or device for mass-killing and ultimate population suppression, and can have the same effect as mass-trapping (Lanier 1990; Phillips 2006). When a synthetic female sex pheromone is used to lure male moths in an attract-and-kill strategy, a large number of male moths must be killed over extended periods of time to reduce matings and reproduction, and ultimately to suppress the pest population. The attract-and-kill approach may be more practical than mass-trapping because no trap-servicing or other frequent maintenance would be required. The major sex pheromone of the Indianmeal moth is a strong attractant for males, and earlier work has suggested it can be used for population control with attract-and-kill techniques (Suss and Trematerra 1986, Trematerra and Capizzi 1991). A gel-based attract-and-kill formulation that contains synthetic female sex pheromone and Permethrin was found to be active against adult males of *P. interpunctella* in simulated field trials (Nansen and Phillips 2004). This same type of insecticide-gel formulation was also effective against adult males of *Grapholita molesta* (Evenden and McLaughlin 2004) with that species' pheromone. However, the attract-and-kill gel formulation required a high density application rate and field persistence of the attractant in the gel was limited. The efficacy of residual contact

insecticides for protecting grain and controlling stored-product insects in structures has been studied and reviewed (Arthur 1996, Arthur and Phillips 2003), but development of an attract-and-kill method for storage moths requires further research on the residual activity of short-term time contact of adult males with insecticides.

The objective of the study reported here was to evaluate the contact toxicities of thirteen insecticides with potential for use in attract-and-kill formulations to control *P. interpunctella* males subjected to brief contact times. The most active materials were further evaluated for their residual toxicity for up to eight weeks after application on different surfaces. The reproductive success of treated *P. interpunctella* males was determined after pairing with female moths and assessment of eggs laid and larvae produced.

MATERIALS AND METHODS

Insects. *P. interpunctella* adults from a laboratory culture at Oklahoma State University were reared on a diet containing corn meal, chick starter/grower crumbles, chick laying crumbles and glycerol (4:2:2:1 by volumetric ratio) in 450-ml glass jars maintained at 28 °C, 60-70 % R.H., and under a 16:8 hr (L:D) photoperiod. Pupae were isolated from colonies by placing 1.0-cm-wide rolls of single-faced corrugated cardboard into culture jars that contained wandering-stage larvae, which then pupated inside the corrugations. Pupa were separated by sex and placed individually into 1.0 dram ventilated vials (Fisher Scientific, Pittsburg, PA) and held until they emerged as adults (Nansen and Phillips 2004). Experiments utilized 1-2 day-old virgin adults, and these adults were only used once.

Insecticides. Table 1 lists the thirteen commercial insecticides, grouped by type of active ingredient, used in the initial toxicity test. Insecticides were applied at concentrations prescribed on the label (the so-called label rate, LR), on 9-cm-diameter plastic Petri dishes (Fisher Scientific, Canada). Further experiments included commercial formulations containing Cyfluthrin (Bayer, Kansas City, MO), Permethrin (FMC Corp. Philadelphia, PA) and Pyrethrin (McLaughlin Gormley King Co., Minneapolis, MN) at label rates (Table 1) and a higher rate (HR) of 2.0% A.I. in water mixtures. The mixtures were applied to plastic-coated paper, metal, paint, plastic, and wood surfaces.

Contact toxicity experiment. The interior surfaces (lid and bottom) of 9-cm-diameter plastic Petri dishes were sprayed with 0.25 ml of water solution with one of the thirteen insecticides using an artist's "air-brush" (Paasche Airbrush Co., Hardwood Heights, IL). Water only was sprayed as the non-treated controls. Petri dishes were allowed to dry in

the laboratory hood for three hours before use. An adult male was transferred from a vial and confined inside a treated Petri dish (lid and bottom) for two seconds. The male moth was then put into a 950-ml glass jar with a single virgin female and 15 g of wheat kernels; the jar was covered with a ventilated paper lid. The wheat kernels were used as a female oviposition substrate. Jars were placed in a growth chamber held at 28 °C, approximately 60% R.H., with a 16:8 (L:D) photoperiod. After 24 hours the mortality of the male and female in each jar was assessed and recorded. Wheat kernels from each jar were sifted with a U.S. No. 14 sieve (Seedburo Equipment Co., U.S.A.) and to collect and count eggs. Eggs were placed on double-sided tape on a 9-cm-diameter black filter paper (Ahlstrom, Mt. Holly Springs, PA) in the bottom of a plastic Petri dish and put into the growth chamber for five days, after which the number of hatched eggs was determined. Each treated dish was exposed to 10 separate males and there were four dishes (replicates) of each insecticide, for a total of 40 males tested for each insecticide and the control in the initial contact toxicity study.

Residual toxicity experiment with different surfaces. The following surfaces were treated inside a 9-cm-diameter dish bioassay arena with solutions of insecticides: uncoated plastic (same Petri dishes as used in the initial contact toxicity experiment; Fisher Scientific, Canada); plastic Petri dishes coated with a layer of white latex house paint (Marketed by Wal-Mart, Bentonville, AR); customized Petri dishes (similar in interior diameter and depth as plastic Petri dish bottoms and lids) made with non-painted plywood; non-painted metal (sheet of aluminum, 3.0 mm thick); and a circular piece of plastic-coated paper fitted into the plastic Petri bottoms and lids. Insecticide formulations were diluted in water and a volume of 0.25 ml was sprayed on the interior surfaces (water

only for non-treated controls) with the artist's air-brush (same as used in the initial contact toxicity experiment) and dried in a fume hood for three hours. We tested Cyfluthrin, Permethrin, and Pyrethrin that lacked the synergist "PBO", piperonyl butoxide, based on results of the initial contact toxicity experiment, describe above. The first bioassays began after the dishes were dried and this set was designated time 0. Petri dishes were stored at room temperature between bioassay periods. A HOBO data logging unit (Onset Computer Co., Bourne, MA) was placed with the dishes and used to monitor the temperature and relative humidity, which were found to vary between 22 and 25 °C, and 40-60% R.H., respectively. This experiment followed the same procedures as in the initial contact toxicity experiment, described above. However, five males were bioassayed separately and consecutively per Petri dish with four different dishes as replicates per treatment, or 20 males per treatment. Residual toxicity of the insecticides on the different surfaces was evaluated by conducting bioassays with the original Petri dishes at 0, 4, and 8 weeks post-treatment.

Statistical analysis. Three response variables were observed in both experiments: percent of mortality of treated adults, number of eggs laid, and percentage of the eggs that hatched. Proportions (percentages) were transformed by the arcsine-square root function prior to analysis. The experimental design used for the initial toxicity test of thirteen insecticides was a completely randomized design with four replicates per treatment. Ten males were observed for each of the four treated Petri dishes. A protected least significant difference procedure (Gomez and Gomez 1984) was used for separating means at the $\alpha = 0.05$ level. The persistence of the insecticides on the different surfaces through the eight-week period was analyzed as a randomized complete block design, with

a factorial arrangement (type of surface material and dose of active ingredient as factors) and repeated four times. Data were analyzed with the PROC MIXED procedure using the REPEATED option (SAS Institute 2003). Treatment differences within week were analyzed with pair-wise t-tests and comparisons were protected by examining the SLICE option within the LSMEANS statement at the $\alpha = 0.05$ level.

RESULTS

Initial contact toxicity experiment.

Cyfluthrin, Permethrin, Deltamethrin, Pyrethrin alone, and Pyrethrin + PBO were the treatments with the highest percent mortality and were statistically similar as a group, with averages of 73 to 95% mortality of *P. interpunctella* adult males following 2.0-second exposures to insecticides and 24 hr of recovery time. These were significantly different from mortality caused by the other insecticides tested and from the non-treated controls (Fig. 1; $F_{13,42} = 21.70$, $P < 0.0001$). This initial study revealed that 2.0-second exposures of males on the insecticide-treated Petri dishes had significant effects on oviposition by females that were paired with these males for 24 hrs (Table 2; $F_{13, 42} = 4.42$, $P = 0.0001$). There was an average of more than 76 eggs laid per female paired with males from non-treated control dishes, and this was significantly different from eggs laid following pairings involving males from insecticide-treated dishes. Males treated with Permethrin, Deltamethrin, and organic Pyrethrin (lacking PBO), had mates with an average of less than eight eggs laid per female; these were statistically similar to each other and considered as the best treatments for suppressing reproduction. However, these three insecticides did not differ statistically in their effect on oviposition from Cyfluthrin, Fipronil, Imidacloprid, Abamectin and Pyrethrin + PBO, which averaged 20 to 25 eggs laid per female. The remaining five insecticides, Malathion, Chlorpyriphos, Dichlorvos, Azadirachtin and Spinosad, had the least effects on oviposition and were not significantly different from each other. Statistical analysis for egg hatching (Table 2) found there was a significant treatment effect on egg hatching ($F_{13,42} = 7.22$, $P < 0.0001$). Eggs derived from pairings with males from non-treated Petri dishes had the highest percent of eggs

hatched, 92.0%, which was statistically similar to eight of the insecticide treatments and different only from Chlorpyriphos, Dichlorvos, Permethrin, Abamectin and Pyrethrin + PBO.

Residual toxicity on several surfaces

Results for the residual toxicity of insecticides applied to different surfaces are presented below within the contexts of a given surface type within a given number of weeks after treatment, times 0, 4, and 8 weeks following application of the insecticides. **Mortality.** The residual toxicity of the three insecticides at prescribed label rates, LR, and at the 2% high rate, HR, against *P. interpunctella* adult males on several surfaces varied substantially according to surface and active ingredient (Figure 2). There was a significant three-way interaction among surface, insecticide and weeks ($F_{48, 210} = 3.41$, $P < 0.0001$).

Mortality on the plastic-coated paper surface at time 0 was above 95 % for the treatments of Cyfluthrin HR, Permethrin LR and HR, and Pyrethrin LR and HR, and these differed statistically from mortality on Cyfluthrin LR, which was about 60% (Fig. 2). All these treatments on the plastic-coated paper were significantly different from the non-treated controls at 0% mortality. Similar levels of mortality were observed at week 4, except for Pyrethrin LR, which dropped to 5% and was statistically similar to the control. Only Permethrin HR kept a high level of mortality (90%) at week 8, which was statistically different from the other treatments. Lower mortality (30 to 50%) was observed for Cyfluthrin LR and HR, Permethrin LR and Pyrethrin HR that were similar

statistically. Furthermore, Pyrethrin LR did not kill any adult males at week 8 and it was similar statistically to non-treated with 0% mortality.

Residual toxicity on the metal surface at week 0 showed that Permethrin HR and Pyrethrin LR and HR were similar statistically with 100% mortality to adult males. Permethrin LR and Cyfluthrin HR were not significantly different and showed lower kill, at 85% mortality, compared to the others. Furthermore, these treatments elicited significantly higher mortality than Cyfluthrin LR and the non-treated control, with 25% and 0% mortality, respectively. A similar trend was exhibited at week 4 on metal, except the efficacy of Pyrethrin LR dropped to 10% and was similar statistically to the non-treated control at 0% mortality. Also, Permethrin LR, Cyfluthrin HR and Cyfluthrin LR were similar statistically and showed lower effectiveness with 60, 45 and 20% mortality, respectively. However, Cyfluthrin LR also was not different statistically from Pyrethrin LR. At 8 weeks the Permethrin HR showed the highest effectiveness in killing adult moths, with 85% mortality, and this was significantly higher than mortality on all the other treatments.

The residual toxicity of the insecticides on the painted surface was overall very poor (Fig. 2). Pyrethrin HR killed 100% of the adult moths at week 0 and was significantly higher in activity than others tested, which were low and mostly similar to the non-treated control. Residual toxicity of all the tested compounds on the painted surface was less than 20% on average at the 4 and 8-week bioassays.

The adult mortality response for the treated plastic surface showed that Pyrethrin HR, Permethrin HR and Cyfluthrin HR were statistically similar to each other and killed over 95% of the adult moths tested at week 0. However, Permethrin HR and Cyfluthrin HR

were similar statistically to Permethrin LR and Cyfluthrin LR, with 80 and 85% mortality. Pyrethrin LR killed only about 35% of adult moths tested, but this was significantly greater than the 0% mortality of moths exposed to non-treated plastic. At week 4 the Permethrin HR had 85% mortality, which was similar statistically to Permethrin LR and Pyrethrin HR. However, Permethrin LR and Pyrethrin HR were not significantly different from Cyfluthrin LR and HR at this time period. Pyrethrin LR elicited only 5% mortality at 4 weeks, which did not differ statistically from the non-treated control. At 8 weeks the Permethrin HR on plastic killed about 80% of adult moths treated, which was similar statistically to Cyfluthrin HR at 60% morality. However, Cyfluthrin HR was not significantly different from Permethrin LR with about 45% mortality. Furthermore, Permethrin LR did not differ statistically from Pyrethrin HR and Cyfluthrin LR, at 45% and 20% mortality, respectively. Additionally, Pyrethrin HR on plastic exhibited low efficacy at 8 weeks and was similar statistically to the rest of the treatments and to the non-treated control.

Residual activity of insecticides tested on the bare wood surface was generally poor (Fig. 2). Permethrin HR killed an average of 80% of adult moths at time 0, which was statistically higher than response to Cyfluthrin LR and HR at 35% and 40% mortality, respectively. These treatments were significantly different from the rest of the treatments and a similar trend was observed at weeks 4 and 8, during which only Permethrin HR elicited a high mortality, 75% and 60%, respectively, but when the other treatments had very low activity.

Egg laying. The mean number of eggs laid per female *P. interpunctella* (Fig. 3) paired with treated males varied significantly among insecticides ($F_{6, 105} = 18.14$; $P <$

0.0001) and there was a significant interaction between insecticide and weeks ($F_{12, 210}$ = 1.89, P = 0.0367). However, there were no significant differences among the surface types ($F_{4, 105}$ = 0.68, P = 0.6071), a marginal significance was detected among weeks ($F_{2, 210}$ = 2.80, P = 0.0630), and no significant interaction between surface and insecticide ($F_{24, 105}$ = 0.70, P < 0.8373), surface and weeks ($F_{8, 210}$ = 0.55, P = 0.8182), or surface and insecticide and weeks ($F_{48, 210}$ = 0.77, P < 0.8559). As expected, females paired with males from non-treated controls laid more eggs throughout the experiment compared to females paired with insecticide-treated males. Generally, those females paired with males from treatments that caused high male mortality laid the lowest numbers of eggs.

The mean number of eggs was over 17 per female for Non-treated, Pyrethrin HR, Permethrin LR and HR, and Cyfluthrin LR on the plastic-coated paper surface at week 0, and these responses were statistically similar to each other (P > 0.05). However, these same treatments, excluding the non-treated control, were not significantly different from the remaining treatments. At week 4, all treatments were statistically similar to each other on the plastic-coated paper, except for Pyrethrin HR (4.9 eggs laid per female), which was significantly different from Non-treated and Permethrin LR (35 and 35.4 eggs laid per female, respectively). At week 8 the non-treated, Pyrethrin HR, Permethrin LR and Permethrin HR did not differ statistically. These treatments, except for the non-treated control, were statistically similar to Pyrethrin LR and Cyfluthrin LR (10 and 15.9 eggs laid per female, respectively). A low and statistically similar mean number of eggs were laid by females paired with males treated with Cyfluthrin LR and HR, Permethrin HR, Pyrethrin LR and HR. However, these treatments were not significantly different from Cyfluthrin LR, Permethrin HR, Pyrethrin LR and HR. These treatments were

statistically similar to Permethrin HR and Pyrethrin HR for which the females laid higher number of eggs.

The mean number of eggs laid by females paired with males from treated metal surfaces at week 0 was more than 34 eggs per female for the non-treated control, Pyrethrin HR, Permethrin LR and HR, which were not significantly different from each other. However, Pyrethrin HR and Permethrin HR were also statistically similar to Pyrethrin LR, which averaged 15.5 eggs per female. Moreover, these treatments, except for Pyrethrin HR, were statistically similar to the rest of the treatments, which averaged fewer than 20 eggs laid per female. A similar trend was observed at week 4, except for Pyrethrin HR, for which the egg laying dropped to an average of 8.5 eggs per female. The egg laying in response to males treated with Permethrin HR was 40.4, which was similar statistically to Pyrethrin LR, Cyfluthrin LR and HR. However, these treatments, except Permethrin HR, did not differ from the rest of the treatments. At week 8, non-treated and Permethrin LR showed high averaged egg laying (61.2 and 35.6, respectively). Response to Permethrin was significantly higher than to the other insecticide treatments.

On the painted surface at week 0, all the insecticide treatments were similar statistically. However, Pyrethrin LR and HR, and Permethrin LR did not differ from non-treated control which averaged the highest number (47.1) eggs laid per female. At week 4 there was greater than 22.85 eggs laid per female for the non-treated control, Permethrin LR and HR, and Cyfluthrin HR, which were statistically similar to each other. These treatments, except for the non-treated paint surface, did not differ from Pyrethrin LR, which averaged 16.8 eggs laid. Moreover, these treatments, except Permethrin LR, were not significantly different from the rest of the treatments. At week 8, the egg laying

was over 17.3 eggs per female for all treatments, except Cyfluthrin LR, which elicited an average of 9.35 eggs laid. All treatments were similar statistically, except non-treated, which was significantly different from Cyfluthrin LR.

