THE SUBLETHAL EFFECTS OF ECDYSONE AGONISTS ON THE ATTRACTIVENESS, RESPONSIVENESS, FERTILITY AND FECUNDITY OF ORIENTAL FRUIT MOTH, AND A COMPARATIVE EXAMINATION WITH CODLING MOTH ON LARVAL FEEDING DAMAGE

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THE SUBLETHAL EFFECTS OF ECDYSONE AGONISTS ON THE ATTRACTIVENESS, RESPONSIVENESS, FERTILITY AND FECUNDITY OF ORIENTAL FRUIT MOTH, AND A COMPARATIVE EXAMINATION WITH CODLING MOTH ON LARVAL FEEDING DAMAGE

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ABSTRACT

The ecdysone agonists methoxyfenozide and tebufenozide are designed to affect the larval stage of Lepidopteran pests. Recent studies have reported sublethal effects on the adults of several tortricid apple tree pests exposed to these insect growth regulators. These effects include changes in mean fecundity and fertility and reduced mate-finding abilities. Here we report sublethal effects of these insect growth regulators on the oriental fruit moth, *Grapholita molesta* (Busck). Wind tunnel assays showed a reduction in mate finding capabilities of the moths when exposed to methoxyfenozide. Mating assays showed a reduction in mean fecundity but, generally, not mean percent fertility when females were exposed to either tebufenozide or methoxyfenozide. Median female longevity was also reduced. In another study presented here the feeding patterns of the oriental fruit moth were compared to that of codling moth, *Cydia pomonella* (L.). Differences in sites of entry as well as visible tunneling and frass were observed. Differences were also detected in the internal fruit damage between the two moth species.

Objectives

1. Determine the sublethal effects of methoxyfenozide-treated surfaces on the attractiveness and responsiveness of adult oriental fruit moth, *Grapholita molesta* (Busck).

2. Determine what effects adult exposure to tebufenozide- and methoxyfenozide-treated surfaces would have on the fecundity and fertility of the oriental fruit moth.

3. Compare and contrast the larval feeding patterns and damage of oriental fruit moth and codling moth, *Cydia pomonella* (L.).

Chapter 1: Literature Review

A. Insect Growth Regulators

Since the advent of crop cultivation, the need to find effective strategies for the control of agricultural pests has been a constant struggle. The earliest record of such efforts appears to be the use of sulfur by the Sumerians as early as 2500 B.C. By the 1600's the insecticidal properties of some soaps, botanicals, and inorganic compounds such as arsenic had been discovered. The modern insecticide era began in the 1930's with the synthesis of new insecticidal compounds such as DDT (dichlorodiphenyltrichloroethane). These broad spectrum insecticides targeted physiological processes found across many higher animal taxa. The early 1960's saw public pressure for the development of control strategies that would reduce the amount of broad spectrum synthetic insecticides in pest control (Pedigo 2002).

One group of compounds that has received much attention over the past several years, because of their environmental safety and relative target specificity, are the synthetic insect growth regulators (IGR's). Such compounds tend to disrupt the target pest's natural physiology and development, such as the molting process, while being relatively safe to non-target organisms (Pedigo 2002).

Insects undergo periodic shedding of their exoskeleton during their immature stages. The molting process begins with an increase of the hormone 20-hydroxyecdysone. The insect ceases feeding and goes through apolysis (the

separating of the epidermis from the old cuticle), which forms an ecdysial space filled with fluid. The epidermal cells then reorganize to form new cuticular layers. As ecdysone levels begin to decline in the insect, the procuticle is digested and recycled for new cuticle formation. Once ecdysone levels reach basal levels ecdysis occurs. This is when the insect sheds the old cuticle. The levels of another key hormone, juvenile hormone, during the molting process dictate the next developmental stage. The lower the juvenile hormone titer, the more advanced the life stage the insect will develop into (Dhadialla et al. 1998).

Synthetic IGR's disrupt several steps in an insect's molting process. Some are targeted at juvenile hormone activity within the insect prior to molting. Others are designed to disrupt chitin synthesis at the end of a molt. Some compounds impact the ecdysone receptors in the molting process. The fact that IGR's can disrupt chemical pathways specific to some insect types is their greatest advantage over conventional insecticides. They are considered third generation insecticides because of their greater environmental safety when compared to first-generation (stomach poisons) and second-generation (contact poisons) insecticides (Pedigo 2002).

Because of their relative specificity, IGR's do have limitations. For example, if they are effective only upon certain developmental stages of the target pest, then the timing of application is critical for effective use. In addition, IGR's are most effective against insects with short life cycles. If an application misses the critical developmental stage an entire generation of pests may be unaffected. Another limitation is that most IGR's are slow-acting. Consequently,

plant injury from a pest can continue for a longer period of time than when conventional insecticides are used. IGR's are of value against stored product and secondary damage pests, but show little promise for crops where visual fruit quality is of importance to the consumer (Mondal and Parween 2000, Pedigo 2002, Abo-Elghar et al. 2004, Dallaire et al. 2004).

1. Juvenile Hormone Mimics

Within the insect juvenile hormone influences many key physiological activities including development, reproduction, behavior, pheromone production, adult diapause and caste determination (Wilson 2004). The first to suggest the potential exploitation of juvenile hormone as an insect control agent was Carol Williams in 1967 (Retnakaran et al. 1985). Unfortunately, the use of natural juvenile hormone was not feasible due to its environmental instability and the difficulties involved with its synthesis. The major breakthrough came a short time later when William Bowers, in 1969, discovered a way to synthesize a juvenile hormone analog several hundred times more active than natural juvenile hormone. There was immediate interest in this new technology. Within 15 years of Bowers' discovery more than 500 different mimics with varying degrees of insecticidal activity had been developed (Retnakaran et al. 1985).

A standard larva-to-pupal molt is induced by the presence of 20-hydroxyecdysone in the absence of juvenile hormone. Juvenile hormone mimics disrupt this life stage by causing an extra, typically lethal, larva-to-larva molt (Dhadialla et al. 1998). In recent years, juvenile hormone has also been shown to

be instrumental in stimulating vitellogenesis and oocyte development in many insects (Cruz et al. 2003). Addition of juvenile hormone mimics into an insect's system usually results in adult sterility or the inability to successfully mate.

2. Juvenile Hormone Antagonists

Some botanicals were discovered that inhibit juvenile hormone production. These juvenile hormone antagonists can block the manufacture of juvenile hormone in the corpora allata. This has the effect of inducing a premature larva-to-pupal molt which results in miniature, sterile adults. William Bowers was the first to discover juvenile hormone antagonists when he isolated the chemicals from the bedding plant *Ageratum houstonianum* (Retnakaran et al. 1985). Most of the resulting synthesized compounds used today are known as precocenes (Retnakaran et al. 1985). Because juvenile hormone has the ability to affect many insect activities including development, reproduction, behavior, pheromone production, adult diapause and caste determination (Wilson 2004), as well as maternal behavior (Rankin et al. 1997, Kight 1998), these antagonists can be highly effective in disrupting key physiological activities.