The plastic surface at week 0 resulted in egg laying that was greater than 17 eggs for the non-treated control, Pyrethrin HR, Permethrin LR and HR. However, Permethrin LR and Pyrethrin HR were statistically similar to the rest of the treatments. At week 4 the non-treated plastic, Permethrin LR and HR showed more than 41.9 eggs per female. These treatments did not differ statistically from each other. However, Permethrin HR was statistically similar to Cyfluthrin LR and Pyrethrin LR (11.4 and 16.1 eggs laid, respectively). These treatments, except Permethrin HR, were not significantly different from the rest of the treatments. At week 8 there was substantial variation and all the treatments were statistically similar to each other.

On the wood surface at week 0, the average egg laying was greater than 23.5 eggs per female for non-treated control, Pyrethrin LR, Permethrin LR and HR, which were similar statistically. These treatments, except for the non-treated wood and Permethrin HR, were not significantly different from the rest of the treatments. A similar trend was observed at week 4, except that Pyrethrin LR, with a mean of 22.5 eggs, was significantly different from non-treated wood and Permethrin HR. At week 8, the egg laying was not statistically different among treatments, except Pyrethrin HR and Cyfluthrin LR, which elicited averages of 13.4 and 9.8 eggs, respectively, and these were statistically lower than eggs laid by females paired with males from non-treated wood (mean of 52.5 eggs laid).

Egg hatching . The three insecticides and five surfaces analyzed impacted the proportion of *P. interpunctella* eggs hatching over time (Figure 4). The analysis of variance of the arcsine transformed proportion of eggs hatching differed significantly among insecticides ($F_{6, 105} = 17.78$, $P < 0.0001$), among the surface types ($F_{4, 105} = 29.42$, $P < 0.0001$) and among weeks ($F_{2, 210} = 60.79$, $P < 0.0001$). There was significant interaction between surface and insecticide ($F_{24, 105} = 5.01$, $P < 0.0001$), surface and weeks ($F_{8, 210} = 2.74$, $P = 0.00668$), and insecticide with weeks ($F_{12, 210} = 3.21$, $P = 0.0003$), but there was no significant three-way interaction of surface, insecticide and weeks ($F_{48, 210} = 1.14$, $P < 0.2679$).

On the plastic-coated paper surface at week 0, the average percent of egg hatching was 95% for non-treated control, which was significantly different from the rest of the treatments. A similar trend was observed at week 4 and 8, except the Pyrethrin LR and Cyfluthrin LR, which were statistically similar to non-treated control.

The metal surface had an average of greater than 95% egg hatching for non-treated control at week 0, which was significantly different from the rest of the treatments. At week 4, non-treated and Cyfluthrin LR were statistically similar (95.1 and 52%, respectively). However, Cyfluthrin LR was not significantly different from Pyrethrin LR and HR, and Permethrin LR. These treatments, except for Cyfluthrin LR, were similar statistically to the rest of the treatments. At week 8, the average percent egg hatching was greater than 56% for non-treated, Pyrethrin LR, Permethrin LR and Cyfluthrin LR, which did not differ significantly. These treatments, except for non-treated, were not significant different from Pyrethrin HR and Cyfluthrin HR, which were 48.8 and 46.7%

hatch, respectively. Additionally, Pyrethrin HR and Cyfluthrin HR did not differ from the rest of the treatments.

The paint surface at week 0 had a percent egg-hatch rate that was greater than 82% for non-treated control and Pyrethrin LR, and these were similar statistically. However, Pyrethrin LR was also statistically similar to Permethrin LR (46%). These treatments were statistically different from the rest of the treatments, except Permethrin LR, which did not differ from the rest of the treatments. At week 4, the percent egg hatching was greater than 58% for non-treated, Pyrethrin LR, Permethrin HR and Cyfluthrin LR, which were not significantly different. These treatments, except for the non-treated metal, did not differ from the rest of the treatments. At week 8, the percent egg hatching was greater than 74% for non-treated, Pyrethrin LR, Permethrin HR, Cyfluthrin LR and HR, which did not differ statistically. However, Pyrethrin LR and Cyfluthrin HR, with 74.1 and 85.1% hatch respectively, were also similar statistically to Pyrethrin HR (46.2% hatch). These treatments, except Pyrethrin HR, were significantly different from the rest of the treatments.

The plastic surface showed greater than 96% egg hatching at week 0 for the non-treated control, which was significantly different from the rest of the treatments. At week 4, non-treated, Pyrethrin LR and Cyfluthrin LR, at 55.2 and 74.9% hatch, respectively, were similar statistically. These treatments, except for the non-treated plastic, did not differ from Cyfluthrin HR at 45% hatch. However, Pyrethrin LR and Cyfluthrin HR were not statistically different from the rest of the treatments. At week 8, the percent average egg laying was greater than 66.5% for non-treated, Pyrethrin LR and Cyfluthrin LR. These treatments, except Cyfluthrin LR, were not significant different from

Pyrethrin HR, Permethrin LR and Cyfluthrin HR, with 41.6, 28.0 and 33.1% hatch, respectively.

On the wood surface at week 0, the non-treated control (97.8%) and Pyrethrin LR (76.8%) averaged the highest percent of egg hatch and were statistically similar. These treatments differed from the rest of the treatments, except Permethrin LR (46.7%) which was statistically similar to Pyrethrin LR. At week 4, the non-treated wood showed the highest percent egg hatch (97.7%) and was not significantly different from Permethrin LR (67.1). However, Permethrin LR did not different from the rest of the treatments, except Pyrethrin LR (19.9%). At week 8, the percent average egg hatching was greater than 95.7% for the non-treated control, Pyrethrin and Permethrin both at LR and did not differ from Cyfluthrin and Pyrethrin both at HR (85.5% and 66.6%, respectively). These treatments, except Pyrethrin HR were significantly different from Cyfluthrin LR (41.5%) and Permethrin HR (21.0%).

DISCUSSION

Results from 2.0-second contact toxicity tests aimed at preventing adult male *P. interpunctella* from mate-finding and reproduction clearly identify the types of toxins and the types of surfaces that would perform best in attract-and-kill pest management methods for this serious pest. Initial screening of a range of active ingredients identified synthetic pyrethroid and natural pyrethrin insecticides as more effective than several other insecticides known to be effective in other contexts, and eight-week residual toxicity was best for higher application rates of Permethrin on most surfaces except the painted plastic surface.

The low activity of compounds other than pyrethroids and pyrethrins may be explained by inherent factors of these compounds related to the application method and the mode of action. There was low percentage or no mortality to the organophosphates (OP) treatments, perhaps due to the presumed inherent, genetically-based resistance to organophosphates by the laboratory culture of *P. interpunctella* used in our experiments. OP resistance by *P. interpunctella* is widespread and well documented (Arthur 1996, Fields and White 2002). Low mortality was also observed in the treatments with Fipronil, Imidacloprid, Abamectin, Spinosad and Azadirachtin, and was perhaps due to the brief contact by the males with the treated Petri dishes or with specific details of the modes of action for these compounds. Previous work that demonstrated contact toxicity of *P. interpunctella* to these or similar compounds had much longer contact times, usually several days to weeks (Huang *et al*. 2004, Yue *et al*. 2003) and did not utilize adults. The 2.0-second contact in the bioassays conducted here clearly limited the types of insecticides that would be effective. Synthetic pyrethroids and naturally-derived

pyrethrins are known for having rapid "knock-down" mortality for various insects, including Lepidoptera (Amin and Knowles 2001, DeSouza *et al*. 1992, Evanden and McLaughlin 2004), so the effectiveness of these active ingredients against *P. interpunctella* adult males in our 2.0-second contact bioassays was not unexpected.

Variation in male reproductive success, which was measured by the number of eggs laid by females paired with treated males and the percentage of these eggs that hatched, thus the fertility of the eggs, is noteworthy and can also be explained by the nature of the brief contact bioassay used. Some experimental units with dead males at 24-hr post-treatment also had dead females and few or no eggs (female mortality data not reported), which suggests that insecticide-contaminated males were able to transfer a lethal dose of toxin to a female during courtship and copulation. Unmated *P. interpunctella* females are known to lay no or only a few unfertilized eggs over several days (Bell 1981); however, unmated or chemically intoxicated female *P. interpunctella* were observed laying eggs in the laboratory. The highest oviposition levels in the experiments reported here were associated with non-treated control males; however, some treatments that resulted in high male mortality also had high levels of oviposition. Interactions of males and females were not observed or recorded during the 24-hr holding period after treatment of males, but it is likely mating occurred in some cases prior to death of the treated male, which then resulted in substantial oviposition by the associated female. Subsequent low levels of egg-hatch for some treatments, such as 50% to 70% hatch for females paired with insecticide-treated males compared to 92% hatch for females from non-treated controls (Table 2), may have been due to incomplete fertilization resulting from male intoxication. In some cases the females may have laid substantial numbers of unfertilized eggs, which

did not hatch, as a spontaneous abortion effect in response to stress from insecticide intoxication before death.

The type of material comprising the surface treated with a given insecticide significantly impacted the residual toxicity of insecticides at 0, 4, and 8 weeks after application of the toxicants. Such negative effects on residual activity may be attributed to physical or chemical reactions of the surface with the insecticides to cause degradation or loss of material from the surface. It was demonstrated that pyrethroids and pyrethrins are stable at acid and neutral pH, but they begin to hydrolyze under alkaline conditions (Laskowski 2002). This study showed that the painted surface and the bare wood surface had the lowest performance for residual toxicity of most test compounds after time 0. Only Pyrethrin at the high rate had any activity on the painted surface against adult males at time 0, but this activity was lost in subsequent bioassay times. Low toxic activity was also associated with the treatments on the bare wood surface at all three testing times, except for the high rate of Permethrin, which persisted with greater than 50% mortality after 8 weeks. Treatment surfaces have been shown to be important in variation of toxic activity for other insects (Giga and Caunhao 1991) and this may be attributed to a physical or chemical reaction of the substrate with the insecticide that lowers the activity. Further research is needed to determine the basis for surface effects on toxicity of these compounds against *P. interpunctella* males. In any case, it is clear that painted or wooden surfaces should be avoided in developing attract-and-kill technology for *P. interpunctella*.

The synthetic pyrethroids Cyfluthrin and Permethrin, and the naturally derived pyrethrins lacking PBO, applied to plastic-coated paper, metal or plastic surfaces at

various concentrations had the highest mortality against adult male Indianmeal moths in these studies compared to other insecticides. Permethrin applied at the high rate of 2.0% A.I. in the final spray suppressed adult males of *P. interpunctella* and prevented substantial reproduction for up to 8 weeks when applied to surfaces of plastic-coated paper, bare metal, bare plastic and to a lesser degree on bare wood. Residual activity of pyrethroids and pyrethrins was very poor when applied to a painted surface, and this maybe was from a lipophilic action that can result following addition or mixing of the active ingredient to plastic, the material under the paint in this formulation. Wheelock *et al*. (2005) showed that up to 50% of the pyrethroids can adsorb to plastic containers in 24-hr, and reduce toxic effect by 50% in 4-hr in *Ceriodaphnia dubia*.

The study clearly shows that attract-and-kill formulations to control *P. interpunctella* for up to 8 weeks can be developed using adequate application doses of Permethrin to a variety of surfaces. The attract-and-kill method is desirable for reduced input of insecticides in food storage areas because the specific pest is targeted via the pheromone lure to contact a small amount of an effective, locally contained, killing agent.

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Table 1. Insecticide active ingredients used in the initial test of contact toxicity against adult male *P. interpunctella* for attract-and-kill formulations, listed by insecticide classification.

Active Ingredient	Percent (%) [A.I] at label rate[1]	Manufacturer or Supplier
Organophosphates		
Malathion	2.2	Drug and Chemical Co.; Floral Park, NY
Chlorpyriphos methyl	1.0	Gustafson, McKinney; TX
Dichlorvos	1.0	Biotech Co., Painesville; OH
Pyrethroids		
Cyfluthrin	0.05	Bayer Crop Sciences; Kansas City, MO
Permethrin	0.5	Gustafson; McKinney, TX
Deltamethrin	0.06	Gustafson; McKinney, TX
Fiproles		
Fipronil	0.12	Aventis; Montvale, NJ
Nicotinoids		
Imidacloprid	0.1	Gustafson; McKinney, TX
Microbials		
Abamectin	0.2	Novartis; Greensboro, NC
Spinosad	0.17	Gustafson; McKinney, TX
Botanicals		
Azadirachtin	0.01	AMVAC; Los Angeles, CA
Pyrethrin “organic”	0.2	MGK Co.; Minneapolis, MN
Pyrethrin +PBO	0.01	Whitmire Micro-Gen; St. Louis, MO

[1] Amount reported is concentration in the final spray mix derived from product label instructions for mixing and application to surfaces of a given area.

PBO = Piperonyl Butoxide

Table 2. Oviposition and the percentage of eggs that hatched per *P. interpunctella* female following pairing with males that had been treated for 2.0-second in the initial contact toxicity test with 13 insecticides.

Treatments	Mean No. Eggs Laid (±SE)[1]	Mean % Egg-Hatch (±SE)[1, 2]
Malathion	31.5 ± 7.1 b	64.1 ± 15.3 ab
Chlorpyriphos methyl	37.7 ± 3.4 b	59.5 ± 9.8 b
Dichlorvos	32.5 ± 13.4 b	45.7 ± 7.0 b
Cyfluthrin	24.5 ± 2.9 bc	72.8 ± 6.5 ab
Permethrin	7.8 ± 1.3 c	53.5 ± 9.7 b
Deltamethrin	7.3 ± 2.1 c	73.7 ± 4.7 ab
Fipronil	19.9 ± 6.6 bc	71.0 ± 13.5 ab
Imidacloprid	22.7 ± 9.3 bc	66.8 ± 9.7 ab
Abamectin	24.4 ± 8.1 bc	57.1 ± 12.9 b
Spinosad	31.9 ± 4.9 b	65.9 ± 7.3 ab
Azadirachtin	33.7 ± 14.9 b	74.9 ± 15.7 ab
Pyrethrin “organic”	7.1 ± 2.7 c	67.3 ± 11.2 ab
Pyrethrin + PBO	25.3 ± 10.4 bc	60.2 ± 10.3 b
Non-treated control	76.4 ± 8.1 a	92.0 ± 2.3 a

[1]Means within a column having the same letter are not significantly different. Treatment means were compared by protected pair-wise t-test, $\alpha = 0.05$; egg laying ($F_{13, 42} = 4.42$, $P = 0.0001$), and egg hatching ($F_{13, 42} = 7.22$, $P < 0.0001$).

[2] Percentage data for egg hatching were arcsine square-root transformed prior to analysis.

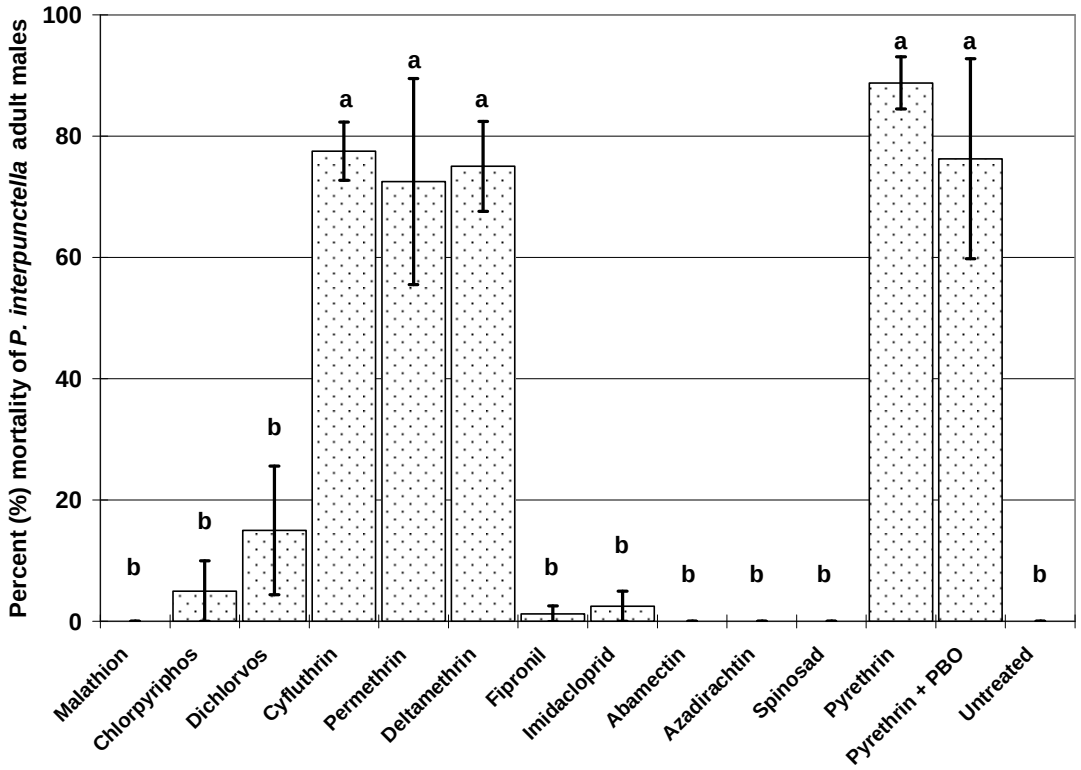

Fig. 1. Mean percentage (%) mortality of *P. interpunctella* adult males after 2.0-second contact with the treated surface of a plastic Petri dish. Bars with the same letter are not significantly different. Percentage data were transformed by the arcsine square-root method and analyzed by ANOVA; treatment means were compared by protected pair-wise t-test, $\alpha = 0.05$. $n = 10$ ($F_{13, 42} = 21.70$, $P < 0.0001$).

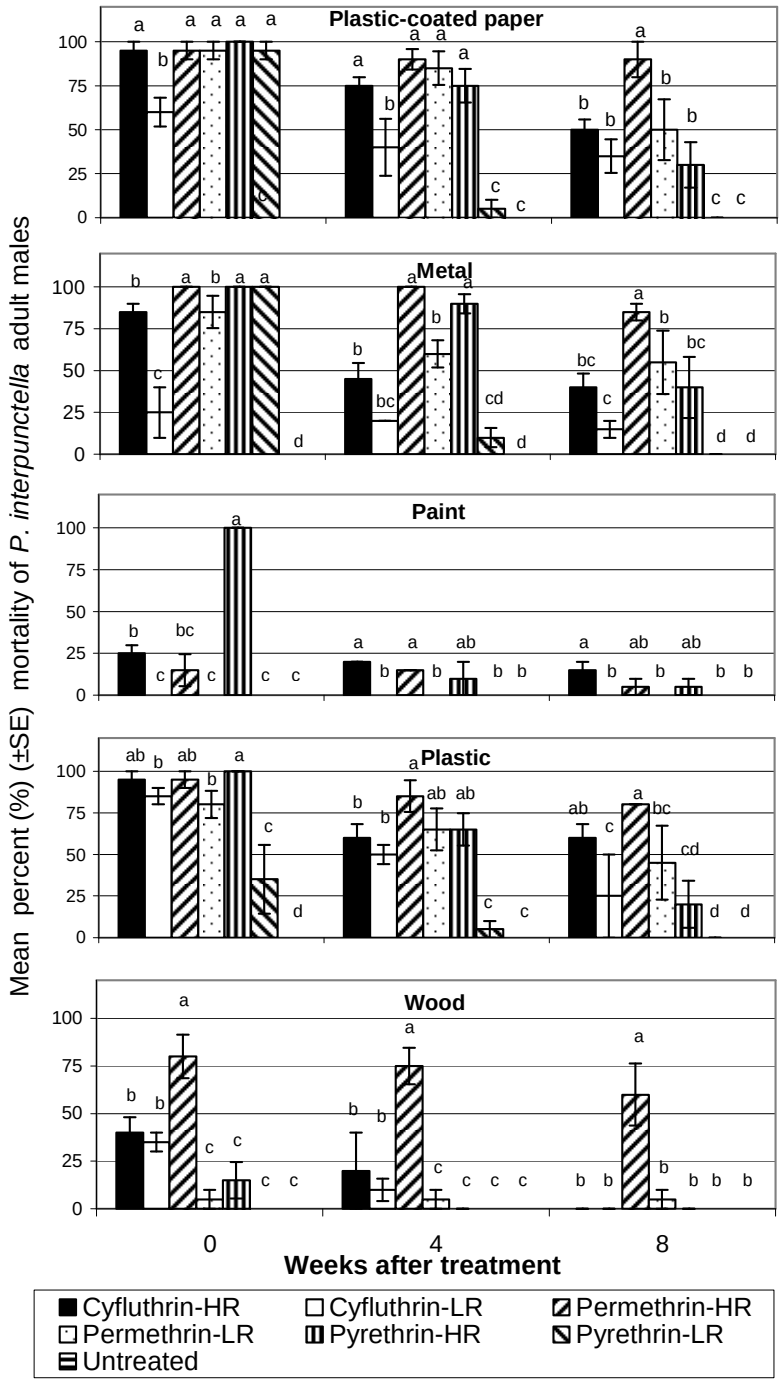

Fig. 2. Mean percent (%) (±SE) mortality of *P. interpunctella* adult males after 2.0-second contact tests with Cyfluthrin, Permethrin or Pyrethrin (no PBO) at label rates (LR) and at a higher rate (2.0% A.I., HR) on plastic-coated paper, metal, painted plastic, unpainted plastic, and bare wood surfaces at three time periods after treatment. Percentage data were transformed by the arcsine square-root method. Means within a time period on a given surface with the same letter are not significantly different (pair-wise t-test protected by the SLICE option in a LSMEANS statement, $F_{48, 210} = 3.41$; $P < 0.0001$).

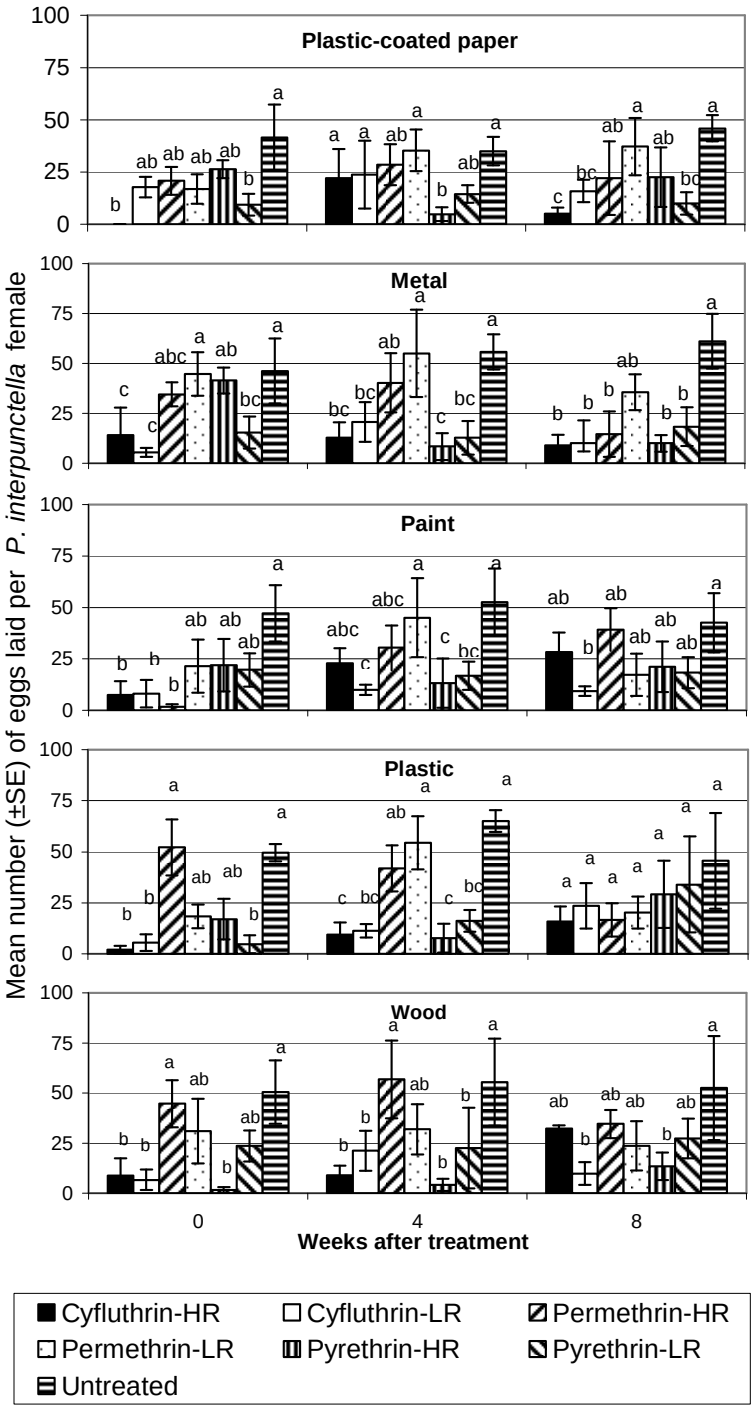

Fig. 3. Mean number (±SE) eggs laid per *P. interpunctella* female after pairing with males from the contact test of active ingredients Cyfluthrin, Permethrin or Pyrethrin (no PBO) at label rate (LR) and at a higher rate (2.0% A.I., HR) on plastic-coated paper, metal, painted plastic, unpainted plastic, and bare wood surfaces. Means within a time period on a given surface with the same letter are not significantly different within weeks (pair-wise t-test protected by the SLICE option in a LSMEANS statement, $F_{6, 105}=18.14$; $P < 0.0001$).

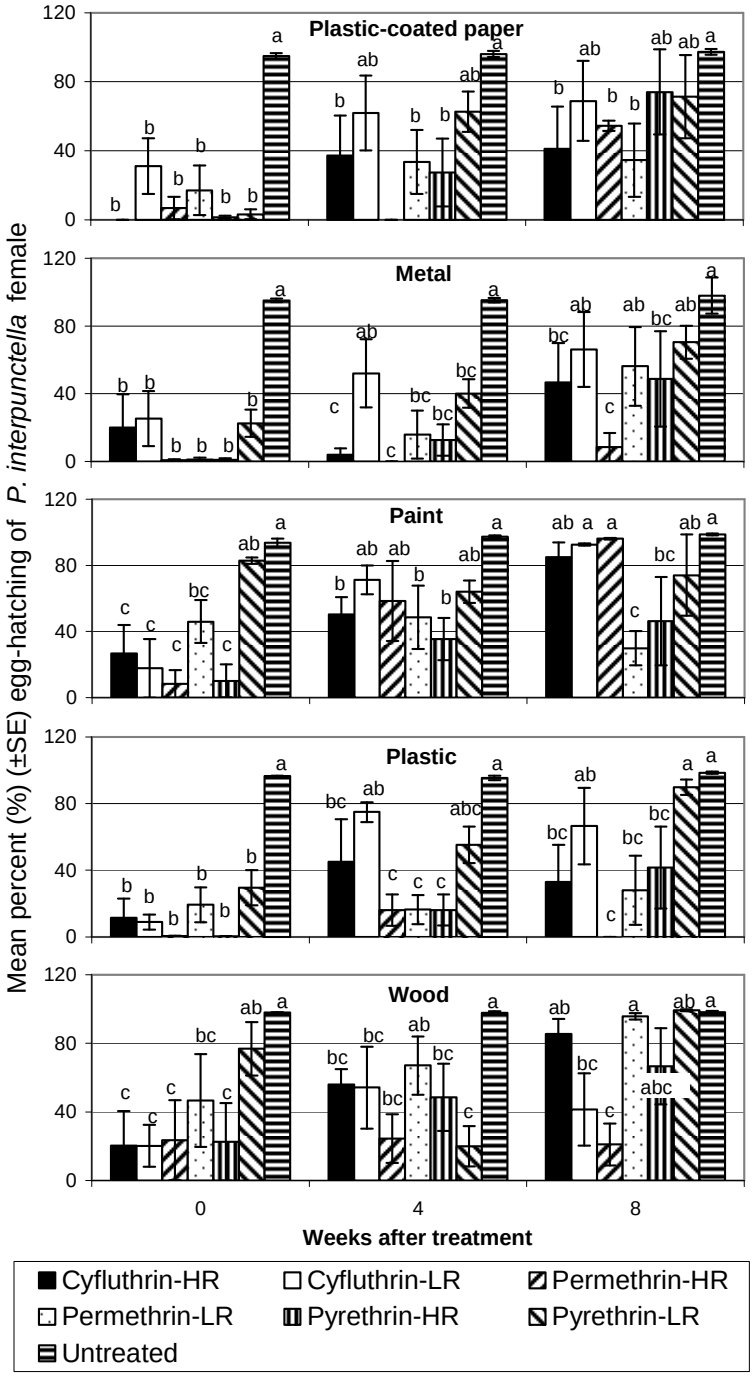

Fig. 4. Mean percent (%) (±SE) eggs hatched from those laid by *P. interpunctella* following pairing with males in the contact test of active ingredients of Cyfluthrin, Permethrin or Pyrethrin at label rate (LR) and at a higher rate (2.0% A.I., HR) on plastic-coated paper, metal, painted plastic, unpainted plastic, and bare wood surfaces. Percentage data were transformed by the arcsine square-root method. Means with the same letter are not significantly different within weeks (pair-wise t-test protected by the SLICE option in a LSMEANS statement, $F_{6, 105}$= 17.78; $P < 0.0001$).

CHAPTER III

LABORATORY EVALUATION OF ATTRACT-AND-KILL FORMULATIONS AGAINST INDIANMEAL MOTH, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

Abstract

The responses of Indianmeal moth males (IMM) *Plodia interpunctella* (Hübner) to attract-and-kill formulations (a gel, a flat wax panel, and a plastic cylinder device), mixed or sprayed with the pyrethroid insecticides permethrin and cyfluthrin, and organically compliant natural pyrethrin, combined with the synthetic female sex pheromone "ZETA", were evaluated in a laboratory wind tunnel. The wax panel and cylinder, which utilized controlled-release pheromone lures, were more attractive to IMM males over the course of an eight-week aging period than was the gel, which had the pheromone incorporated into the gel matrix. The contact time of responding males was higher on the wax panel and plastic cylinder than on the gel formulation. The percentage of mortality of males was higher with wax panels formulated with Cyfluthrin 6.0% [AI], Permethrin 6.0% [AI] and the cylinder formulated with Cyfluthrin 2.0% [AI], compared to the gel over the eight-week study. These same formulations had the greatest impact on egg laying by females paired with treated males and on the percent of eggs that hatched. Of all the attract-and-kill formulations tested, the most promising for field applications to suppress IMM pest populations was the wax panel containing 6.0% of either cyfluthrin or permethrin.

Key words: Wind tunnel, attract-and-kill, pheromone, stored-products, longevity

INTRODUCTION

Pheromone-based pest management techniques like mating disruption, mass trapping and attract-and-kill have been developed as alternatives to traditional insecticides applications to control important pest Lepidoptera. All of these techniques use synthetic sex pheromones to attract males or otherwise interrupt male mating behavior. However, the most common use of synthetic pheromones for stored product moths is for monitoring populations, and this has become part of the integrated pest management programs for these pests (Burkholder and Ma 1985, Vick *et al*. 1981, 1986; Mullen *et al*. 1991, Phillips *et al.* 2000). The predominate female pheromone of *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) is *(Z,E*) -9,12 tetradecadienyl acetate, commonly referred to as ZETA (Brady *et al*. 1971, Kuwahara *et al*. 1971, Kuwahara and Casida 1973, Sower *et al*. 1974, Soderstrom *et al*. 1980, Teal *et al*. 1995 and Zhu *et al*. 1999). Studies with pheromones have used wind tunnels as helpful tools to observe and understand behavior of moths. Schofield *et al*. (2003) showed in wind tunnel studies that the concentration of female sex pheromone influenced the flight track, course and drift angles of *Cadra cautella* males. Upwind flight, landing and wing fanning of Brazilian apple leaf roller moth males, *Bonagota cranodes*, were observed up to 72% in response to calling females in a wind tunnel (Coracini *et al*. 2003). Furthermore, behavioral effects such as wing-fanning, take-off, upwind flight, landing and touching the odor source were the subject of a recent study of responses to attract-and-kill formulation for the Indianmeal moth, *P. interpunctella* (Nansen and Phillips 2004), Oriental fruit moth, *Grapholita molesta* (Busk) and Codling moth, *Cydia pomonella* (Evenden and McLaughlin, 2005).

The attract-and-kill, or "attracticide", method of pest control incorporates an attractant of a target insect with an insecticide in order to kill large numbers of insects and ultimately reduce a pest population (Lanier 1990). A wind tunnel bioassay can be valuable in the assessment of the impact of attract-and-kill formulations on male mortality and reproduction. The LastCall® gel (IPM Tech, Inc., Portland, OR), which is a combination of pheromone with the synthetic pyrethroid Permethrin in a gel matrix, was formulated to control Oriental fruit moth, *Grapholita molesta* (Evenden and McLaughlin 2004, 2005, Evenden *et al*. 2005), Codling moth, *Cydia pomonella* (L.) (Krupke *et al*. 2002, Evenden and McLaughlin 2005) and Indianmeal moth, *Plodia interpunctella* (Nansen and Phillips 2004). Formulations against codling moth, *Cydia pomonella* (L.) with Codlemone at 0.065% reached maximal catch rates, and the percentage of mortality was up to 100% with Cyfluthrin at 0.1 to 10% [AI] in the gel (Lösel *et al*. 2000). Phelan and Baker (1987) used a wind tunnel for studies with navel orangeworm, *Amyelois transitella* females and found that upwind attraction to crude almond oil was reduced as result of increasing the percentage of the insecticides Permethrin and Cypermethrin at 1.0% each, but not with Fenvalerate at 2.5% [AI], for which the efficacy was 90% mortality. The density of point sources of attracticides was tested in a wind tunnel by Miller *et al*. (1990), who observed that high densities of Nomate pheromone (16 fibers per m^2) caused higher mortality of pink bollworms than 1 to 4 fibers per m^2.