3. Chitin Synthesis Inhibitors

Chitin is one of the major components in an insect's exoskeleton. For example, it is responsible for giving the exoskeleton its rigid characteristics the insect needs for internal support and water conservation. Chitin synthesis inhibitors disrupt the molting process through inhibition of chitin synthesis,

endocuticular deposition, egg hatch, and DNA synthesis, as well as disruption of ecdysis and alteration of carbohydrases and phenyloxidases (Retnakaran et al. 1985, Mondal and Parween 2000, Medina et al. 2002). The majority of the chitin synthesis inhibitors belong to the group benzoylphenyl ureas. They were discovered around 1970 by the Philips-Duphar Company when they combined the herbicide dichlobenil with the urea herbicide diuron (Retnakaran et al. 1985).

4. Ecdysone Agonists

Ecdysone agonists are one of the most recently developed groups of IGR's. They were discovered by the Rohm and Haas Company in 1983. Ecdysone agonists mimic the molting hormone, 20-hydroxyecdysone, resulting in premature molting. The lethal effects are a multi-step process. First, the larva ceases to feed within 4-16 hours of ingestion. Within 24 hours the larva goes through apolysis, most noticeable at the head capsule. The larva, however, is unable to complete ecdysis. With the old head capsule not being completely shed, the new head and mouthparts are unable to sclerotize. In addition, the hind guts are extruded, resulting in loss of hemolymph and molting fluid. Desiccation and death soon occur (Dhadialla et al. 1998).

Soon after the discovery of these ecdysone agonists, Rohm and Haas developed the first analog (RH-5849) to be extensively tested in the field (Dhadialla et al. 1998). It had activity over a small range of Lepidoptera, Coleoptera and Diptera (Smagghe and Degheele 1994a, Dhadialla et al. 1998, Mondal and Parween 2000). RH-5849 was eventually replaced by the more

selective, potent, and cost-effective analogs, tebufenozide (RH-5992) and methoxyfenozide (RH-2485) (Smagghe and Degheele 1994a, Dhadialla et al. 1998, Beckage et al. 2004).

a. Tebufenozide

Tebufenozide (RH-5992), the first successor of RH-5849 was released in 1992 (Dhadialla et al. 1998). It is a more directed ecdysone agonist with a higher toxicity to lepidopteran insects and a lower toxicity to other insect orders, making it a desirable tool for integrated pest management regimes (Smagghe and Degheele 1994b, Dhadialla et al. 1998). There have been isolated examples of higher effectiveness of tebufenozide to non-lepidopteran insects when compared to RH-5849 (Beckage et al. 2004). Tebufenozide's high level of specificity allows for adequate control of the target pests without negatively affecting any biological control agents and other beneficial organisms (Rodriguez et al. 2001). It is also possible to couple it with a mating disruption tactic for enhanced control (Trimble and Appleby 2004). However, recent studies have demonstrated a potential development of cross resistance among some pests with conventional insecticides such as azinphosmethyl (Smirle et al. 2002). Tebufenozide was the first ecdysone agonist on the commercial market and is now sold under the names Confirm®, Mimic®, and Romdan® (Dhadialla et al. 1998).

b. Methoxyfenozide

Methoxyfenozide (RH-2485) is the most recent addition to the ecdysone agonist family. It was introduced by the Rohm and Haas Company in 1996. As with tebufenozide, it is highly specific to lepidopteran pests, but has a low toxicity towards other insect orders. Unlike tebufenozide, methoxyfenozide has root systemic activity, especially with monocotyledonous plants like rice. Methoxyfenozide, however, is not systemic through leaves. Depending on the target pest, application rates can vary between 20 and 200 g/ha, approximately half the required rates of tebufenozide (Carlson et al. 2001), reflecting its greater effectiveness to susceptible insects over tebufenozide. One example of this is with mosquitoes (Beckage et al. 2004). As with tebufenozide, recent studies have reported that pests resistant to conventional insecticides, such as azinphosmethyl, can also be resistant to methoxyfenozide (Smirle et al. 2002). Gore and Adamczyk (2004) went so far as to artificially create resistance in a population of beet armyworms, *Spodoptera exigua* (Hübner), to help in the development of a resistance management program. Methoxyfenozide is currently being sold under the trade names Intrepid®, Runner®, and Prodigy® (Carlson et al. 2001).

B. Sublethal Effects of Insecticides

The behavior of all animals, including insects, is governed through proper communication between the hormonal and nervous systems. Insecticides have been developed to exploit these physiological systems. Thus, it is fair to assume

if an insect is exposed to a sublethal dose of an insecticide the behavior and physiology of the insect would probably be affected. For example, mate location and courtship, oviposition, oogenesis, ovulation, spermatogenesis, sperm mortality, and physiological effects on egg fertilization could all be negatively influenced through a sublethal exposure to an insecticide (Haynes 1988).

1. Neurotoxic Insecticides

Neurotoxic insecticides make up the majority of what are commonly known as “conventional broad spectrum insecticides.” They disrupt normal neurological functions within the insect. These chemicals provide an effective, inexpensive, and reliable means of insect control (Haynes 1988). The three most common classes of insecticides in this group are the organophosphates, carbamates (synaptic poisons) and the synthetic pyrethroids (axonic poisons). Synaptic poisons interrupt the normal cell-to-cell signal transmission of the nervous system. Axonic poisons inhibit signal transmission along individual cell axons (Pedigo 2002).

Bariola (1984) determined that two organophosphates, azinphosmethyl and methyl parathion, were capable of reducing mating occurrences of pink bollworms, *Pectinophora gossypiella* (Saunders), as well as reducing oviposition when treated with sublethal doses as freshly emerged adults. Alford and Holmes (1986) demonstrated that sublethal doses of the organophosphate fenitrothion reduced the fecundity of the spruce budworm, *Choristoneura fumiferana* (Clemens), when both sexes had been placed on treated artificial diet as fourth

instar larvae for seven days. Nuessly and Hentz (2004) observed that when the organophosphates chlorpyrifos and methyl parathion where sprayed at field rates they were able to induce sublethal activities described as “uncoordinated movement, uncontrolled twitching and hyperextension of mouthparts and ovipositor” in adult corn silk fly, *Euxesta stigmatias* Loew, within one hour of treatment. Nuessly and Hentz (2004) observed these same effects with two carbamates, methomyl and thiodicarb on adult corn silk fly.

Alford and Holmes (1986) showed that when both sexes of spruce budworm had been placed on treated artificial diet as fourth instar larvae for seven days sublethal levels of carbaryl and aminocarb extended the larvae’s developmental period , and aminocarb also caused a reduction in fecundity. Carbaryl appears to also have an effect on pink bollworms, reducing mating and oviposition in treated adults (Bariola 1984).

Pink bollworm physiology, while affected by both the organophosphates and carbamates, was most influenced by pyrethroids. For example, the pyrethroids cyfluthrin, flucythrinate, fenpropathrin fenvalerate and permethrin all were capable of reducing mating and oviposition in treated adults (Bariola 1984). Sublethal rates of cyfluthrin was also shown to slow the growth rate of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Bernard and Lagadic 1993). In adult corn silk fly Nuessly and Hentz (2004) found that the pyrethroids esfenvalerate, cyfluthrin, and lambda-cyhalothrin could all cause “uncoordinated movement, uncontrolled twitching and hyperextension of mouthparts and ovipositor” within one hour of treatment.

2. Juvenile Hormone Mimics

Juvenile hormone mimics (JHM) induce the insect to proceed through an extra, lethal larva-to-larva molt. By far, the most commonly studied JHM is fenoxycarb. For example, fenoxycarb has been shown to have sublethal effects on tufted apple bud moth, *Platynota idaeusalis* (Walker). When larvae were fed treated diet the subsequent adults displayed a reduction in fecundity and fertility and an increase in female development time (Biddinger and Hull 1999). Hicks and Gordon (1992) and Gordon (1995) both proved fenoxycarb treated adult eastern spruce budworm, *Choristoneura fumiferana* (Clemens), developed a substantial reduction in fertility. Fenoxycarb has also been reported to reduce the number of oocytes in codling moth, *Cydia pomonella* (L.), females when treated as pupae and as freshly emerged adults (Webb et al. 1999). Additional studies have shown fenoxycarb to reduce fertility in the California fivelined ips beetle, *Ips paraconfusus* Lanier, the pear psylla, *Cacopsylla pyricola* Foerster, and the oriental cockroach, *Blatta orientalis* (L.) (Chen and Borden 1989, Evans et al. 1995, Higbee et al. 1995). Reductions in fecundity through sublethal exposure of fenoxycarb also occur in the oriental cockroach the migratory grasshopper, *Melanoplus sanguinipes* (F.), the differential grasshopper, *Melanoplus differentialis* (Thomas), and the red imported fire ant, *Solenopsis invicta* Buren (Glancey and Banks 1988, Capinera et al. 1991, Evans et al. 1995).

3. Juvenile Hormone Antagonists

Juvenile hormone antagonists work against the normal effect of juvenile hormone in the molting process. The result of exposure to juvenile hormone antagonist is a premature larva-to-pupa or nymph-to-adult molt. While the juvenile hormone antagonist's function is counteractive to JHM's, it has similar sublethal effects such as a reduction in fecundity (Connat and Nepa 1990). Numerous studies have reported that sublethal exposure to precocene I or precocene II can reduce fecundity in the fruit fly, *Drosophila melanogaster* Meigen, the black blowfly, *Phormia regina* Meigen, and the brown planthopper, *Nilaparvata lugens* (Stal) (Wilson et al. 1983, Yin et al. 1989, Ayoade et al. 1996). In addition, precocene can also reduce the mating and sex attractancy in the migratory locust, *Locusta migratoria* (L.) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Chang et al. 1984, Shalom and Pener 1986).

4. Chitin Synthesis Inhibitors

Chitin synthesis inhibitors prevent the insect from synthesizing chitin and completing the molting process. Sublethal doses of diflubenzuron, the most widely studied chitin synthesis inhibitor, has been reported to delay ecdysis in codling moth larvae, *Cydi pomonella* (L.) (Soltani and Soltani-Mazouni 1992). When applied to boll weevil, *Anthonomus grandis grandis* Boheman, larvae it reduces cuticle hardness (Haynes and Smith 1994, Villavaso et al. 1995). The sublethal effects of diflubenzuron exposure are not limited to the molting process. For example, adult boll weevils suffered from reduced fertility and flight activity

when exposed to diflubenzuron as larvae (Haynes and Smith 1994, Villavaso et al. 1995). When larvae are reared on diet containing sublethal doses of diflubenzuron, a reduction in fertility in the corn earworm, *Helicoverpa zea* (Boddie), results (Carpenter and Chandler 1994). Rup and Chopra (1985) reported reduced fertility as well as fecundity and adult longevity of diflubenzuron-treated banana fruit fly, *Zaprionus paravittiger* (Godble and Vaidya). Kim et al. (1992) also reported a reduction in fecundity and adult longevity of diflubenzuron-treated adult alydids, *Riptortus clavatus* (Thunberg). Interestingly, two *Brachymeria* parasites of gypsy moth pupae develop multiple progeny within a single host when adults are treated with diflubenzuron. Typically only one offspring rears within a single host (Khoo et al. 1985).

5. Ecdysone Agonists

Ecdysone agonists induce molting by emulating 20-hydroxyecdysone. Due to their persistence in the body, however, ecdysone agonists prevent the completion of molting, usually resulting in death of the individual. However, if applied at sublethal doses or at alternate life stages, they can have multiple effects. The most common of these effects is a reduction in fecundity and fertility. When neonate tufted apple bud moth, *Platynota idaeusalis* (Walker), were treated with sublethal doses of tebufenozide the resulting adults had a reduced fertility (Biddinger and Hull 1999). Smagghe and Degheele (1994b) noticed the same effect when fifth instars of the beet armyworm, *Spodoptera exigua* (Hubner), were treated with tebufenozide. Codling moth, *Cydia pomonella* (L.),

redbanded leafroller, *Argyrotaenia velutinana* (Walker), and oblique-banded leafroller, *Choristoneura rosaceana* (Harris), all showed reductions in fecundity and fertility when exposed to field rates of tebufenozide and methoxyfenozide throughout their adult lives (Sun and Barrett 1999, Sun et al. 2000). These same moth species showed a reduction in fertility and fecundity when recently emerged, virgin females were treated with the same levels of tebufenozide and methoxyfenozide for only a 24 h period (Sun and Barrett 1999, Sun et al. 2000). RH-5849 and, to a greater extent, tebufenozide have been determined to reduce fecundity and fertility in the cluster caterpillar, *Spodoptera litura* (F.), most likely due the reduced number of sperm transferred from male to female (Seth et al. 2004).

The negative effects due to sublethal or non-target stage exposure to ecdysone agonists are not restricted to fecundity and fertility. Seth et al. (2004) noticed a reduction in mating success and adult longevity of the cluster caterpillar, *Spodoptera litura* (F.). Smagghe and Degheele (1994b) saw a reduction in weight gain and feeding of *Spodoptera exigua* (Hubner) when treated. Tebufenozide treated larvae of *Choristoneura fumiferana* (Clem) and *Choristoneura rosaceana* (Harris) developed a plethora of problems such as an increase in development time, a decrease in adult weight, a reduction in sperm production, a delay in ovarian maturation, a reduction in mating success and a reduced ability of the males to successfully orient themselves upwind in a wind tunnel (Dallaire et al. 2004).

C. Wind Tunnel Research with Lepidoptera

Studies on orientation responses of insects to visual environmental cues and to host odors have stimulated the use of wind tunnels in insect research. The increased interest in insect sex pheromones that began in the 1970's has made the wind tunnel an invaluable research tool. For example, wind tunnels are being used in several areas of pheromone work including pheromone identifications, blend quality testing, designing of pheromone traps and orientation studies, including the behavioral effects from sublethal insecticide exposure (Baker and Linn 1984).

There are several distinct advantages of using wind tunnels when compared to field flight bioassays. The first is the ability to adjust individual variables. Temperature, humidity, wind speed, light intensity, and plume conditions can all be manipulated or maintained for consistency. Another advantage is the ability to test throughout the year, as there is no dependence on seasonal field conditions. A third advantage is the ease of manipulation of the experimental procedure to develop meaningful studies within a shorter time span (Baker and Linn 1984).

The majority of wind tunnels utilize horizontal air flow, yet vertical ones do exist. Some have fans that push air through the tunnel, which helps maintain consistent plumes. Others pull the air through the tunnel, allowing for the ability to better control the quality of the incoming air. The latter type is usually used in pheromone research, as it makes exhausting the air away from the intake system easier to accomplish (Baker and Linn 1984).