The Indianmeal moth, *Plodia interpunctella* (Hübner), is one of the most serious stored-product insect pests of value-added food products worldwide, and there is substantial interest in developing safe and effective alternatives to traditional residual and

fumigant chemical control of this pest (Arthur and Phillips 2003, Phillips 2006). Efficacy of the attract-and-kill method, in which pest reproduction is impacted after large numbers of males are killed following contact with point-sources that have pheromone combined with an effective contact insecticide, was demonstrated for *P. interpunctella* by Nansen and Phillips (2004). However, that study examined only one attract-and-kill gel-based formulation, and it did not assess the activity of the tested gel formulation over time. A study with aged gel formulations would have helped predict the time period over which it would remain active in practical pest control applications. Recent experiments (Chapter 2 of this dissertation) evaluated a variety of contact insecticides against male *P. interpunctella* for residual activity, and determined that natural pyrethrum and synthetic pyrethroids had very good activity in simple surface-contact bioassays.

In the current study we evaluated the efficacy of three types of attract-and-kill devices, each with a variety of insecticide formulations, for residual activity against *P. interpunctella* males in a wind tunnel. The efficacy of these attract-and-kill formulations was based on male contact with the formulation following upwind flight, male mortality, and egg laying by females paired with these males, and egg hatching. In addition, we evaluated the residual activity of these attract-and-kill formulations at five different times for a period of eight weeks.

MATERIALS AND METHODS

Insects. *P. interpunctella* male and female adults from the laboratory culture at Oklahoma State University were reared on a diet containing corn meal, chick starter/grower crumbles, all mash egg crumbles and glycerol (4:2:2:1 by volumetric ratio) in 425 ml glass jars (Alltrista, Muncie, IN) placed in a growth chamber at 28 °C, 60-70 % R.H., and 16:8 hr (L:D photoperiod). Corrugated cardboard rolls (1 × 5 cm) were placed into the culture jars for the last-stage wandering larvae to crawl into and pupate. The pupae were removed from the cardboard rolls, separated by sex and placed individually into 1-dram vials with ventilated plastic caps (Fisher Scientific, Pittsburg, PA) and returned to the growth chamber until they emerged as adults. For these experiments, 1-2 day-old virgin adults were used and each adult was used only once.

Wind tunnel. The wind tunnel used consisted of a wood frame (W = 91 cm, H = 91 cm, L = 182 cm) with side walls and roof made of rigid Plexiglass®. The floor of the tunnel was an aluminum sheet and the two ends of the tunnel were covered by conventional window fly screening to prevent escape of moths. The down-wind end of the tunnel had a plenum that reduced the 91- × 91-cm square opening to a 38 cm diameter circular opening with an exhaust fan driven by an electric motor equipped with a rheostat to adjust exhaust wind speed. Air was exhausted from the tunnel via a 38-cm-diameter pipe directly out of the room and to the exterior of the building so that contaminated air could not re-enter the tunnel. Room air was drawn into the tunnel at the upwind end by the suction of the exhaust fan and passed through an activated charcoal-impregnated filter to provide relatively clean air to the tunnel for flight assays. Wind speed in the tunnel was measured with smoke tests using titanium tetrachloride and was set at 60 cm/sec for all

tests, which was observed to give relatively even laminar flow through the central core of the tunnel from upwind to downwind end. Insects and test materials were prepared in a separate room and only brought into the wind tunnel room when a specific test was to be conducted, to minimize contamination of room air between assays. Controlled conditions maintained in the wind tunnel room were 26-28 °C, 50-60% R.H., and lights provided by four fluorescent tubes, 60 W each, suspended over the tunnel roof.

Formulations Tested and Experimental Procedures. Three sets of experiments, each one with a different type of attract-and-kill formulation, were conducted. The first tested was LastCall® gel (IPM Tech, Inc., Portland, OR) with the following formulations that each contained the synthetic female pheromone "ZETA" at 0.16% by weight: Permethrin 6.0% [AI], Pyrethrin 6.0% [AI], and gel with no insecticide but with ZETA only as an attractant to serve as a non-insecticide control (blank). These formulations were tested as droplet sizes of 50- or 100-mg applied to the surface of a glass microscope slide (7.6 × 2.5 cm, Sargent-Welch, U.S.A.) and held in place at the upwind end of the tunnel with a small binder clip (ACCO, U.S.A.) suspended from a laboratory stand. The second attract-and-kill formulation was a wax panel (20 × 13 cm; Suterra, Bend, OR.) that contained the active ingredient cyfluthrin at 0.01, 0.1, 1.0 and 6.0% [AI] or permethrin 6.0% [AI] and deployed with a controlled release pheromone lure containing the pheromone "ZETA" (Biolure® by Suterra, Bend, OR) placed in the center of the wax panel; and a control wax panel, with no insecticide, deployed with the pheromone lure. The material structure of the wax panel was a paper fiberboard panel that was coated with a mixture of paraffin and oil that contained the insecticide. The Biolure® pheromone release device was a sealed, thin foil pouch for which the bottom and most of the top

surface were impermeable film that contained a reservoir of liquid pheromone, and the pheromone was evaporated through a semi-permeable membrane that controlled the release rate by its size and structure. Finally, the third device was a plastic mesh cylinder (7 mm mesh; 35-cm height ×10-cm dia.; Uniek Co., U.S.A.). Insecticides were sprayed onto the cylinders until run-off with an artist's air brush (Paasche, U.S.A.), and were either permethrin (FMC Co., Philadelphia, PA), cyfluthrin (Bayer, Kansas City, MO) or organically-compliant pyrethrin without PBO (Pyperonyl Butoxide; McLaughlin Gormley King Co., Minneapolis, MN), each at 2% [AI] in the final mix and deployed with a Suterra Biolure® in the middle of the cylinder. A cylinder without insecticide, but with a pheromone lure was used as a control. Attract-and-kill devices were suspended on a laboratory stand at the mid-point of the upwind end of the wind tunnel.

Two-day old virgin adult male *P. interpunctella* were released from a cage held on a laboratory stand at the middle of the downwind end of the tunnel. Each male moth was given five minutes to take flight and respond upwind to the device and contact it. Moths that did not touch the device in five minutes were considered as "no response" and scored 0 for purposes of analysis; and those males that contacted the device were scored as responders. The percentage of moths in a test group contacting each device, and time each male was in contact with a device, were recorded. Once a male finished contact and flew away from the device it was captured and placed into a glass jar with a virgin female and 15 g of wheat kernels as a substrate for egg laying. Every male-female pair was kept for 24-hr in a growth chamber at 28 °C, 60-70 % R.H., and a 16:8 hr (L:D) photoperiod. Male mortality was recorded after 24 h. Eggs laid in the wheat were carefully separated from the wheat using a U.S. no. 14 sieve (Seedburo Equipment Company, U.S.A),

counted and placed on double-sided tape on a 9-cm-diameter black filter paper (Ahlstrom, Mt Holly Springs, PA.) in a 9-cm-diameter Plastic Petri dishes (Fisher Scientific, Canada). The eggs were placed into a growth chamber at 28 °C, 60-70 % R.H., and 16:8 hr (L:D) photoperiod for five days, after which the number hatched was recorded.

Statistical Analysis. Data for each of the three attract-and-kill formulations, which were the gel, the wax panel and the plastic cylinder, were analyzed as three separate experiments within a time period, and comparisons were made for each specific formulation (e.g., applied insecticide concentration of a particular device type) across time periods. Each device formulation type was treated with different concentrations of insecticides and four replicates of each device type-insecticide concentration were established. A group of five adult males were released in the wind tunnel and bioassayed against each replicate of each device type, for a total of up to 20 males being tested against each device type. Each adult male in a group of five was released individually and used only once. The attract-and-kill formulations were tested in the wind tunnel at 0, 4, 6, and 8 weeks after being established, and they were held and aged in a room separate from the wind tunnel between testing times. The experimental design used for each attract-and-kill formulation was a randomized complete block design with four replicates. The observations assessed were the percentage of released males that landed on and made contact with the device, the time in seconds each adult male was in contact with a given device (contact time), the percentage of male mortality of those that made contact, the number of eggs laid per female, and the percentage of these eggs that hatched per female. Proportions (percentages) were transformed by the arcsine-square root function prior to

analysis. Data were analyzed with the procedure PROC MIXED in SAS/STAT 9 for Windows (SAS Institute 2005), and the repeated measures option assuming an autoregressive covariance structure was used. Every attract-and-kill device type was analyzed separately. Every treatment was compared across the test period times (0, 2, 4, 6, or 8 weeks) and treatment differences were compared within each time period. Treatments compared across and within each time period were analyzed with pair wise t-tests and comparisons were protected by examining the SLICE OPTION within the Least Square Means statement at $\alpha = 0.05$ level.

RESULTS

Device Contact. Table 1 reports the mean percentage of *P. interpunctella* adult males that contacted the attract-and-kill devices in five minutes. Statistical analyses of the LastCall® gel formulations across the entire eight-week experiment did not show significant differences for contact behavior in the wind tunnel among the two insecticide active ingredients and blank gels ($F_{2, 567} = 1.82$; $P = 0.1633$), the amount tested, 50 mg or 100 mg each, for Permethrin and Pyrethrin in the gels ($F_{1, 567} = 0.04$; $P = 0.8492$), interactions of insecticide treatment and weeks ($F_{8, 567} = 1.69$; $P = 0.0970$), interactions of amounts of the two insecticide gels and weeks ($F_{4, 567} = 0.58$; $P = 0.6781$), and interactions among insecticide active ingredient, amount of the gel used and weeks of aging of the gel formulations ($F_{8, 567} = 1.24$; $P = 0.2750$). However, there was a significant interaction effect of active ingredient tested and the two amounts of gel for each AI ($F_{2, 567} = 4.73$; $P = 0.0092$). Landing and contact responses of moths varied significantly in some cases when compared across gel types within a given bioassay week, and also across weeks within a particular gel type. At week 0, the highest percentage of landing by male moths was 55% for the Blank 100 mg and Permethrin 50 mg, and the lowest was on Permethrin 100 mg at 20%. At week 2, 4, and 6, there was no significant difference in percent contact among treatments. By week 8 responses to the gels were very low, but with some difference among treatments (Table 1). When comparing across bioassay times the maximum landing by males on the gel formulations was observed at week 2, when responses ranged from 70 to 85%. Analysis of the percentage of males landing on the wax panel devices showed no statistical difference among treatments for the whole experiment ($F_{5, 567} = 1.01$; $P = 0.4117$), nor for

treatments within weeks ($F_{20, 567} = 1.30$; $P = 0.1733$). The comparison of the moths that contacted the device across the eight-week period shows that at week 0, less than 25% of moths landed on the device. This response increased from 90 to 100% from week 2 to the end of the experiment. At week 0, the formulations Blank and Cyfluthrin at 0.01 and 0.1% elicited 25% or less of landing, which were statistically different from the Cyfluthrin 1.0 and 6.0%, and Permethrin 6.0%, at 5% landing for each (Table 1). However, Cyfluthrin 0.1% was statistically similar to Cyfluthrin 1.0%. At week 2, all treatments reached a 90 to 100% of landing and it was similar up to the end of the experiment at week 8. The percentage of moths landing on the plastic cylinder was observed to be significantly different among treatments overall ($F_{3, 377} = 3.74$; $P = 0.0113$) and treatments within weeks ($F_{12, 377} = 2.18$; $P = 0.0121$). At week 0, Blank and Cyfluthrin 2.0% showed attractiveness of 50 and 60% respectively, significantly greater than the other treatments. At week 2, Cyfluthrin and Pyrethrin 2.0% elicited 85 and 80% landing, respectively, and were statistically similar. Pyrethrin 2.0% did not differ from Blank (65% landing). However, these treatments differed from Permethrin 2.0%, which showed the lowest landing rate of 45% in week 2. From week 4 to the end of the experiment at week 8, all treatments elicited 100% landing by tested males onto the plastic cylinder devices. These plastic cylinder devices used the same commercial pheromone lures as the wax panel formulations, and similar patterns of response were observed during other weeks for the two devices. At week 0, there was low response and from week 4 to the end of the experiment there was 100% landing of all 20 males (5 males in replicates) for all wax panel and cylinder devices.

Contact time. The contact time, which was the time in seconds that adult males were in contact with devices tested, is shown in Table 2. The gel-like formulations all had relatively short contact times and did not show significant differences among amounts of gel (i.e., 50 mg vs. 100 mg; $F_{1, 570} = 0.19$; $P = 0.6594$), in the interaction of gel amount and week of the bioassay ($F_{4, 570} = 0.96$; $P = 0.4300$), or in the interaction among treatment AI, amount of gel and week ($F_{8, 570} = 1.66$; $P = 0.1059$) for the whole experiment. However, the AI treatments were significantly different ($F_{2, 570} = 3.69$; $P = 0.0255$), AI treatments within weeks among gel types ($F_{8, 570} = 2.20$; $P = 0.0259$) and amount of gel within weeks ($F_{2, 570} = 5.67$; $P = 0.0036$) for moth contact time over the whole experiment. All treatments, when analyzed across the eight-week period, showed the highest contact time at week 2, and they were significantly different from the rest of the weeks. At week 0 the gel formulations Blank 100 mg and Permethrin 50 mg were statistically similar and showed the highest contact time (0.8 and 0.75 seconds, respectively), but they differed statistically from Pyrethrin 50 mg and Permethrin 100 mg, which had the lowest contact times. All these treatments were statistically similar to the rest of the treatments. At week 2 Permethrin 50 mg showed the longest contact time, with a mean of 1.5 seconds, and it was significantly different from the formulations with Pyrethrin 50 and 100 mg. At week 4, all treatments were statistically similar, and at weeks 6 and 8 the contact times were very brief and differences were slight among gel types, though statistically significant. Male contact time on the wax panel formulations revealed that there was significantly different among AI treatments overall ($F_{5, 570} = 2.23=$; $P = 0.0498$) and among treatments within weeks ($F_{20,570} = 3.44$; $P < 0.0001$). At week 0, all treatments had very short contact times when compared to the rest of the

weeks and were statistically similar. At week 2, Cyfluthrin at 6% had the longest mean contact time of 13.8 seconds, while Permethrin at 6.0% had the shortest time of 7.1 seconds. Conversely, at week 4 Permethrin at 6.0% had the longest contact time at 12.4 sec, while Cyfluthrin at 0.01% had the lowest contact time at 5.6 sec. At week 6, Cyfluthrin 0.01%, Cyfluthrin 0.1% and Permethrin 6.0% did not differ statistically, but they were significantly different from Cyfluthrin 1.0% and 6.0%. Cyfluthrin 6.0% and Permethrin 6.0% did not differ statistically from the rest of the treatments at week 6. Contact times on wax panels at week 8 were statistically similar to those observed at week 6. Contact times for the plastic cylindrical device formulations showed a significant difference among AI treatments overall ($F_{3, 380} = 8.58$; $P < 0.0001$) and AI treatments within weeks ($F_{12, 380} = 1.82$; $P = 0.0436$). As with wax panels, contact times on plastic cylinders were short at time 0 and then were longer in most cases from bioassay time 2 weeks through 8 weeks, with the longest mean contact time observed for males on cylinders with Cyflthrin at 2.0% AI.

Male mortality after contact. Table 3 shows the percentage mortality of adult male *P. interpunctella* 24-hr after contacting the attract-and-kill devices. For the gel formulation there were no significant difference among amounts of gel ($F_{1, 567} = 0.60$; $P = 0.4380$), interaction of gel amounts within week ($F_{4, 567} = 0.14$; $P = 0.9664$), interaction of AI treatment by gel amount ($F_{2, 567} = 1.96$; $P = 0.1422$), or AI treatment by gel amount by week ($F_{8, 567} = 1.62$; $P = 0.1152$). However, there were significant differences among treatments ($F_{2, 567} = 35.86$; $P < 0.0001$) and treatments within weeks ($F_{8, 567} = 6.99$; $P < 0.0001$). Regardless of overall differences, the highest mortality was observed only in week 2 with gel containing 6.0% Permethrin, at 70%, and in subsequent

bioassay times the male mortality levels were relatively low, ranging from 0% to 40%. The wax panel formulation revealed significant differences among AI treatments ($F_{5, 567}$ = 196.37; $P < 0.0001$) and for the interaction of AI treatments by weeks ($F_{20, 567}$ = 12.11; $P < 0.0001$). At week 0, all AI treatments were statistically similar with very low mortality. However, from week 2 to the end of the experiment at week 8 the formulations based on Cyfluthrin and Permethrin both at 6.0% AI, which were statistically similar, killed over 85% of the adult males, followed by Cyfluthrin 1.0%, which differed statistically from the rest of the treatments, which had only 0% to 10% mortality. The attract-and-kill formulations based on the plastic cylinder showed a significant difference in male mortality among AI treatments ($F_{3, 380}$ = 78.15; $P < 0.0001$), but the interaction of AI treatments by weeks was not significantly different ($F_{12, 380}$ = 1.38; P = 0.1732). The cylinder device sprayed with Cyfluthrin 2.0% elicited significantly higher levels of mortality compared to the other treatments, and it killed 75% or more of the adult males during the whole experiment, except for week 0 in which it killed 50% on average.