Several orders of insects are regularly studied in wind tunnels. The most common of which is Lepidoptera, as many aspects of lepidopteran behavior revolve around wind as a stimulus or as a carrier of stimulating odors. For example, a wind tunnel was utilized to accurately gauge flight distances and dispersal patterns of the fall webworm, *Hyphantria cunea* (Drury) (Yamanaka et al. 2001). Reddy et al.(2004) showed the diamondback moth, *Plutella xylostella* (L.), was able to orient towards favored host plants via the wind tunnel. The moths *Tuta absoluta* (Meyrick), a pest of tomatoes, and *Tyta luctuosa* (Denis and Schiffermuller), a potential biological control agent of field bindweed, both became subjects in wind tunnel studies determining trapping efficiency of sex pheromone components (Ferrara et al. 2001, Cao et al. 2003). Nansen and Phillips (2004) looked at combining permethrin with an established synthetic sex pheromone attractant for control of the indianmeal moth, *Plodia interpunctella* (Hubner).

Some wind tunnel work in recent years have provided a better understanding regarding pheromone-mediated communication and host-finding processes of tortricid pests. Willis and Baker (1994) used a video recording device to map and quantify the orientation behavior of a male oriental fruit moth, *Grapholita molesta* (Busk), toward the female. They found that as the male approached the female his ground speed decreased, angle of tack with respect to the female increased, and rate of counterturning increased, giving the overall result of the male track slowing and narrowing as he approached. Valeur and Lofstedt (1996) used the wind tunnel to compare synthetic pheromone blends for

oriental fruit moth by placing blend components in series or parallel to each other and monitoring the attraction to them by the male. The ultimate result was that males were more attracted to complete blends rather than just blend components. Evenden and McLaughlin (2004) compared the combination of pheromones at several rates mixed and unmixed with the pyrethroid permethrin to see if permethrin affected the attractiveness of the pheromone, ultimately showing no effect. Natale et al.(2004) utilized flight chambers to quantify oriental fruit moth attraction to host plant odors. Three tests were performed: no-choice, short-term (5 min) individual insect choice and long-term (14 h) multiple insect tests. All tests showed positive responses to the host volatiles. Hoelscher and Barrett (2003a) reported a reduction in responsiveness of male redbanded leafroller, *Argyrotaenia velutinana* (Walker), and oblique-banded leafroller, *Choristoneura rosaceana* (Harris), treated with methoxyfenozide when exposed to female sex pheromone.

Much research has been conducted concerning the makeup of the sex pheromone of female codling moth, *Cydia pomonella* (L.), and its role in moth courtship. For example, studies comparing the moth's main pheromone component, (E,E)-8-10-dodecandienol (codlemone), with other isomers and other secondary components have been conducted (Arn et al. 1985, Preiss and Priesner 1988, McDonough et al. 1995, Ebbinghaus et al. 1998, El-Sayed et al. 1998). A complimentary study showed that males do not respond as well to synthetic codlemone as they do to calling females or female gland extracts (El-Sayed et al. 1999). Castrovillo and Carde (1979) used a wind tunnel to show that

females exhibit the same calling periodicity to which they were reared, even under constant photophase or scotophase conditions. Castrovillo and Carde (1980) were able to prove codling moth males use visual cues as well as pheromones to locate females by showing that males spent more time close to a dead female than to a pheromone lure. Wind tunnel studies were used to determine codlemone concentrations in an attracticide formulation containing cyfluthrin (Losel et al. 2000). Other studies were able to determine that mated female codling moths were attracted to apple odors, with enhanced attraction to apples already infested with moths (Reed and Landolt 2002).

Several other tortricid moths have also been the subject of wind tunnel studies. Mating disruption, behavior, habituation and pheromone component studies have been performed on the European grapevine moth, *Lobesia botrana* Den. & Schiff. (Hurtrel and Thiery 1999), European grape moth, *Eupoecilia ambiguella* Hubner (Rauscher et al. 1984), light-brown apple moth, *Epiphyas postvittana* (Walker) (Foster and Harris 1992, Rumbo et al. 1993), spruce budworm, *Choristoneura fumiferana* (Clemens) (Sanders 1985, Dallaire et al. 2004), smaller tea tortrix moth, Adoxophyes sp. (Kainoh et al. 1984, Hiyori et al. 1986), redbanded leafroller, *Argyrotaenia velutinana* Walker (Miller and Roelofs 1978) and obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Evenden et al. 2000, Dallaire et al. 2004).

D. Experimental Organisms

1. Oriental Fruit Moth

The oriental fruit moth, *Grapholita molesta* (Busck), is a member of the family Tortricidae. It is a native of northwest China. The earliest documentation of its spread outside of China was to Japan in 1901, but by the 1930's every continent had proof of its presence (Rothschild and Vickers 1991). The oriental fruit moth was introduced to North America from Japan in 1913 through the importation of flowering cherry trees (Rothschild and Vickers 1991, Beers et al. 1993).

Common host plants include various species of *Prunus* and *Pyrus,* as well as fruiting myrtle (*Eugenia myrianthus*), rose (*Rosa sp.*), loquat (*Eriobotrya japonica*), and apple (*Malus silvestris*) (Rothschild and Vickers 1991, Beers et al. 1993). The preferred wild host is fruiting quince (*Cydonia vulgaris*), but the primary cultivated hosts are peach and nectarine (Summers 1966, Rothschild and Vickers 1991, Beers et al. 1993).

Beginning in Brazil in 1982 (Reis Fo et al. 1988), reports throughout the world indicated oriental fruit moth moving into commercial apple orchards, but the reasons for this host shift are, as yet, unclear (Usmani and Shearer 2001, Hughes and Dorn 2002).

a. Biology

Oriental fruit moth eggs are small (0.8 mm in diameter), flat, oval discs that are white in color. They turn amber in color just before hatch (Rothschild and

Vickers 1991, Beers et al. 1993). Eggs are typically laid on smooth surfaces of leaves or young twigs (Reis Fo et al. 1988, Rothschild and Vickers 1991, Beers et al. 1993). A single female can produce up to 200 eggs (Beers et al. 1993), but there is great variation between individuals (Rothschild and Vickers 1991). Egg incubation is typically 4-8 days (Rothschild and Vickers 1991, Beers et al. 1993).

Freshly hatched larvae are approximately 2 mm long and 0.5 mm in diameter with a cream-colored body and a black head (Beers et al. 1993). The Oriental fruit moth typically goes through four larval instars with five instars possible under certain conditions. The mature larvae are pink/white in color with a brown head capsule and are between 8 and 13 mm long. Larval development typically takes two to three weeks (Rothschild and Vickers 1991, Beers et al. 1993).

Prior to pupation, the mature larva leaves the host's shoot or fruit and finds a protected site to spin a cocoon, such as under the tree's bark or in brush or ground litter (Beers et al. 1993). Early generations split their choices between in-tree sites and ground litter sites. Later generations, including the overwintering generation, primarily leave the tree to pupate, which takes about 10-16 days (Rothschild and Vickers 1991).

Adults are approximately 5 mm long and are gray with indistinct light and dark bands, giving the oriental fruit moth a salt-and-pepper appearance (Beers et al. 1993). Adult life expectancy is between 12 and 15 days. Both sexes are attracted to plant volatiles related to host plants, presumably for host-finding (Natale et al. 2004). Mate-finding is pheromone mediated, but the process can be

regulated by light and temperature. Adults become sexually active within two days of emergence. Mating procedures begin with the female moving to the upper canopy of the tree to begin “calling.” This consists of the female releasing sex pheromone from glandular tissue in the intersegmental membrane between the posterior abdominal segments. Females begin calling approximately 3 hours before the onset of scotophase and will continue 1 hour into scotophase, but onset of calling could be much earlier on cool days (Rothschild and Vickers 1991).

Male moths show similar activity patterns to the female, but some data suggests they remain sexually responsive later into scotophase. Three of the four components that make up the female sex pheromone are responsible for arousal and upwind flight of the male, while the fourth is believed to be critical in the final stages of courtship. Once the male finds the female he everts his hair pencils (hair-like structures at the tip of the abdomen) that release a blend of components used to enhance female receptivity. Once in the mating posture insemination occurs via a spermatophore. Males will typically mate only once per night, but may mate with several females on successive nights. One mating is enough for the female to lay her full complement of eggs (Rothschild and Vickers 1991).

The oriental fruit moth overwinters as a mature larva within a silken cocoon (Beers et al. 1993). Depending on latitude two to seven generations can be expected per year (Rothschild and Vickers 1991). Missouri typically sees five

generations per year, with a partial sixth in the southern portions of the state (Sarai 1970).

b. Pest Status and Control

The oriental fruit moth modifies its food preference throughout the year. First generation larvae primarily bore into young shoot tips of the cultivated host plant, but can also feed upon young, developing fruit. The proportion of larvae attacking shoots versus those attacking fruit shifts toward the fruit with each subsequent generation (Reis Fo et al. 1988, Rothschild and Vickers 1991, Beers et al. 1993). Damage of the young fruit is relegated to near the surface, but as the fruit matures the larvae burrow further into the ripening flesh. Larval presence in fruit is indicated by tiny entrance holes near the stem containing fine, brown frass (Rothschild and Vickers 1991, Beers et al. 1993).

Before the advent of synthetic organic insecticides in the 1940's, inorganic chemicals were largely ineffective against the oriental fruit moth. Farmers were relegated to numerous forms of cultural control. This prompted the successful development of biological control programs. Most of this work has revolved around the larval parasitoid *Macrocentrus ancylivorus* Rohwer and several *Trichogramma* egg parasites (Merritt 1933, Rothschild and Vickers 1991).

With the advent of DDT (dichlorodiphenyltrichloroethane), chemical control became an effective tool against newly hatched larvae. This continued with the development of several organophosphate and carbamate compounds (Rothschild and Vickers 1991). Despite the long-term and widespread use of

these insecticides, resistance did not show up until the mid 1990's (Kanga et al. 1999, Usmani and Shearer 2001). To limit resistance development, some current management programs suggest utilizing organophosphates to control first generation larvae, and pyrethroids or mating disruption for all subsequent moth generations (Kanga et al. 1999, Trimble et al. 2001).

The process of mating disruption includes releasing synthetic sex pheromone in concentrations high enough to create a background of pheromone which masks the natural pheromone released by the female. Mating disruption programs were established in the 1970's. By the 1980's mating disruption showed enough potential to reach mass-market. In some areas where the oriental fruit moth is a key pest mating disruption has been shown to be as effective, or more effective, than conventional insecticide programs (Rothschild and Vickers 1991). In recent years synthetic sex pheromones have been combined with insecticides such as permethrin to create attracticides where the oriental fruit moth comes into contact with the insecticide (Evenden and McLaughlin 2004).

In recent years IGR's, like methoxyfenozide, have been added to spray regimes, with applications occurring at the egg or neonate stage (Borchert et al. 2004a).

2. Codling Moth

The codling moth, *Cydia* pomonella (L.), is a member of the family Tortricidae. It originated in Asia Minor and was first recorded by Theophrastus, a

favorite pupil of Aristotle, in 371 B.C. (Smotavac 1963). Emigrants from the region spread the moth into Europe from which it has radiated out into all apple growing regions of the world. The codling moth has been a well known pest of apples since the mid 1700's (Slingerland 1898), and considered a serious pest of apples for 250 years. Other known hosts include various *Pyrus* and *Prunus* species as well as, quince (*Cydonia sp.*) and walnut (*Juglans sp.*) (Barnes 1991, Beers et al. 1993, Howitt 1993).

a. Biology

Codling moth eggs are laid singly and are transparent (Beers et al. 1993). They are flat, oval and measure 1 to 1.2 mm in diameter (Beers et al. 1993, Howitt 1993). A single female can deposit as many as 100 eggs, which can hatch within six to 14 days (Beers et al. 1993, Howitt 1993). Greater than eighty percent of codling moth eggs are laid on apple leaves in the immediate vicinity of fruit (Jackson 1979). Studies have demonstrated that oviposition is stimulated by volatiles released by growing and ripening apple fruit (Vallat and Dorn 2005, Witzgall et al. 2005).

Neonate larvae are approximately 2 mm long and 0.5 mm in diameter with a cream-colored body and black head (Beers et al. 1993, Howitt 1993). Since they are incapable of developing on a diet of leaves alone (Pszczolkowski et al. 2002), newly hatched larvae are attracted to apple volatiles released by the fruit (Landolt et al. 1998). Upon reaching the fruit they promptly enter it via the side or calyx end and feed on the inside flesh and seeds. Mature larvae are white or pink

in color with brown heads. They reach up to 13 mm long by the end of their fifth and final larval instar. Mature larvae leave the interior of the apple in search of an appropriate pupation site, usually under bark on the trunk or branches of the tree or in brush or litter on the ground (Beers et al. 1993, Howitt 1993). A recent study by Jumean et al. (2005) has identified a larval aggregation pheromone used to locate suitable pupation sites.

Codling moth pupae are brown and approximately 12 mm in length. They are contained within a loose cocoon made of silk spun by the mature larva, and remain enclosed for two to three weeks during the summer generations (Beers et al. 1993, Howitt 1993).

Adults are 9 mm long with a wingspan of 19 mm. They are gray-brown with criss-crossing fine bands of alternating white and gray. The forewings are tipped with bronze scales (Beers et al. 1993, Howitt 1993). Like larvae, mated adults are attracted to apple odors. This host-finding is augmented by odors released from infested or damaged fruit. The location of an infested apple tree could show proximity to other, uninfected trees or fruits (Reed and Landolt 2002).

Mate-finding is pheromone mediated, and is regulated by light, temperature, humidity and air movement (Borden 1931). There is some debate over the time of day at which peak flight activity occurs: one hour before to one hour after sunset (Castrovillo and Carde 1979), beginning at sunset for 30 minutes (Howell et al. 1990), or 0-1 hour after sunset and again at the onset of sunrise (Knight et al. 1994b). Moth flight is mainly limited to temperatures ranging from 13^0C to 27^0C (Castrovillo and Carde 1979). The act of mate finding begins

with the female getting into the releasing posture, head down, abdomen up, and wings slightly apart, and emitting the pheromone. The male begins by flying upwind in response to the sex pheromone. Once close to the female, he lands and begins walking towards her while fanning his wings. The male then touches her with his head and positions himself beside her to probe her abdomen with his. Once the male's valves grasp the female's genitalia wing fanning ceases and the male rotates 180^0 to the female (Castrovillo and Carde 1980).

The codling moth overwinters as a mature larva (Beers et al. 1993, Howitt 1993). One to four generations occur a year in North America, dictated by latitude and elevation (Riedl and Croft 1978). A typical Missouri climate produces three broods a year (Jenkins et al. 1942).

b. Pest Status and Control

The codling moth is considered a direct pest, as it attacks the harvestable portion of the plant, the fruit. This damage comes in two forms, "stings" and "entries." Stings are areas of damage where a larva has chewed through the skin but died or gave up and moved on before getting to the flesh. Entries occur when the larva chews through the skin and into the flesh. Entries are usually accompanied by brown frass, a mixture of fruit particles and insect excrement forced out the opening (Beers et al. 1993).