Egg laying. Table 4 shows the mean egg laying per female *P. interpunctella* that were paired for 24-h with males that had contacted attract-and-kill devices in wind tunnel bioassays. The statistical analysis for the gel formulation showed that there was no significant difference among AI treatments ($F_{2, 570}$ = 2.75; P = 0.6877), amount of gel ($F_{1, 570}$ = 0.20; P = 0.6558), interaction of AI treatment by amount of gel ($F_{2, 570}$ = 0.008; P = 0.9247), interaction of AI treatment by week ($F_{8, 570}$ = 0.70; P = 0.6877), interaction of amount of gel by week ($F_{4, 570}$ = 0.41; P = 0.7981) and interaction of AI treatment by amount of gel by week ($F_{8, 570}$ = 0.28; P = 0.9721). Treatment differences were found

only for week 2, in which the formulations with Pyrethrin 100 mg and Permethrin 100 mg showed the lowest averages of egg laying and were significantly different from the Blank formulations (50 and 100 mg). However, all the remaining formulations did not differ from each other and the numbers of eggs laid by females paired to males that had contacted gels were relatively high. In the case of the wax panel, there was a significant difference among AI treatments ($F_{5, 570} = 35.85$; $P < 0.0001$) and with the interaction of AI treatments by weeks ($F_{20, 570} = 3.28$; $P < 0.0001$). At week 0, there was no significant difference among treatments. From week 2 to week 8 the wax panel formulations with Cyfluthrin 1.0% and 6.0%, and Permethrin 6.0% were statistically similar and elicited low egg laying averages compared to Blank and the formulations with low percentage of Cyfluthrin (0.01 and 0.1% AI), which averaged over 35 eggs laid per female. Females paired with males that had been bioassayed against the cylinder devices showed a significant difference in egg laying among the AI treatments ($F_{3, 380} = 28.98$; $P < 0.0001$), but there was no significant interaction effect of the AI treatments by weeks ($F_{12, 380} = 0.87$; $P = 0.5746$). In the whole experiment, the Blank treatment showed the highest egg laying and was significantly different from the rest of the treatments, except at week 8, in which it was similar to Permethrin 2.0%. The Cyfluthrin 2.0% generally had the most suppressive effect on number of eggs laid per female.

Egg hatching. Responses to the gel formulations revealed a significant difference among AI treatments ($F_{2, 569} = 10.21$; $P < 0.0001$) in the percentage of eggs that hatched from those laid by females paired with males from bioassays (Table 5). However, there were no significant differences among gel amount ($F_{1, 569} = 0.01$; $P = 0.9492$), interaction of AI treatment by gel amount ($F_{2, 569} = 1.79$; $P = 0.1679$), interaction of AI treatment by

week ($F_{8, 569} = 1.82$; $P = 0.0706$), interaction of gel amount by week ($F_{4, 569} = 0.40$; $P = 0.8098$) and interaction of AI treatment by gel amount by week ($F_{8, 569} = 0.38$; $P = 0.9299$). There were no significant differences among AI treatments at weeks 0 and 8. In the other weeks there were statistically significant reductions in egg hatching in clutches from insecticide-treated gels, but these were not substantial. The experiment with the wax panel showed significant differences in egg hatch among AI treatments ($F_{5, 570} = 45.57$; $P < 0.0001$) and in the interaction of AI treatments by week ($F_{20, 570} = 4.05$; $P < 0.0001$). Permethrin 6.0% and Cyfluthrin 1.0% and 6.0% were the treatments with lower percentage of hatched eggs in most of the dates and these three were statistically similar at the eight-week period. In general, high concentrations of Cyfluthrin and Permethrin on wax panels were associated with lower percent of egg hatching compared to the Blank and low percent AI of Cyfluthrin. The cylinder formulation showed experiment-wide significant differences in egg hatching among AI treatments ($F_{3, 377} = 37.38$; $P < 0.0001$). However, there was no significant interaction of AI treatments by weeks ($F_{12, 377} = 1.12$; $P = 0.3419$). The percentage of hatching of the insecticide treatments was significantly lower than Blank in weeks 4, 6, and 8. Hatch rates were the lowest resulting from AI treatments of Cyfluthrin 2.0% in week 2 and 4, being 8.4% and 8.0 %, respectively, and hatching ranged from 16.9% to 67.7% in other AI treatments.

DISCUSSION

The experiments reported here were conducted to determine what device design, pheromone release technology and insecticide formulation might be effective to pursue further for development of an attract-and-kill technology for *P. interpunctella*. Therefore the purpose of the eight-week period studied here was to examine a realistic time period in which a pest control company might apply a treatment to a facility requiring Indianmeal moth control. Since the ultimate goal of the attract-and-kill strategy is to kill enough males in a population to cause a negative impact on reproduction, these experiments provided an estimate of reproductive impact by killing or otherwise incapacitating male moths so that mating and reproduction with females could be reduced. The reproductive fitness of individual males that had contacted an attract-and-kill device was manifested by how many eggs were laid and 1st instar larvae (percentage of eggs that hatched) produced when they were paired with a virgin female immediately after treatment. Device design, pheromone release formulation and insecticide formulation all affected efficacy of the treatments in the experiments reported here.

For all three designs investigated here, the gel, the wax panel and the cylinder, the percentage of males landing on and maintaining contact with the device was consistently low at time 0, but then improved in subsequent weeks as the formulations aged. This delayed activity was probably due to the pheromone dispensing system being newly exposed to air at time 0; at the moment that they were opened from sealed packages (Biolure® lures) or applied from tubes (LastCall® gel), there was a relatively high release of pheromone. It has been shown that initial high release of pheromone can cause a repellency effect, or lack of complete response, instead of full attractive response and

sustained contact with the source (Hussain *et al*. 1994, personal observations). The data presented here show the gel formulations elicited very low contact response (20-55%) at time 0, peak responses at the 2-week bioassay (70-85%), and then a sharp decline in activity from week 4 to week 8. Thus, the gel formulation could not sustain activity for substantial male-killing through the eight-week study, and would probably be ineffective in a practical application for 8 weeks. Alternatively, the Biolure® pheromone lures used with the wax panel and cylinder devices had characteristic low activity for contact at time 0, but showed increased and sustained activity for male response from week 2 onward, with essentially 100% male contact and contact times of several seconds. Contact time with the devices was similarly much higher for the wax panels and cylinders that were baited with Biolure®, compared to the gel formulation, and this was maintained from week 2 until the 8-week end of the study.

The pheromone-based upwind responses of males and the geometric designs of the attract-and-kill devices seemed to have a substantial impact on male mortality and reproductive fitness. The low percent of landing and low contact time on the gel formulations with Permethrin and Pyrethrin killed only a modest number of adult males, but mortality was much higher for certain formulations of the wax panel and the cylinder device over the same time periods. Higher and sustained mortality levels for certain formulations of wax panels and plastic cylinders can be attributed to the more effective pheromone lure system, but also probably to the overall larger surface area of the device itself, compared to the small amount of material presented by the gel formulations. High contact times were recorded for moths responding to wax panels and cylinders, and it was observed that during these times the male moths would move around over the surface of

the device, which probably contributed to better contact with insecticide and the ultimate toxicity. Higher male mortality levels, specifically on the wax panels with 6.0% Cyfluthrin and 6.0% Permethrin, and on the plastic cylinder with 2.0% Cyfluthrin, corresponded to low levels of egg laying and low hatch rates of those eggs. These results suggest that the wax panel formulation would be very effective for Indianmeal moth suppression in practical applications. The results clearly indicate that higher concentrations, greater than 1.0%, of the synthetic pyrethroids Cyfluthrin and Permethrin result in the most effective attract-and-kill devices when the wax panel and plastic cylinder were used. Organically-compliant natural Pyrethrin at 2.0% was not effective enough on the plastic cylinder at any bioassay time during the eight-week period to pursue further applied research. Permethrin at 2.0% on the cylinder was also not effective compared to 2.0% Cyfluthrin, and this may have been due to physical or chemical interaction with the substrate that resulted in lowered activity compared to that of the same compound on another substrate (see Chapter 2). Future research will need to involve studies with formulations of high concentration Cyfluthrin or Permethrin on wax panels, or Cyfluthrin on plastic cylinders with *P interpunctella* populations in experimental or commercial food establishments.

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Table 1. Mean percentage (%) of *P. interpunctella* adult males (±SE) that landed onto three attract-and-kill devices in a wind tunnel during an eight-week period.

Formulation	Doses	Mean percentage (±SE) of *P. interpunctella* male moths that landed onto the device														
		Weeks after treatment														
		0			2			4			6			8		
Gel	Blank 50 mg	40	±11.2	B, ab	80	±9.2	A,a	20	±9.2	BC,a	25	±9.9	B,a	0	±0.0	C,b
	Blank 100 mg	55	±11.4	AB,a	70	±10.5	A,a	45	±11.4	AB,a	40	±11.2	B,a	30	±10.5	B,ab
	Pe 6% 50 mg	55	±11.4	B,a	85	±8.2	A,a	30	±10.5	B,a	20	±9.2	B,a	0	±0.0	B,b
	Pe 6% 100 mg	20	±9.2	B,b	75	±9.9	A,a	20	±9.2	B,a	15	±8.2	B,a	5	±5.0	B,ab
	Py 6% 50 mg	30	±10.5	BC,ab	70	±10.5	A,a	45	±11.4	AB,a	15	±8.2	C,a	35	±10.9	BC,a
	Py 6% 100 mg	30	±10.5	BC,ab	75	±9.9	A,a	45	±11.4	B,a	20	±9.2	BC,a	15	±8.2	C,ab
Wax panel	Blank	25	±9.2	B,a	90	±6.9	A,a	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a
	Cy 0.01 %	20	±9.2	B,ab	95	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a
	Cy 0.1 %	20	±6.9	B,ab	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a
	Cy 1 %	10	±5.0	B,bc	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a
	Cy 6 %	5	±9.9	B,c	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a
	Pe 6 %	5	±5.0	B,c	90	±6.9	A,a	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a
Plastic Cylinder	Blank	50	±11.5	B,a	65	±10.9	B,b	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a
	Cy 2%	60	±11.2	B,a	85	±8.2	A,ab	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a
	Pe 2%	30	±10.5	B,b	45	±11.4	B,c	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a
	Py 2%	30	±10.5	C,b	80	±9.2	B,b	100	±0.0	A,a	100	±0.0	A,a	100	±0.0	A,a

Means within rows followed by the same letter (Upper case) are not significantly different at $P < 0.05$. Means within columns for each formulation followed by the same letter (Lower case) are not significantly different at $P < 0.05$ by use of DIFF option in an LSMEANS statement in PROC MIXED in SAS. Percentage data were transformed by the arcsine square-root method. Analysis was conducted separately for each attract-and-kill formulation. Py = Pyrethrin, Pe = Permethrin, Cy = Cyfluthrin.

Table 2. Mean times, in seconds (±SE), that P. interpunctella adult male were in contact with three attract-and-kill devices in a wind tunnel during an eight-week period.

Formulation	Doses	Mean time (±SE) seconds that *P. interpunctella* male moths were touching the device													
		Weeks after treatment													
		0			2			4			6			8		
Gel	Blank 50 mg	0.6	±0.2	B,ab	1.4	±0.2	A,ab	0.3	±0.1	BC,a	0.3	±0.1	BC,ab	0.0	±0	C,b
	Blank 100 mg	0.8	±0.2	AB,a	1.2	±0.2	A,ab	0.6	±0.2	B,a	0.6	±0.2	B,a	0.6	±0.2	B,a
	Pe 6% 50 mg	0.8	±0.2	B,a	1.5	±0.2	A,a	0.3	±0.1	C,a	0.2	±0.1	C,ab	0.0	±0	C,b
	Pe 6% 100 mg	0.2	±0.1	B,b	1.1	±0.2	A,ab	0.4	±0.2	B,a	0.2	±0.1	B,b	0.1	±0.1	B,b
	Py 6% 50 mg	0.3	±0.1	BC,b	0.8	±0.2	A,b	0.7	±0.2	AB,a	0.2	±0.1	C,ab	0.4	±0.1	ABC,ab
	Py 6% 100 mg	0.4	±0.2	B,ab	1.0	±0.1	A,b	0.5	±0.1	B,a	0.3	±0.1	B,ab	0.2	±0.1	B,ab
Wax panel	Blank	0.8	±0.5	B,a	10.7	±2.3	A,abc	10.7	±1.4	A ,a	11.9	±1.4	A,bc	12.0	±1.2	A,ab
	Cy 0.01 %	2.5	±1.3	C,a	9.7	±1.9	B,bc	5.6	±0.9	C,c	14.9	±1.6	A,ab	12.7	±1.8	AB,ab
	Cy 0.1 %	1.3	±0.7	D,a	10.8	±2.3	B,abc	6.6	±1.3	C,bc	16.2	±2.1	A,a	14.6	±1.7	AB,a
	Cy 1 %	0.2	±0.1	C,a	13.6	±1.9	A,ab	7.4	±1.0	B,abc	5.7	±1.2	B,d	9.2	±1.1	B,b
	Cy 6 %	0.1	±0.1	C,a	13.8	±1.5	A,a	10.2	±1.0	AB,ab	9.7	±1.3	B,cd	10.4	±1.3	C,b
	Pe 6 %	0.1	±0.0	C,a	7.1	±1.2	B,c	12.4	±1.4	A,a	12.5	±1.6	A,abc	12.9	±1.0	A,ab
Plastic Cylinder	Blank	4.7	±1.7	C,a	12.4	±2.4	AB,a	15.7	±2.3	A,a	9.4	±1.5	BC,b	15.8	±2.7	A,a
	Cy 2%	5.0	±1.3	C,a	9.0	±1.2	C,ab	16.2	±1.9	AB,a	11.4	±1.8	BC,ab	18.2	±2.6	A,a
	Pe 2%	1.3	±0.6	C,a	4.5	±1.6	BC,b	13.2	±2.4	A,a	7.1	±1.4	B,b	6.2	±1.4	BC,b
	Py 2%	1.0	±0.4	B,a	11.5	±2.5	A,a	13.6	±1.9	A,a	16.9	±3.3	A,a	15.9	±2.4	A,a

Means within rows followed by the same letter (Upper case) are not significantly different at $P < 0.05$. Means within columns for each formulation followed by the same letter (Lower case) are not significantly different at $P < 0.05$, by use of DIFF option in an LSMEANS statement in PROC MIXED in SAS. Analysis was conducted separately for each attract-and-kill formulation. Py = Pyrethrin, Pe = Permethrin, Cy = Cyfluthrin.

Table 3. Mean percentage (%) mortality of *P. interpunctella* adult males (±SE) 24 hours after response to three different attract-and-kill formulations in a wind tunnel during an eight-week period.