The codling moth has been the target of chemical control strategies since the late 1800's. Over the years changes in control measures have been required due to its penchant for developing resistance. In 1900 only one to three arsenical

sprays per year were required to control the moth. By the 1940's that number had jumped up to six to 10 sprays a season. Within a few years of the introduction of DDT (dichlorodiphenyltrichloroethane) the codling moth was already showing signs of resistance (Cutright 1954). By the mid 1950's organophosphates were already widely used for codling moth control (Dunley and Welter 2000). Carbamate and pyrethroid use soon followed. Unfortunately, the moth began showing resistance to these as well (Knight et al. 1994a, Chapman and Barrett 1997, Dunley and Welter 2000). Resistance management strategies have included the use of alternative methods of control: granulosis virus (Glen and Clark 1985), biological control (Falcon and Huber 1991), mating disruption (Pfeiffer et al. 1993), and particle films (Unruh et al. 2000). Another recent area of interest has been the use of IGR's. These somewhat specialized compounds are currently effective at lower rates than their conventional predecessors (Pasquier and Charmillot 2004), but care needs to be taken as some evidence of cross resistance with conventional insecticides has been observed (Bouvier et al. 2002). Several studies have reported the lethal and sublethal efficacy of tebufenozide and methoxyfenozide on all life stages of the codling moth (Sun and Barrett 1999, Knight 2000, Smagghe et al. 2004).

Chapter 2: Materials and Methods

A. Insects

All oriental fruit moth, *Grapholita molesta* (Busck), and codling moth, *Cydia pomonella* (L.), used in the studies came from laboratory colonies maintained at the University of Missouri, Horticulture and Agroforestry Research Center (HARC), New Franklin, Missouri. These colonies were started in 2004 with egg masses obtained from a laboratory colony at the Department of Entomology, Rutgers State University, New Jersey, and larvae from a laboratory colony at the Yakima Agricultural Research Laboratory, USDA-ARS, Wapato, Washington, respectively. Periodic stocking of the HARC colonies from both parent colonies were performed throughout the season to maintain colony vigor.

All moth colonies were kept in environmental chambers set at 24^{o}C with a photoperiod of 16L:8D.

B. Chemical Treatments

The chemicals used in the studies were methoxyfenozide (Intrepid® 2F), tebufenozide (Confirm® 2F), both produced by Dow Agrosciences (Indianapolis, Indiana), and Latron B-1956® (a resin-based nonionic surfactant), produced by UAP-Loveland Industries Inc. (Greeley, Colorado). Small volume quantities (1000 ml) of methoxyfenozide and tebufenozide were prepared at concentrations corresponding with recommended field rates (300 ppm and 360 ppm, respectively.) This included 1.25 ml/1000 ml of water and 1.50 ml/1000 ml of

water, respectively. Latron B-1956® (or a similar spreader sticker) is recommended to be included in field applications of both methoxyfenozide and tebufenozide. As such, a proportionate field rate of the surfactant (0.125% vol./vol.) was added to both treatment solutions (1.25 ml/1000 ml of water). Hereafter, Latron B-1956® will be referred to only as Latron.

Due to some inconclusive results of Latron's sublethal effects on previously tested tortricid moth pests (Sun and Barrett 1999, Hoelscher and Barrett 2003a) some of the treatment combinations in this study included Latron. For example, a Latron-Latron treatment was established as a positive control, and water alone was used as a negative control treatment.

The treatment solutions were stored in Pyrex® glass flasks (wrapped in aluminum foil) in the dark at room temperature when not in use. Fresh solutions were prepared every 7 days.

C. Wind Tunnel Setup

The wind tunnel used in these studies was located at the University of Missouri, Horticulture and Agroforestry Research Center (HARC), New Franklin, Missouri. The wind tunnel was housed in a basement room that was connected with the building's central air conditioning and heating system. In addition, the room had two wall vents (input and exhaust) with fans to access outside air.

The wind tunnel consisted of three main parts: the air input section, the main tunnel body, and the air exhaust section (Figure 1). The air input section consisted of a 15-inch, 120 volt, rotary-blade fan (Patton® high velocity fan,

model U2-1487) connected to the main body of the tunnel by a 0.9 m long tube of plastic sheeting. The input end of the tunnel was near a central air conditioning/heating vent and the outside air intake vent. This allowed the appropriate combination of air temperature and humidity to be maintained during the experiments.

Air flow from the rotary fan first entered a ‘mixing chamber’ on the tunnel body that consisted of two sheets of sheer, 100% polyester cloth stretched tightly across the tunnel’s opening and separated from each other by 10 cm. The purpose of this mixing chamber was to dampen the air turbulence created by the rotary fan, thus creating a laminar flow of air throughout the tunnel body. Laminar flow of air through a wind tunnel is crucial when predicting the path of a pheromone plume from a point source. Smoke plumes of titanium tetrachloride ($TiCl_4$) were utilized to confirm that laminar flow was occurring in the desired pattern. Small pieces of cotton were saturated in a $TiCl_4$ solution and placed in cages and positions in the tunnel identical to those used in the experiments. After each use of $TiCl_4$, usually once a month, the inside of the wind tunnel was washed thoroughly with soap and water to remove any contaminants.

The main tunnel body consisted of a 2.9 m length of white polyvinyl chloride (PVC) pipe with an interior diameter of 60 cm. On the top of the tunnel, and just off-center, running the length of the tunnel body, were three observation windows measuring 39 x 14 cm each. Below each window was a hinged door measuring 60 x 18 cm also with windows. All windows were covered with clear

Plexiglas sheets attached from the inside to maintain even, smooth walls on the interior of the tunnel.

The air exhaust portion of the wind tunnel consisted of 18 gauge sheet metal forming a cone that tapered from the 60 cm diameter of the wind tunnel body to 30.5 cm diameter. Flexible ductwork connected the cone to the exhaust wall outlet, accompanied by a fan used to help pull air from the wind tunnel out of the building. Between the PVC tunnel body and the sheet metal ductwork was a single layer of nylon tulle cloth stretched tightly across the opening to prevent moths from escaping the tunnel body.

D. Wind Tunnel Assays

1. Non-choice Flight Assay

Exposure Cage- The treatment exposure cages were constructed from clear plastic vials 9.2 cm long with an internal diameter of 4.5 cm (Fisher Scientific® Polystyrene Containers) (Figure 2). The closed end of the vial was removed and replaced with wire mesh screen measuring 1.5 x 1.5 mm. The center portion of the snap cap lid was also removed and replaced with mesh screening. The cages were then lined with a removable plastic mesh (2 x 2 mm). The vials and lids and plastic mesh liners were submerged and agitated in a treatment solution for one minute then allowed to air dry.

Insects and Equipment- Four to five recently emerged virgin oriental fruit moth (0-24 h old), of the same sex were placed in a treatment exposure cage. All cages containing the same sex were placed in an 18 x 31 cm Sterilite® plastic

sealable container for 48 h. All cages were stored inside an environmental chamber set at 24^{o}C with a 16L:8D photoperiod.