Formulation	Doses	Mean percent (±SE) mortality of *P. interpunctella* male moths													
		Weeks after treatment													
		0			2			4			6			8		
Gel	Blank 50 mg	0	±0.0	A,b	0	±0.0	A,d	0	±0.0	A,c	0	±0.0	A,a	0	±0.0	A,b
	Blank 100 mg	0	±0.0	A,b	0	±0.0	A,d	0	±0.0	A,c	0	±0.0	A,a	0	±0.0	A,b
	Pe 6% 50 mg	25	±9.9	B,a	70	±10.5	A,a	20	±9.2	B,b	15	±8.2	BC,a	0	±0.0	C,b
	Pe 6% 100 mg	10	±6.9	B,ab	50	±11.5	A,b	15	±8.2	B,bc	5	±5.0	B,a	5	±5.0	B,b
	Py 6% 50 mg	0	±0.0	C,b	30	±10.5	AB,c	40	±11.2	A,a	15	±8.2	BC,a	30	±10.5	AB,a
	Py 6% 100 mg	15	±8.2	B,a	50	±11.5	A,b	40	±11.2	A,a	15	±8.2	B,a	10	±6.9	B,b
Wax panel	Blank	0	±0.0	A,a	0	±0.0	A,c	0	±0.0	A,c	0	±0.0	A,c	0	±0.0	A,c
	Cy 0.01 %	0	±0.0	A,a	0	±0.0	A,c	0	±0.0	A,c	5	±5.0	A,c	0	±0.0	A,c
	Cy 0.1 %	0	±0.0	A,a	5	±5.0	A,c	0	±0.0	A,c	10	±6.9	A,c	5	±5.0	A,c
	Cy 1 %	5	±5.0	C,a	35	±10.9	B,b	50	±11.5	AB,b	55	±11.4	A,b	60	±11.2	A,b
	Cy 6 %	5	±5.0	B,a	95	±5.0	A,a	85	±8.2	A,a	85	±8.2	A,a	95	±5.0	A,a
	Pe 6 %	0	±0.0	B,a	85	±8.2	A,a	95	±5.0	A,a	95	±5.0	A,a	100	±0.0	A,a
Plastic Cylinder	Blank	0	±0.0	A,b	0	±0.0	A,c	0	±0.0	A,c	0	±0.0	A,c	0	±0.0	A,c
	Cy 2%	50	±11.5	B,a	75	±9.9	A,a	90	±6.9	A,a	95	±5.0	A,c	90	±6.9	A,a
	Pe 2%	15	±8.2	C,b	30	±10.5	BC,b	55	±11.4	A,b	40	±11.2	AB,b	20	±9.2	BC,bc
	Py 2%	15	±8.2	B,b	25	±9.9	B,b	50	±11.5	A,b	35	±10.9	AB,b	30	±10.5	AB,b

Means within rows followed by the same letter (Upper case) are not significantly different at $P < 0.05$. Means within columns for each formulation followed by the same letter (Lower case) are not significantly different at $P < 0.05$, by use of DIFF option in an LSMEANS statement in PROC MIXED in SAS. Percentage data were transformed by the arcsine square-root method. Analysis was conducted separately for each attract-and-kill formulation. Py = Pyrethrin, Pe = Permethrin, Cy = Cyfluthrin.

Table 4. Mean number (±SE) of eggs laid per *P. interpunctella* female after being paired for 24 hours with a male that had responded to one of three different attract-and-kill formulations in a wind tunnel during an eight-week period.

Formulation	Doses	Mean number (±SE) egg laying per *P. interpunctella* female													
		Weeks after treatment													
		0			2			4			6			8		
Gel	Blank 50 mg	57.0	±11.4	A,a	61.2	±12.5	A,a	48.7	±8.0	A,a	53.7	±10.8	A,a	53.5	±7.0	A,a
	Blank 100 mg	53.0	±9.9	A,a	62.7	±17.4	A,a	48.0	±9.1	A,a	46.7	±10.3	A,a	44.6	±11.2	A,a
	Pe 6% 50 mg	49.5	±11.3	A,a	47.9	±15.1	A,ab	35.7	±9.6	A,a	47.9	±9.3	A,a	51.1	±10.3	A,a
	Pe 6% 100 mg	54.4	±9.4	A,a	31.2	±9.9	A,b	40.7	±10.1	A,a	52.7	±8.2	A,a	47.3	±9.0	A,a
	Py 6% 50 mg	49.2	±11.0	A,a	44.7	±13.2	A,ab	21.5	±7.9	A,a	46.1	±8.8	A,a	48.9	±11.2	A,a
	Py 6% 100 mg	44.7	±9.1	A,a	30.8	±10.0	A,b	35.1	±10.3	A,a	43.9	±9.4	A,a	54.9	±8.4	A,a
Wax panel	Blank	56.3	±11.4	C,a	64.5	±12.3	B,a	58.4	±10.3	AB,a	86.7	±9.5	AB,a	118.3	±11.2	B,a
	Cy 0.01 %	53.9	±9.7	B,a	41.7	±7.3	B,b	56.9	±11.8	AB,a	80.6	±13.0	A,a	54.1	±7.9	B,b
	Cy 0.1 %	48.3	±8.2	A,a	45.1	±8.2	A,ab	44.0	±8.8	A,ab	35.2	±6.4	A,a	42.1	±5.5	A,bc
	Cy 1 %	43.7	±9.2	A,a	14.6	±5.6	B,c	28.1	±9.2	AB,bc	22.3	±6.9	AB,bc	13.0	±4.9	B,d
	Cy 6 %	50.9	±8.7	A,a	7.1	±3.3	B,c	21.8	±7.1	B,bc	15.1	±6.0	B,bc	16.7	±5.5	B,d
	Pe 6 %	35.3	±7.8	A,a	17.3	±8.1	AB,c	9.0	±4.6	B,c	9.7	±5.6	B,c	25.6	±11.7	AB,cd
Plastic Cylinder	Blank	56.3	±9.0	A,a	52.1	±11.8	A,a	56.7	±8.8	A,a	64.5	±10.2	A,a	52.1	±6.7	A,a
	Cy 2%	18.6	±6.1	A,b	5.2	±2.3	A,b	4.3	±3.1	A,c	10.5	±4.2	A,c	19.8	±7.3	A,b
	Pe 2%	22.0	±7.2	AB,b	19.9	±5.2	B,b	22.2	±6.8	AB,bc	41.4	±8.3	A,b	38.7	±12.8	AB,ab
	Py 2%	31.1	±8.0	AB,b	24.3	±6.5	AB,b	27.0	±6.9	AB,c	37.1	±6.9	A,b	14.5	±5.2	B,b

Means within rows followed by the same letter (Upper case) are not significantly different at $P < 0.05$. Means within columns for each formulation followed by the same letter (Lower case) are not significantly different at $P < 0.05$, by use of DIFF option in an LSMEANS statement in PROC MIXED in SAS. Analysis was conducted separately for each attract-and-kill formulation. Py = Pyrethrin, Pe = Permnethrin, Cy = Cyfluthrin.

Table 5. Mean percentage (%) of eggs hatched (±SE) from those laid by a *P. interpunctella* female after being paired for 24 hours with a male that had responded to one of three different attract-and-kill formulations in a wind tunnel during an eight-week period.

Formulation	Doses	Mean percent (±SE) hatching per *P. interpunctella* female													
		Weeks after treatment													
		0			2			4			6			8	
Gel	Blank 50 mg	63.0	±7.9	A,a	66.3	±9	A,a	76.3	±6.7	A,a	69.9	±8.4	A,ab	82.1	±4.6	A,a
	Blank 100 mg	66.5	±8.3	A,a	51.6	±9.8	A,ab	69.3	±8.1	A,a	56.8	±8.6	A,ab	69.0	±9.2	A,a
	Pe 6% 50 mg	59.6	±9.3	AB,a	25.0	±8	C,c	44.2	±9.3	BC,b	68.5	±8.2	A,ab	68.3	±7.9	AB,a
	Pe 6% 100 mg	64.3	±8.7	AB,a	39.2	±9.4	B,bc	52.8	±9.9	AB,ab	75.9	±7.4	A,a	60.2	±9.1	AB,a
	Py 6% 50 mg	52.5	±8.5	AB,a	36.0	±9.4	B,bc	35.7	±9.3	B,b	57.7	±9.8	AB,ab	60.0	±8.3	A,a
	Py 6% 100 mg	64.6	±8.9	AB,a	37.8	±9.7	C,bc	42.5	±9.8	BC,b	49.4	±10.3	ABC,b	66.7	±8	A,a
Wax panel	Blank	69.7	±9.4	B,a	65.5	±8.8	B,a	80.0	±7.8	B,a	77.6	±6.2	AB,a	95.9	±1	A,a
	Cy 0.01 %	65.9	±8.8	B,a	65.6	±7.8	B,a	49.2	±8.5	B,b	66.5	±7	AB,a	83.3	±8	A,a
	Cy 0.1 %	68.2	±6.9	AB,a	68.3	±8.1	AB,a	48.8	±9.2	B,b	64.9	±8.8	B,a	82.8	±8	A,a
	Cy 1 %	65.8	±9.1	A,a	35.6	±8.9	BC,b	14.2	±5.5	C,c	42.3	±9.8	B,b	22.5	±8.9	BC,b
	Cy 6 %	69.4	±8.1	A,a	11.1	±5.6	B,c	5.9	±2.5	B,c	18.2	±8	B,c	8.7	±6	B,b
	Pe 6 %	61.1	±9.9	A,a	12.4	±6.8	B,c	19.9	±8.1	B,c	27.1	±8.8	B,bc	7.9	±4	B,b
Plastic Cylinder	Blank	75.3	±8.7	A,a	58.4	±11	B,a	74.7	±8.6	A,a	88.9	±5.6	A,a	94.3	±1.2	A,a
	Cy 2%	35.2	±9.9	AB,b	8.4	±5.1	AB,b	8.0	±5	B,c	16.9	±7.1	A,c	28.7	±9.7	A,b
	Pe 2%	39.9	±10.1	A,b	43.7	±9.3	A,a	37.2	±10.5	A,b	56.1	±9.2	A,b	43.0	±10.9	A,b
	Py 2%	51.7	±9.8	AB,ab	43.2	±10.1	AB,a	42.7	±9.9	B,b	67.7	±9.1	A,b	37.9	±10.7	B,b

Means within rows followed by the same letter (Upper case) are not significantly different at $P < 0.05$. Means within columns for each formulation followed by the same letter (Lower case) are not significantly different at $P < 0.05$, by use of DIFF option in an LSMEANS statement in PROC MIXED in SAS. Percentage data were transformed by the arcsine square-root method. Analysis was conducted separately for each attract-and-kill formulation. Py = Pyrethrin, Pe = Permethrin, Cy = Cyfluthrin.

CHAPTER IV

PHEROMONE-BASED SUPPRESSION METHODS TO CONTROL INDIANMEAL MOTH, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) IN COMMERCIAL ESTABLISHMENTS

Abstract

Three attract-and-kill formulations for *Plodia interpunctella* Hübner, a gel, a wax panel and a plastic cylinder were tested in simulated warehouse rooms at three densities of devices and at three densities of moths per room. The wax panel and the plastic cylinder formulations suppressed all the densities of moths with only one device per room. A second study with two field experiments was conducted during 2005 and 2006 in replicated commercial pet food and grocery stores that harbored natural populations of *P. interpunctella*. In the summer of 2005 the wax panel formulation suppressed adult male responses to traps and numbers of larvae in food bait cups after the first month of being established, and suppression was maintained until the third month compared to non-treated buildings. The second experiment in 2006 compared several pheromone-based methods of control in commercial food retail buildings. Numerically, the mass-trapping treatment showed the lowest adult moth capture after the first month of the experiment until the end of the third month. However, this treatment was similar statistically to that of the attract-and-kill panels, mating disruption and non-treated control establishments in most of the weeks. Monitoring of larvae in food cups revealed the pheromone-based methods were not significantly different from each other, but that

they suppressed moth populations in most of the weeks compared to non-treated control buildings. This research shows potential for successful pheromone-based suppression methods for Indianmeal moths in commercial food storage applications.
Keywords: semiochemicals, attract-and-kill, mass-trapping, mating disruption, stored products.

INTRODUCTION

Since the female-produced sex pheromone of *Plodia interpunctella* (Hübner) was identified as *(Z,E)*-9,12 tetradecadienyl acetate, referred to here as "ZETA" (Brady *et al*. 1971, Kuwahara *et al*. 1971, Kuwahara and Casida 1973, Sower *et al*. 1974, Soderstrom *et al*. 1980, Teal *et al*. 1995 and Zhu *et al*. 1999), its use in management of this important pest has been implemented in different ways. The main use of pheromones for stored-product pests is as attractant lures in traps for detection and monitoring of storage pests (Phillips 1997) in food storage areas, processing factories (Hoppe and Levinson 1979; Vick *et al.* 1981, 1986), wheat storage bins (Hagstrum 2000), in and around flour Mills (Doud and Phillips 2000) and feed mills (Roesli *et al.* 2003). Pheromone lures for *P. interpunctella* are often deployed in sticky traps that offer advantages over visual inspections (Mullen and Dowdy 2001), and are valuable tools for determining spatial and temporal distribution and encourage the use of integrated pest management programs (Mueller 1998) and in enclosed environments are useful for making management decisions against insect pests (Burkholder and Ma 1985). Additionally, the longevity of lures enhances trap-catch efficiency (Mullen *et al.* 1991). For example, the efficiency and longevity of *P. interpunctella* sex pheromone was tested in a warehouse where the

attractiveness of Storegard® lures (Trece Inc., Salinas, CA) and Biolure® lures (Consep Membranes Inc., Bend, OR) were up to 40 weeks, a time period beyond manufacturers' claims (Mullen et al. 1991).

The use of pheromones for suppressing insect pests has been studied widely in Lepidoptera with the goal being to reduce the population by killing mainly males or in other ways to prevent mating to females. Mass-trapping is a method based on the catching and killing of as many males as possible, and thus reduces mating by male storage moths in warehouses and flour mills (Mueller and Pierce 1992, Levinson and Buchelos 1981, Trematerra 1994), and other food storage facilities (Chow *et al*. 1977). Mating disruption is another method in which one releases high levels of synthetic pheromone in a treatment area so that the male moth gets confused and is not capable of finding the female, either by "false trail-following" or by some neural disruption in male behavior (Cardé and Minks 1995). Mating disruption for stored-product moths has been evaluated for *Sitrotoga cerealella* (Olivier) (Vick *et al.* 1978) and *Ephestia cautella* (Walker) (Mafra-Neto and Baker 1996, Shani and Clearwater 2001) in corn storage (Fadamiro and Baker 2002). Mating disruption of *P. interpunctella* showed a reduction of up to 93% mating of *P. interpunctella* populations in small-scale plots (Ryne *et al*. 2001). Another type of pheromone-based suppression is the "attract-and-kill" method that is a combination of a sex pheromone and a killing agent, such as a pathogen or insecticide (Lanier 1990), and is also known as "lure and kill", "attract and kill" and "attraction-annihilation". Attract-and-kill may target males, females or both, depending on the system, and this technique has been studied in important Lepidoptera pests in stored-products, such as the navel orangeworm, *Amyelois transitella* (Phelan and Baker

1987) and the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Trematerra and Capizzi 1991). Research on the Indianmeal moth, *Plodia interpunctella*, investigated the attract-and-kill LastCall® gel and was able to suppress oviposition only at the lowest populations density (1 male:1 female) in simulated warehouses of 11.3 cubic meters (Nansen and Phillips 2004). Recent work (Chapters 2 and 3 of this dissertation) studied a variety of contact insecticides and attract-and-kill device designs for suppressing Indianmeal moth populations.

The overall objectives of the work reported in this chapter were to determine the efficacy for attract-and-kill devices for suppressing populations of Indianmeal moth under simulated and actual field conditions, and to compare this method with mass-trapping and mating disruption in field situations. Three experiments were conducted. First, the effectiveness of three attract-and-kill formulations for suppressing small populations of *P. interpunctella* were evaluated under controlled conditions in simulated warehouse rooms. A second study was the assessment of the attract-and-kill formulated into wax panels in true commercial establishments with naturally occurring Indianmeal moth populations. The third study compared the pheromone-based control methods of attract-and-kill, mating disruption and mass trapping in commercial establishments.

MATERIALS AND METHODS

Insects. *P. interpunctella* male and female adults used in simulated warehouse experiments were reared on diet containing corn meal, chick starter/grower crumbles, all mash egg crumbles and glycerol (4:2:2:1 by volumetric ratio) in 460-ml glass jars (Alltrista, Muncie, IN) placed in a growth chamber at 28 °C, 60-70 % R.H., and 16:8 hr (L:D) (Phillips and Strand 1994). Cardboard rolls were placed into the culture jars for the last stage wandering larvae to crawl into and pupate. The pupae were removed from the cardboard rolls, separated by sex and placed individually into 1-dram shell vials with ventilated plastic caps (Fisher Scientific, Pittsburg, PA) and returned to the growth chamber until they emerged as adults. One- to two-day-old virgin adults were used for the simulated warehouse studies, and these adults were only used once.

Simulated Warehouse Experiments. These experiments were conducted using four separate commercial "mini-storage" rooms located near Stillwater, OK. The building was divided into several main sections by halls and doors; and every section contained six to seven individual storage rooms. The dimensions of the storage rooms used were 3.3- × 3.3- ×6.6-m, for a volume of 71.9 m^3. The storage rooms were composed of a concrete floor, sheet-metal walls and a sheet-metal ceiling; the entrance was a metal roll-up over-head door. The storage rooms were equipped with minimal climate control so that the air temperature was kept between 25 and 30 °C in the summer season. The upper side of the sidewalls had a 10-cm-wide gap that was covered with a plastic sheet to prevent insects from escaping or entering the storage rooms. A plastic sheet was hung just inside the door to each room and was sealed with tape and Velcro to the ceiling walls

and floor to prevent adult *P. interpunctella* from flying away when the experimental room was being serviced.