A pedestal for female cage placement was constructed 5 cm downwind from the mixing chamber at the head of the wind tunnel. The pedestal consisted of a 35 cm stainless steel rod with a sheet metal platform adhered at each end. The top platform measured 8 x 15 cm. A small section of PVC tube, cut in half lengthwise, was attached to the top of the platform. This provided a holding position for the exposure cage to rest without rolling off the platform. A paper card measuring 8 x 12 cm, covered with a thin layer of TangleTrap® insect adhesive, was positioned in front of the exposure cage (Figure 2).

One meter downwind from the female platform a second platform of the same height was positioned for the male moth release cage. This cage consisted of a Plexiglas cylinder measuring 9 cm long with an internal diameter of 6.5 cm. Both ends of the cage were fitted with Plexiglas rings covered with mesh screen hinged to the top of the cage (Figure 3). Both ring covers were also attached to each other via a short length of monofilament fishing line. A thin wire was threaded through a small hole in the top of the wind tunnel above the release cage. When hooked onto the fishing line, the wire could be retracted from outside the wind tunnel, opening both ends of the release cage simultaneously. This arrangement allowed the male moths to leave the cage and fly either upwind or downwind with minimal moth or air flow disturbance.

Procedures- Air velocity and temperature within the tunnel were maintained at 0.2-0.3 m/s and 23-25^{o}C (as measured by a Fisher Scientific®

Economy Digital Anemometer), respectively. Illumination was maintained at full light levels (200-300 lux) throughout the duration of the experiments. Willis and Baker (1994) and Valeur and Lofstadt (1996) performed wind tunnel studies under similar light levels, at 250 lux and 400 lux, respectively.

The following treatment combinations, based on exposure to treated cage surfaces (by sex) for 48 h, were established:

Treatment	Females	Males
A	Water	Water
B	Water	Latron
C	Latron	Latron
D	Latron	Water
E	Water	Methoxyfenozide
F	Methoxyfenozide	Water
G	Latron	Methoxyfenozide
H	Methoxyfenozide	Latron
I	Methoxyfenozide	Methoxyfenozide

Prior to the beginning of each treatment trial, an exposure cage containing four to five females was removed from the environmental chamber and placed on the upwind pedestal of the wind tunnel. Two exposure cages containing eight to ten males were also removed from the environmental chamber and placed inside

the release cage on the downwind platform. All moths were then allowed to acclimate to the temperature and airflow within the tunnel for 10 minutes.

After the acclimation period the front and back hinged covers of the release cage were pulled up, releasing the males. Twenty minutes later the number of males captured on the sticky card in front of the female cage was recorded. All moths were then removed from the wind tunnel and the procedure was repeated with new female and male treatment replicates.

Each day the treatment trials began two hours before the onset of scotophase and were terminated one hour after the onset of scotophase (a 3 h testing period). Several studies performing wind tunnel research with oriental fruit moth decided on similar times of day for observation (Lacey and Sanders 1992, Valeur and Lofstedt 1996, Evenden and McLaughlin 2005). Hughes and Dorn (2002) reported that peak oriental fruit moth flight activity occurred during the first hour of scotophase. As a result of these past studies, only 5-6 treatment trials could be completed each day. As such, the experiment was completed in two phases. The first phase compared the effects of Latron 1956® to the water control (treatments A, B, C, D, and I). The second phase compared the effects of methoxyfenozide to the water control (treatments A, E, F, G, H, and I). To avoid any day-to-day variation, all treatments in a given phase were examined each day. The order of treatment testing was changed daily to eliminate the effect of time. Overall, each treatment had 10 replicates, and new moths were used for each replicate.

After each day of testing the treatment cages, lids and liners were cleaned with hot, soapy water before being retreated with the same treatment solution. The wind tunnel was cleaned and rinsed down with water once a week to remove any possible pheromone residue buildup. Upon completion of the two phases, data were compared for equality and pooled for analysis.

Data Analysis- All statistical analyses were conducted using the SAS program (SAS Institute 2004). Differences among treatment combinations in regards to the mean number of male moths trapped on the sticky card in front of the females' cage were determined by analysis of variance (ANOVA) using general linear model (GLM) procedures. If the overall treatment F tests were significant then the treatment means were separated by Fisher's protected least significant differences (LSD). Differences were considered statistically significant at the level of $P<0.05$ unless otherwise noted.

2. Treatment Choice Assay

Exposure Cage- The treatment exposure cages consisted of 5.7 cm long clear plastic vial tubes (Fisher Scientific® Polystyrene Containers) with an interior diameter of 2.5 cm (Figure 4). The closed end of the vial was removed and replaced with a polyester fine mesh fabric. The center of the snap cap lid was removed and replaced with the same polyester mesh. The cages were lined with a removable plastic mesh screen (2 x 2 mm). The vials, removable lids and plastic mesh liners were submerged and agitated in a treatment solution for one minute then allowed to air dry.

Insects and Equipment - Two recently emerged virgin oriental fruit moth (0-24 h old), of the same sex were placed in a treatment exposure cage. All cages holding the same sex were placed in an 18 x 31 cm Sterilite® plastic sealable container for 48 h. All cages were stored inside an environmental chamber set at 24°C with a 16L:8D photoperiod.

A framework made of aluminum pieces (1.6 x 12.7 mm) spanning the width of the wind tunnel was placed at the upper portion of the tunnel 5 cm downwind from the mixing chamber. A platform measuring 8 cm wide was created that spanned the width of the wind tunnel at a height of 30 cm from the bottom of the tunnel. Two female exposure cages were centered in the tunnel on the framework (Figure 5). A white cardboard landing pad measuring 4 x 6.4 cm was suspended directly in front of each cage (Figure 4). The two female cages were placed far enough apart, 21.3 cm, to separate the pheromone plumes emanating from each cage before they intersected approximately 1.5 m downwind (as verified by test smoke plumes of titanium tetrachloride).

A male release cage was placed 1.5 m downwind of the female exposure cages, at the point of plume intersection. This cage consisted of a white polyvinyl chloride (PVC) tube measuring 8 x 15 cm (Figure 5). The downwind opening of the cage was covered with wire mesh screen (1.5 x 1.5 mm) to prevent moths from escaping via that direction. The upwind end of the cage was open. A hole measuring 4.5 cm in diameter was cut into the top of the cage 7 cm from the front to allow for moth placement inside the cage. A pedestal was created from stainless steel rod 26 cm long attached to sheet metal on the bottom and a

length of PVC pipe of similar diameter to the release cage cut in half to serve as a stable support on top. The male release cage was placed on top of this pedestal.

Air velocity and temperature within the tunnel were maintained at 0.2-0.3 m/s and 23-25^{o}C (as measured by a Fisher Scientific® Economy Digital Anemometer), respectively. Illumination was maintained at full light levels (200-300 lux) throughout the duration of the experiments.

Procedures- The following treatment combinations, based on moth exposure to treated cage surfaces for 48 h, were established for the two upwind female cages:

Treatment	Left female cage	Right female cage
A	Water	Water
B	Latron	Water
C	Latron	Latron
D	Methoxyfenozide	Water
E	Methoxyfenozide	Latron
F	Methoxyfenozide	Methoxyfenozide
G	1 mg Septum Lure	Water

The male moths were exposed to the following treated surfaces for 48 h:

Treatment	Male
1	Water
2	Latron
3	Methoxyfenozide

All female treatments were combined with all male treatments to create a total of 21 treatment combinations. Due to the large number of treatment combinations an incomplete block design was utilized. The design separated the treatment combinations into sets of 5 treatment replicates per block. Typically, one to two blocks were completed each day. The incomplete block design changed the order of treatment combinations examined daily to avoid any effects of time on moth calling and responsiveness. Ten replications were completed for each of the 21 treatment combinations. New moths were used for each replicate. To remove any effect of female cage position (right versus left), the incomplete block design included rotating female cage position for the final 5 replicates.