Three attract-and-kill formulations, described also in Chapter 3, were tested in a series of simulated warehouse experiments. The first formulation was the LastCall® gel applied as a 100-mg droplet onto a 4 × 4 cm piece of aluminum foil. The gel contained the pheromone ZETA at 0.16% by weight and the pyrethroid insecticide Permethrin at 6% by weight. The second formulation was the wax panel (20 ×13 cm; Suterra, Bend, OR) impregnated with Permethrin at 6.0% and deployed with a Biolure® controlled release pheromone lure (Suterra, Bend, OR). The third formulation was a plastic mesh cylinder (7 mm mesh; 35-cm height × 10-cm diameter) coated with the pyrethroid Cyfluthrin at 2.0% in the spray and deployed also with a Biolure® lure hung in the center of the cylinder. In treated mini-storage rooms the attract-and-kill devices were held with a small binder clip and hung from the ceiling with a steel wire at about 1.55 m from the floor. Experimental treatments, which were a non-treated control and 1, 2 or 3 attract-and-kill devices, were randomly assigned to each the four mini-storage rooms and deployed on a Monday, and then removed on a Friday for each of the four one-week-long replicates. A Petri dish bottom (15 × 90 mm), containing 15 g of wheat as an egg laying substrate, was placed close to each of the four corners of each room and on top of a wood board (5.0 × 7.5 × 7.5 cm) to avoid direct contact with the floor. Two-day old virgin male and female adults of *P. interpunctella* were released at opposite ends of the room, males at one end and females at the other, within 60 cm of the walls. The four Petri dishes with wheat were retrieved from each room at the end of the 4-day exposure period, labeled and transported to the laboratory for processing. The wheat from each Petri dish

was carefully sifted with a standard U.S. No. 14 sieve and the number of eggs laid in each dish was counted.

The three attract-and-kill formulations (gel, wax panel, and the plastic cylinder) were tested in nine separate 4-week long simulated warehouse experiments at treatments of 0 (non-treated control), 1, 2, or 3 attract-and-kill devices per mini-storage room. Separate experiments for each formulation were conducted at moth densities of 5, 10, or 15 male-female pairs released per room. The response variable observed after each replicate was the number of eggs laid per dish of wheat in each room during a given 4-day study period, or replicate. The overall experiment was a randomized complete block design with a factorial arrangement for which the factors were the density of moths per room, the number of devices per room, and the type of attract-and-kill formulation tested, each conducted four times, or one replicate per week for four consecutive weeks. The data were analyzed with the PROC MIXED procedure (SAS Institute, 2005). Treatment differences were analyzed with pair-wise t-tests and comparisons were protected by examining the SLICE option within the LSMEANS statement at the $\alpha = 0.05$ level.

Commercial-Scale Field Experiments. Two field studies of pheromone-based suppression of naturally occurring *P. interpunctella* populations were conducted during the spring and summer months of 2005 and 2006 in the area of Dallas, TX. The 2005 study compared the attract-and-kill wax panels with non-treated buildings. A total of 8 buildings were used, including 5 pet food stores and 3 small grocery stores, and thus four buildings were randomly assigned to each treatment. The 2006 experiment had 15 buildings: 6 pet food stores, 8 grocery stores and 1 small pet food warehouse. The 2006 study compared attract-and-kill vs. mass trapping vs. mating disruption. For each

building the types of food products were identified and building space measurements were taken to calculate the volume of each building so that treatments could be equitably assigned to buildings across replicates based on building size and perceived moth infestation level. Since simulated warehouse experiments found the wax panel was effective at its lowest deployment density (see below), which was one panel per 71.9 m^3, the treatments were applied to the commercial buildings at that same density for both the 2005 and 2006 field experiments.

In 2005 only the attract-and-kill wax panel (Suterra, Bend, OR) formulated with 6.0% Permethrin and a Biolure® pheromone lure (Suterra, Bend, OR) were used and compared to non-treated buildings. In 2006, a new attract-and-kill panel was studied that was a 20- × 13-cm piece of plastic-coated paper (same material used for diamond-shaped sticky traps, but without the added glue material; Suterra, Bend, OR), sprayed to run-off with a solution of the pyrethroid Deltamethrin at 0.08% (A.I.) and deployed with a Biolure® lure. The mass-trapping treatment used standard diamond-shaped sticky traps (Suterra, Bend, OR), each deployed with a Biolure® lure, at the same density of 1 trap per 71.9 m^3. The mating disruption treatment used only Biolure® lures that were also deployed at a density of one lure per 71.9 m^3 as in the other treatments. These three treatments were compared to each other and to buildings that were non-treated controls.

Moth Population Variables. Adult males were monitored in both the 2005 and 2006 field trials using diamond-shaped sticky traps (Suterra, Bend, OR) deployed with a Biolure® lure. Ten sticky traps were used per store and these were deployed for a 3-day period, from Friday to Monday, every two weeks over the approximate 3-month period of the experiment. The pheromone trap monitoring of males started in every building two

weeks before the treatments were assigned to the buildings. Once the treatments were assigned and deployed they were left in place for the duration of the experiment in each year. Moth reproduction in each building was monitored by counting larvae developing in 10 Styrofoam baits cups (226 ml; Dart, U.S.A.) placed in each building. Each bait cup contained 50 g of laboratory moth rearing diet (see above) and the 10 cups were distributed evenly throughout each building and replaced every two weeks, on a Monday at the end of a 3-day pheromone-trapping period for the course of the experiment.

As with the pheromone traps for monitoring males, the bait cups for monitoring female reproduction were initially deployed in all buildings two weeks before the treatments were assigned, and then monitoring continued on a 2-week cycle for the entire study. The bait cups were returned to the laboratory and placed in a growth chamber at 28 °C, 60-70 % R.H., and 16:8 hr (L:D) photoperiod for another two weeks to allow for egg hatching. Cups were then put in a heated sand bath (55 ± 5 °C) until the larvae crawled up and out of the diet, escaping from heat, and they were counted.

Data Analysis. For the 2005 experiment the wax panel was compared to non-treated controls; it was a balanced, completely randomized design with four buildings assigned the treatment and four non-treated, and observed over a 3-month period. In 2006 the attract-and-kill panel, mass trapping and mating disruption methods were compared to the non-treated control building. Each pheromone-based treatment was assigned to each of four stores, and only three stores were designated non-treated controls, so this was an unbalanced completely randomized design. The variable responses analyzed were the number of males caught per sticky trap and the number of larvae collected per bait cup. The data were analyzed with the PROC MIXED procedure (SAS Institute, 2005) using

the REPEATED option (every two weeks during the three-moth period). Treatment differences within two-week periods were analyzed with pair-wise t-test and comparisons were protected by examining the SLICE option within the LSMEANS statement at the $\alpha = 0.05$ level.

RESULTS

Simulated warehouses. The analysis of variance for eggs laid by female *P. interpunctella* in simulated warehouse studies (Table 1) showed a significant difference among attract-and-kill formulations ($F_{2, 151} = 8.72$, $P = 0.0003$), among the number of attract-and-kill devices assigned to rooms ($F_{3, 214} = 28.37$, $P < 0.0001$) and among density of moths in the rooms ($F_{2, 214} = 5.19$, $P = 0.0063$). However, there were no significant interactions of the device type and the number of devices deployed ($F_{6, 151} = 0.33$, $P = 0.9178$), the device type and the density of moths in each room ($F_{4, 151} = 1.64$, $P = 0.1674$), the number of devices and the density of moths per room ($F_{6, 151} = 0.31$, $P = 0.9299$), nor in the three way interaction of the device type, number of devices and density of moths ($F_{12, 151} = 0.42$, $P = 0.9522$). Only a single wax panel or a single plastic cylinder device per room was needed to significantly suppress the egg laying at all densities (5, 10, and 15 pairs per room) of *P. interpunctella* compared to non-treated control rooms with no attract-and-kill devices. The LastCall® gel was the least effective of those tested as it significantly impacted the egg laying only at the highest rate of 3 devices per room, and this was only at two densities of moths. Eggs laid in the non-treated control rooms were statistically similar to those laid in rooms treated with 1 or 2 gel devices per room at all three moth densities.

Field Experiments. The mean number of *P. interpunctella* adult males per sticky trap is shown in Figure 1 for the 2005 field experiment. There was an overall statistical difference between the attract-and-kill wax panel and non-treated across all weeks ($F_{5, 26.5} = 2.86$, $P = 0.0340$). Trapping on June 10, which was during the pre-treatment period, showed no significant difference between the wax panel and the non-treated control buildings, and this lack of difference was similar to that observed on July 8, just after treatment. However, from July 22 until the end of the experiment (September 2), the buildings with wax panels had significantly lower male moth captures in pheromone traps compared to non-treated buildings (Fig. 1).

Figure 2 shows the mean number of larvae per bait cup for the 2005 experiment in wax panel-treated and non-treated buildings. The overall difference between treatments for the entire experiment was marginally significant ($F_{1, 6.4} = 5.20$, $P = 0.0597$). Analysis of each week separately found that treatments were similar statistically from June 10 through July 22, but from August 5 to the end of the experiment the number of larvae present in the bait cups in buildings treated wax panels was significantly lower than those from bait cups in non-treated buildings. The results for 2005 suggest that the wax panel treatment suppressed *P. interpunctella* male activity and reproduction about 1-2 months after application.

The mean number of *P. interpunctella* adult males responding to pheromone traps in the 2006 experiment is shown in Table 2. There was a significant difference among treatments ($F_{3, 17.7} = 5.52$, $P = 0.0074$) and weeks ($F_{7, 75.9} = 4.11$, $P = 0.0007$), but there was no significant difference in the interaction of weeks by treatments ($F_{21, 74} = 1.57$,

$P = 0.0824$) for male trap captures. The pre-treatment monitoring was made at weeks 0 and 2 (Jun 2 and 16 of 2006). At week 0 there were no significant differences among treatments, and at week 2 the buildings destined to be set-up with the attract-and-kill method showed the higher number of adult males (7.3) caught per trap compared to the other treatments. Treatments were set-up on Jun 19 of 2006. There were less than 0.5 adult males per sticky trap in the mass-trapping treatment for all but one sample period during the whole experiment, which were numerically the lowest male numbers compared to the rest of the treatments. However, males caught in mass-trapping buildings were not significantly different from those for the mating disruption treatment, except in week 6. The attract-and-kill, mating disruption and non-treated did not show significant differences in male activity by the end of the study, except right after the deployment of the pheromone-based devices in the stores on week 4 when mating disruption and mass-trapping were statistically similar and differed from the other treatments.

The mean number of *P. interpunctella* larvae per bait cup in the 2006 experiment is shown in the Table 3. There was an overall significant difference among treatments ($F_{3, 16.4} = 7.62$, $P = 0.0021$), but there were no significant difference among weeks ($F_{6, 60.7} = 0.28$, $P = 0.9444$), nor was there any significant interaction of weeks (time) by treatments ($F_{18, 60.3} = 0.81$, $P = 0.6858$). The pre-treatment monitoring with bait cups was for one two-week period, ending June 16 of 2006, and there were no statistically significant differences at that time among buildings intended for the various treatments. Larval counts dropped dramatically after deploying treatments; the attract-and-kill, mass-trapping and mating disruption methods were not significantly different form each other,

but had significantly lower numbers of moth larvae in food cups compared to non-treated controls in most weeks. Larval numbers in the non-treated buildings were statistically similar to the attract-and-kill buildings at week 4 and 12, and they were similar to the mating disruption at week 10. On these dates, the number of larvae was low in many samples, and this may have been due to the presence of Sawtooth grain beetle, *Oryzaephilus surinamensis*, in some cups, which might have preyed on moth eggs. We also found the larval parasitoid *Bracon hebetor* in food cups on those dates.

DISCUSSION

One possible cause for the low impact on egg laying by LastCall® gel at high population densities in the simulated warehouse experiments was that adult male moths were found stuck on the small surface of the gel drop in some cases, and thus may have prevented other males from contacting the gel and dying before mating. Another possible effect on treatments with low egg laying was that fresh gel, which presumably released large amounts of pheromone initially, repelled or interrupted response of adult male moths instead of attracting them, and these males may have been inhibited to mate females. Apparent effectiveness of the LastCall® gel at the lowest moth density, one male and one female, in the study by Nansen and Phillips (2004) probably resulted from the single male in most replicates being killed after contact with the gel, which preceded his finding the female to mate. In the present simulated warehouse studies the lowest moth density was five males and five females, and it was possible that one or more males successfully located and mated females in the high moth density trials prior to contacting the toxic gel. The wax panel and plastic cylinder devices tested in the simulated warehouse studies were clearly effective at suppressing moth reproduction in these rooms, even at the lowest density of one device per room. The effectiveness of the wax panel and cylinder devices was probably due to their larger surface areas, which facilitated higher contact frequency and greater contact time by males, as demonstrated in results from Chapter 3.

The increases of the *P. interpunctella* populations in the commercial field sites during the 2005 and 2006 field trials probably resulted from the movement of infested merchandise into the establishments and also from normal increases of moth numbers due

to reproduction in warm weather with ample food. The densities of adult males and larvae were clearly suppressed in buildings treated with wax panels in 2005 compared to the control buildings that displayed population increases. Thus the 2005 experiment suggests that attract-and-kill wax panels with pheromone lures and 6.0% Permethrin can be effective suppression tools for managing pest populations of *P. interpunctella*.

The new attract-and-kill plastic-coated paper panels used in 2006 did not show male-suppression results similar to those for wax panels in 2005. One of the causes may be due to the much lower concentration of the active ingredient applied onto panels in the 2006 device (0.08% Deltamethrin sprayed to run-off) compared to the very high level of Permethrin (6.0 % by weight) in the wax panels in 2005. One hypothesis about the success of the wax panel attract-and-kill device is that the high concentration and amount of the active ingredient on the wax surface made it a long-term and highly effective killing device.

The 2006 field study showed that other pheromone-based methods could similarly and significantly suppress *P. interpunctella* reproduction in commercial food storage buildings, as evidenced by low larval counts in bait cups. All three methods tested in 2006, which were attract-and-kill, mating disruption and mass trapping, used the same pheromone release lures, Biolure®, and were all deployed at the same density of one device per 71.9 m^3 in treated buildings. Mating disruption has been recently shown to suppress stored product moth populations at much lower deployment densities of lures than studied here (Ryne *et al*. 2006). The mating disruption treatment had no male-killing component with it, such as a insecticide-treated surface or a sticky trap, it can be concluded that pheromone-releasing devices alone deployed at the density used in this

study can significantly impact moth reproduction without the need to actively kill or otherwise remove males from the population. Thus it follows that a potentially low-cost, low maintenance and relatively safe method like mating disruption using pheromone lures only, may be optimal compared to using traps or toxic panels. Adoption of pheromone-based control methods for *P. interpunctella* by the pest control industry will ultimately depend on consideration of cost-effectiveness of the method and other market or practical factors related to the customer, the practitioner and the stored-product system being managed.

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Table 1. Mean number of eggs laid (±SE) per Petri dish for the three attract-and-kill formulations (gel, wax panel, and plastic cylinder) at densities of 0, 1, 2, or 3 devices per warehouse room and at three densities of moths (5, 10, or 15 pairs).

Formulation	Pairs of moths	Density of devices per warehouse room (71.9 m^3) (±SE)			
		0	**1**	**2**	**3**
Gel	5	15.9 ± 4.8 B,a	7.3 ± 7.2 A,a	5.4 ± 4.0 AB,a	0.6 ± 0.6 A,b
	10	32.3 ± 6.6 AB,a	16.3 ± 9.6 A,a	16.3 ± 9.7 AB,a	13.0 ± 4.8 A,a
	15	37.6 ± 4.6 A,a	17.7 ± 1.9 A,ab	18.1 ± 5.9 A,ab	15.7 ± 9.5 A,b
Wax panel	5	20.9 ± 8.2 AB,a	1.0 ± 0.8 A,b	0.0 ± 0.0 B,b	0.0 ± 0.0 A,b
	10	17.5 ± 8.2 AB,a	2.4 ± 1.5 A,b	0.2 ± 0.1 B,b	0.0 ± 0.0 A,b
	15	25.2 ± 7.5 AB,a	2.0 ± 1.0 A,b	0.1 ± 0.1 B,b	0.0 ± 0.0 A,b
Plastic cylinder	5	15.8 ± 6.0 B,a	3.1 ± 2.4 A,b	0.0 ± 0.0 B,b	0.0 ± 0.0 A,b
	10	32.9 ± 6.8 AB,a	6.3 ± 2.5 A,b	0.6 ± 0.5 B,b	0.0 ± 0.0 A,b
	15	25.8 ± 7.7 AB,a	8.2 ± 1.7 A,b	3.0 ± 1.6 AB,b	0.5 ± 0.4 A,b

Mean within columns followed by the same upper case letter are not significant different at $P > 0.05$. Means within rows followed by the same lower case letter are not significantly different at $P > 0.05$ (ANOVA followed by pair-wise t-test of effect among attract-and-kill formulations, $F_{2, 151} = 8.72$, $P = 0.0003$).

Table 2. Mean number (±SE) of *P. interpunctella* adult males per sticky trap caught using several pheromone-based methods of control in commercial establishments in Dallas, TX. 2006. Treatments were set up on 6/19/2006, after Week 2 of the period 6/2/06 to 9/2/06.

Treatment	6/2/2006 Week 0	6/16/2006 Week 2	6/30/2006 Week 4	7/16/2006 Week 6	7/28/2006 Week 8	8/11/2006 Week 10	8/25/2006 Week 12	9/2/2006 Week 14
Non-treated	0.9 (±0.1)a	2.9 (±1.2)b	3.3 (±1.3)b	4.6(±1.0)a	4.2(±2.7)a	4.3(±4.1)a	4.1(±1.7)ab	4.0(±1.0)a b
Attract-and-kill	3.6 (±1.8)a	7.3 (±2.3)a	5.1 (±1.0)a	2.9(±0.6)a	5.6(±1.5)a	4.3(±1.4)a	5.7(±1.2)a	4.2 (±0.5)a
Mating Disruption	0.4 (±0.2)a	1.6 (±0.2)b	1.2 (±0.5)c	2.4(±1.4)a	2.7(±2.8)ab	2.3(±0.5)ab	2.2(±0.6)b	2.6(±0.3)ab
Mass Trapping	0.6 (±0.5)a	1.8 (±0.7)b	0.5 (±0.2)c	0.2(±0.2)b	0.2(±0.1)b	0.3(±0.2)b	0.3(±0.2)b	0.5(±0.1)b

Means within columns followed by the same letter are not significantly different at $P > 0.05$ (ANOVA followed by pair-wise t-test of the effect among treatments, $F_{3, 17.7} = 5.52$, $P = 0.0074$).

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Table 3. Mean number (±SE) of *P. interpunctella* larvae per bait cup caught using several pheromone-based methods of control in commercial establishments in Dallas, TX. 2006. Treatments were set up on 6/19/2006, after Week 2 of the period 6/2/06 to 9/2/06.

Treatment	6/16/2006 Week 2	6/30/2006 Week 4	7/16/2006 Week 6	7/28/2006 Week 8	8/11/2006 Week 10	8/25/2006 Week 12	9/8/2006 Week 14
Non-treated	7.9 (±4.6) a	13.0 (±5.4) a	13.9 (±7.1) a	16.9 (±7.8) a	15.9 (±6.1) a	13.9 (±4.9)a	15.1 (±6.5) a
Attract-and-kill	3.4 (±1.4) a	5.4 (±2.9) ab	3.6 (±3.0) b	3.1 (±2.8) b	5.4 (±3.9) b	8.5 (±5.5) ab	3.7 (±2.1) b
Mating Disruption	7.8 (±4.0) a	0.0 (±0.0) b	1.5 (±1.5) b	0.0 (±0.0) b	8.3 (±6.4) ab	1.2 (±1.1) b	3.5 (±1.7) b
Mass Trapping	3.4 (±2.0) a	3.0 (±2.6) b	0.8 (±0.6) b	1.0 (±1.0) b	0.6 (±0.4) b	1.0 (±0.9) b	0.2 (±0.2) b

Means within columns followed by the same letter are not significantly different at $P > 0.05$ (ANOVA followed by pair-wise t-test of the effect among treatments, $F_{3, 16.4} = 7.62$; $P = 0.0021$).

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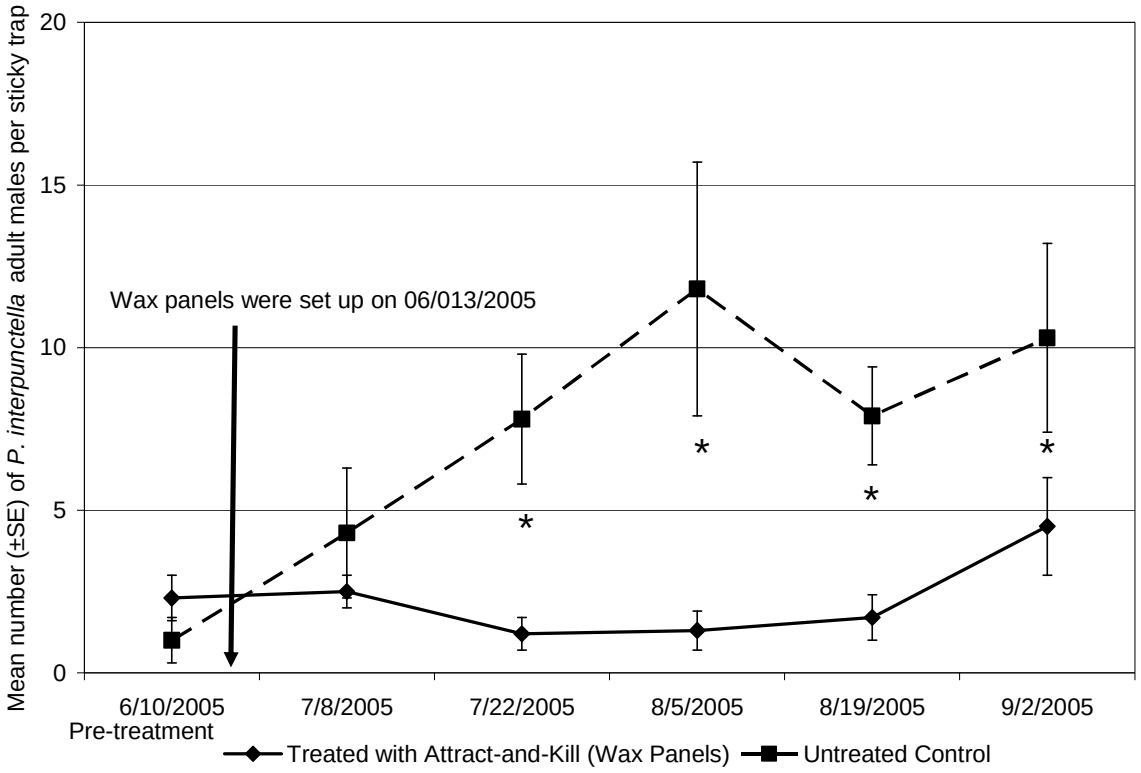

Fig. 1. Mean number (±SE) of *P. interpunctella* adult males caught per sticky trap by effect of the attract-and-kill formulated with wax panels impregnated with Permethrin 6.0% A.I. and deployed with a synthetic female sex pheromone Biolure® in treated and non-treated commercial establishments in Dallas, TX. 2005.

* Treatments are significantly different at $P < 0.05$ (ANOVA followed by pair-wise t-test of the treatments effect within weeks, $F_{5, 26.5} = 2.86$; $P = 0.0340$).

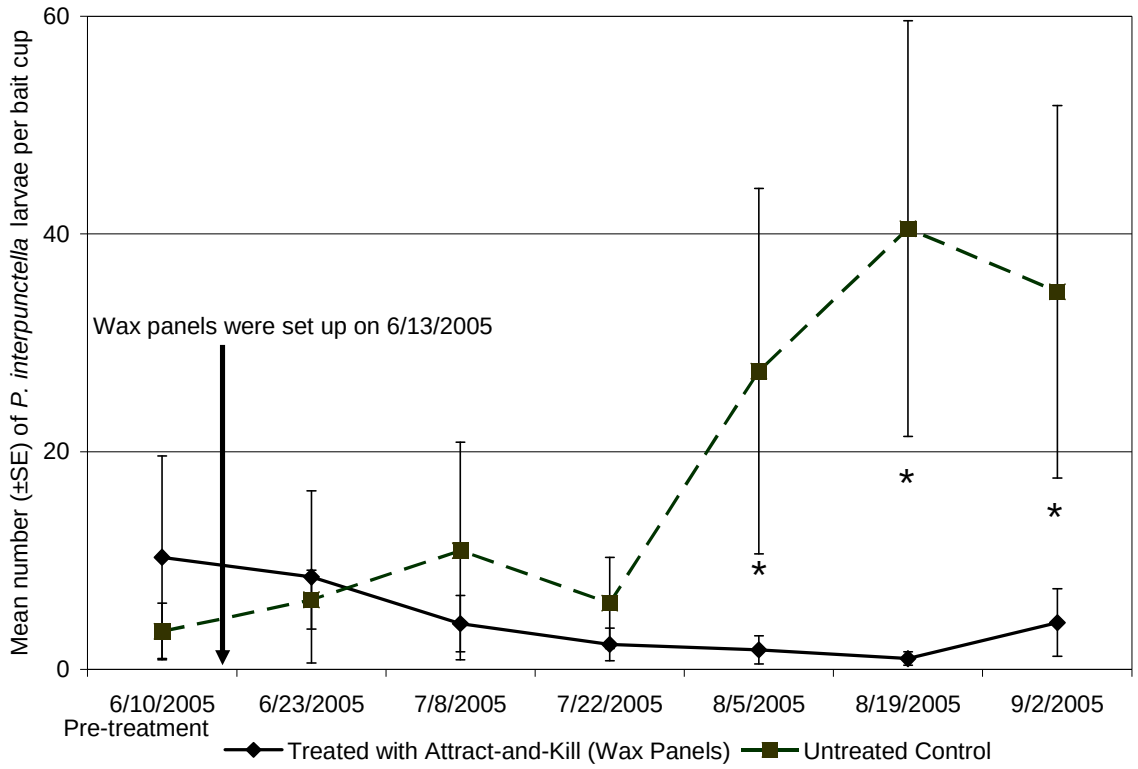

Fig. 2. Mean number (±SE) of *P. interpunctella* larvae per bait cup by effect of the attract-and-kill formulated with wax panels impregnated with Permethrin 6.0% A.I. and deployed with a synthetic female sex pheromone Biolure® in treated and non-treated commercial establishments in Dallas, TX. 2005.

* Treatments are significantly different at $P < 0.05$ (ANOVA followed by pair-wise t-test of the effect among treatments, $F_{1, 6.4} = 5.20$, $P = 0.0597$).

CHAPTER IV

SUMMARY AND CONCLUSIONS

The following is a summary of the research conducted for this dissertation on the evaluation of attract-and-kill formulations to control the Indianmeal moth, *Plodia interpunctella* (Hübner) performed in the laboratory, in simulated warehouses, and in commercial facilities.

Chapter II. Contact toxicity of insecticides

The overall conclusions on the contact toxicity experiments with a variety of insecticides found that pyrethroids and naturally derived pyrethrins, both applied to surfaces at registered label rates, had the highest toxicity against adult male Indianmeal moths in these studies compared with other classes of insecticides. Permethrin applied at the high rate of 2.0% in the final spray suppressed adult males of *P. interpunctella* and prevented reproduction for up to 8 weeks when applied to surfaces of plastic-coated paper, bare metal, bare plastic and to a lesser degree on bare wood. Residual activity of pyrethroids and pyrethrins was very poor when applied to a painted surface, and this maybe due to degradation or reaction of the active ingredient when in contact with the dry paint. The study clearly showed that attract-and-kill formulations could control *P. interpunctella* for up to 8 weeks and can be developed using adequate application doses of Permethrin to a variety of surfaces. The attract-and-kill method is desirable for reduced input of insecticides in food storage areas because the specific pest is targeted via

the pheromone lure to contact a small amount of an effective and locally contained killing agent.

Chapter III. Laboratory evaluation of attract-and-kill formulations

Three attract-and-kill device formulations were evaluated in the laboratory using wind tunnel bioassays to assess contact of males after flight, and toxicity and reproduction up to eight weeks after formulation. For all the three experiments, at week 0 the percentage of landing and contact time was low, presumably because the pheromone dispensers were brand new and at the moment that they were opened, they released high volume of pheromone plume. These presumably relatively high levels of pheromone caused a repellency or arresting effect, instead of direct attraction and contact. The gel-like formulation had more than 70% of the adult males landing on the formulation at time 2. However, the percent of males landing and their contact time was lower after week 4 up to the end of the experiment at week 8. Thus, the gel did not release an optimal amount of pheromone to attract adult males. On the other hand, the wax panel and cylinder formulations deployed with the Biolure® pheromone dispenser showed up to 100% of landing and more than 6 seconds in average of contact time.

The low percent of landing and contact time of the attract-and-kill formulations in the wind tunnel resulted in a low impact over mortality of adult males. The gel formulated with Permethrin 50 and 100 mg, and Pyrethrin 100 mg killed more than 50% adult males only at week 2. Better suppression of adult males was made by the Cyfluthrin 6.0% and Permethrin 6.0% impregnated in the wax panel and Cyfluthrin 2.0% in the cylinder device. The wax panel and plastic cylinder formulations impacted the adult males with

more than 75% mortality from week 2 and persisted to the end of the experiment at week 8. The formulations that impacted the adult male populations also impacted the egg laying and the percent of egg hatching. Thus, wax panels and plastic cylinders treated with Cyfluthrin and Permethrin, both at 6.0% [A.I.] and deployed with the Biolure® slow release pheromone lure that lasts up to 8 weeks, offer a good suppression of adult males of *P. interpunctella* in wind tunnel conditions.

Chapter IV. Pheromone-based methods in commercial establishments

The attract-and-kill devices were tested further under controlled field-like conditions in mini-storage rooms, which acted as simulated food warehouses. In the controlled simulated warehouses experiment the wax panel and the cylinder formulations suppressed the populations of the *P. interpunctella* at 1 panel per room, regardless of moth population density. This success was also in part due to the effectiveness of attraction by the Biolure® deployed on these formulations. This controlled experiment eliminated the LastCall gel from further consideration and it was a baseline on which to design the field experiments in commercial buildings.

In the field experiments done in commercial establishments the wax panel treated with 6.0% Permethrin was very effective at suppressing *P. interpunctella* populations during 2005. However, in 2006 a new attract-and-kill panel using 0.08% Deltamethrin sprayed on plastic-coated paper, did not work as well as the wax panel in the previous year, presumably due to the lower amount of active ingredient that was adsorbed to the plastic film (see chapter 2). Mating disruption using the same Biolures at the same density as attract-and-kill panels was very effective. Mass trapping was similar to mating disruption

and more effective than attract-and-kill in the 2006 study because it truly removed large numbers of males form the breeding population by trapping them, as opposed the possible ineffective male-killing with new attract-and-kill formulation. Given the proper formulations, either method of control like mating disruption, mass-trapping or attract-and-kill, may be implemented to control this important pest, and their success will depend in the concentration of active ingredient for the attract-and-kill formulations, and combination of the source of pheromone to be used. Success could also be enhanced by limiting the movement of infested merchandise from store to store, and a good recommendation would be to apply the method of control consistently to a whole set of stores and their source of merchandise.

Conclusions

The impact of the studies reported here is that pheromone-based techniques may be adopted by pest control operators and the food industry for managing stored product moths. This dissertation can be used as baseline about how often the devices and lures have to be set up and when they have to be changed. More research is needed in order to test the longevity of the active ingredients. The ultimate adoption of attract-and-kill or other pheromone based methods will rely in part on government regulatory approval, user acceptance through proven efficacy, and cost-benefit analyses by the user.

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Candidate for the Degree of

Doctor of Philosophy

Thesis: ATTRACT-AND-KILL METHODS FOR CONTROL OF INDIANMEAL MOTH, *Plodia interpunctella* (Hübner) (LEPIDOPTERA: PYRALIDAE), AND COMPARISONS WITH OTHER PHEROMONE-BASED CONTROL METHODS

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Pages in Study: 114 Candidate for the Degree of Doctor of Philosophy

Major Field: Entomology

Scope and Method of Study: The approaches were to test attract-and-kill formulations in laboratory conditions, simulated warehouses and commercial establishments to control *Plodia interpunctella* populations.

Findings and Conclusions: Adult males of *Plodia interpunctella* were impacted by pyrethroids and naturally derived Pyrethrins in contact toxicity tests, where mortality was more than 70%. Permethrin might be an alternative to be used in an attract-and-kill formulation for up to eight weeks. Is has adequate residual active ingredient, and adequate material and doses can be sprayed on to plastic-coated paper, bare metal, or bare plastic. The dispenser of pheromone is important in the attraction of adult males. An optimal release gradient will attract males; otherwise improper gradients will repel or do not elicit any attraction. Wax panels and plastic cylinders, both with 6.0% active ingredient (permethrin and cyfluthrin, respectively), that are deployed with a Biolure® strips suppressed adult populations in wind tunnel experiments. Similar impact was demonstrated in simulated warehouses, except that cyfluthrin was used at 2.0% active ingredient. In 2005, a field experiment was conducted in commercial establishments, and wax panels with 6.0% permethrin that were deployed with Biolures® significantly suppressed *P. interpunctella* populations. In 2006, pheromone-based methods were tested in commercial establishments. Among methods tested, the attract-and-kill formulation using 0.08% deltamethrin sprayed on plastic-coated paper. This method was not as effective as the wax panels utilized during the previous year. Mating disruption method with Biolures® only was as equally as effective as mass-trapping. Either of these methods can be used by pest control operators if applied at the proper concentration and formulation.

ADVISER'S APPROVAL: Dr. Thomas W. Phillips