Experiments were started three hours before the onset of scotophase and terminated one hour after the onset of scotophase. Prior to each experimental trial two exposure cages containing females were removed from the environmental chamber and placed on the framework at the front of the wind tunnel. An exposure cage containing two males was placed on the floor of the tunnel. All moths were allowed to acclimate to the tunnel temperature and airflow for 10 minutes.

After the acclimation period, the males were gently placed into the release cage by carefully shaking them out of the exposure cage into the opening at the top of the release cage. Males were then observed for 10 minutes. Of the two males released only data on the behavior of the most active individual was recorded. Careful measurements were taken on when the male left the release cage, when and how long it acquired a pheromone plume, which female

treatment plume it acquired, and when, which and how long it was in contact with a female cage assembly. At the termination of each 10 minute observation period, both males and all females were removed from the tunnel. The study was then repeated with another treatment replicate.

Data Analysis- All statistical analyses were conducted using the SAS program (SAS Institute 2004). Differences within treatment combinations in regards to the pheromone plume and cage choices and differences among treatment combinations in regards to the mean presence of males in the pheromone plume or on one of the female cages were individually determined by analysis of variance (ANOVA) using generalized linear model (GENMOD) procedures. If the overall treatment Chi-square tests were significant then the treatment means were separated by individual chi-square tests. Differences were considered statistically significant at the level of $P<0.05$ unless otherwise noted.

E. Fecundity and Fertility Assays

Mating/oviposition cages were constructed from 10 cm long sections of white polyvinyl chloride (PVC) pipe with an interior diameter of 7.6 cm. Two lids were constructed of 9 mm wide PVC rings with inner diameters of 8.8 cm (equal to the outer diameter of the cage) covered with 1.5 x 1.5 mm wire mesh screen. Each cage was lined with 2 x 2 mm plastic mesh screen (Figure 6). The purpose of the plastic mesh liners and wire mesh covered lids was to remove any unintended smooth surfaces (preferred oviposition sites). Moths were exposed to removable thin, clear plastic discs 8.8 cm in diameter positioned between the

upper lid and the cage that served as oviposition sites. The lids, plastic oviposition discs and plastic mesh liners were submerged and agitated in one of the following treatment solutions for one minute then allowed to air dry:

	Treatment
A	Water
B	Latron
C	Tebufenozide
D	Methoxyfenozide

Data Analysis- All statistical analyses were conducted using the SAS program (SAS Institute 2004). Differences among treatment combinations in regards to the number of replicates with eggs laid and eggs hatched were separated by the Continuity Adjusted Row by Column Chi-square Test. Rank transformations of the fecundity and fertility data, as outlined by Conover and Iman (1981), were performed before being analyzed. Differences among treatment combinations in regards to number of eggs laid and percent of eggs hatched were determined by analysis of variance (ANOVA) using general linear model (GLM) procedures. If the overall treatment F tests were significant then the treatment means were separated by Fisher's protected least significant differences (LSD). Differences among treatment combinations in regards to the survivability of the females were determined by the Kaplan-Meier method of survival analysis (LIFETEST) procedures. Differences between survival curves were determined using the Log-Rank chi-square test. Differences were considered statistically significant at the level of $P<0.05$ unless otherwise noted.

1. Continuous Exposure Assay: both sexes

Procedures- One female and two male oriental fruit moth, all less than 24 hours old and virgin, were placed in a treatment mating/oviposition cage. Ten replications were conducted for each treatment. After moth introductions all cages were stored inside an environmental chamber set at 24^{o}C with a 16L:8D photoperiod.

Every 24 hours the cages were inspected for adult mortality and oviposition. The number of eggs found on each plastic disc was recorded and replaced with a clean, freshly treated disc. The discs with eggs were sealed in a plastic container (Tupperware®) measuring 15 x 27 x 6 cm with a moist paper towel (to retain high humidity). The egg discs were stored inside an environmental chamber set at 24^{o}C with a 16L:8D photoperiod. The number of eggs hatched on each disc was recorded every 24 hours. Each disc was observed for 10 days.

2. 24-hour Exposure Assay: Treated/Nontreated Pairings

Exposure Cage- The treatment exposure cages were constructed from clear plastic vials 9.2 cm long with an internal diameter of 4.5 cm (Fisher Scientific® Polystyrene Containers). The closed end of the vial was removed and replaced with wire mesh screen measuring 1.5 x 1.5 mm. The center portion of the snap cap lid was also removed and replaced with mesh screening. The vials, lids and plastic mesh liners (2 x 2 mm) were submerged and agitated in a treatment solution for one minute then allowed to air dry.

Procedures- One female or two male oriental fruit moth, depending on the study, all less than 24 hours old and virgin, were placed in a treated exposure cage for 24 hours. The untreated sex was immediately placed in a mating/oviposition cage. After 24 hours the treated sex was added to the mating/oviposition cages. Ten replications were conducted for each treatment. All cages were stored inside an environmental chamber set at 24^{o}C with a 16L:8D photoperiod.

Every 24 hours the cages were inspected for adult mortality and oviposition. The number of eggs found on each plastic oviposition disc was recorded and the disc was replaced with a clean, freshly treated disc. The discs with eggs were sealed in a plastic container (Tupperware®) measuring 15 x 27 cm with a moist paper towel (to retain high humidity). The eggs were stored inside an environmental chamber set at 24^{o}C with a 16L:8D photoperiod. The number of eggs hatched on each disc was recorded every 24 hours.

F. Codling Moth and Oriental Fruit Moth Feeding Comparison Assay

Feeding Cage- The feeding cages were constructed from opaque, soft plastic cups with removable lids (Fisher Scientific® Multipurpose Containers). The cups measured 11 cm tall with internal diameters at the base and top of 6.5 cm and 9 cm, respectively. Several pin-size holes were pierced into the lids to allow for gas exchange. Three lengths of 12 gauge wire approximately 9 cm long were punctured through the cup approximately 3.5 cm from the base and equidistant from each other to create a wire platform (Figure 7).

Insects and Equipment - Organic, pesticide-free Jonathan apples were purchased from a local grocery store (Hy-Vee, Columbia, MO). The apples were soaked in warm water for five minutes then dried with paper towels to remove any dirt or other contaminants. The apples were then placed inside the cage on top of the wire supports. The purpose of the wire supports was to raise the apples off the bottom of the cage to allow the larvae free access to all parts of the apple.

First instar larvae of oriental fruit moth and codling moth (less than 24 hours old) were placed inside the cages. Each cage received two larvae of the same species. All larvae were placed on the top of the apples, away from the stem. The cages were then stored inside an environmental chamber set at 24^{o}C with a 16L:8D photoperiod.

Procedures- Observations on fruit damage in each cage were taken every three to four days for two weeks. Data collection on feeding damage included the presence and size of surface and subsurface damage, presence, size and moisture content of expelled frass (Figure 8), and any noticeable activity of the larvae. To easily separate and quantify the location of surface damage on the apple, each fruit was separated into six external regions. Regions one and six were located at the stem and calyx ends of the apple, respectively. Regions two through four were evenly separated transverse rings around the apple (Figure 9).

Two weeks after introduction of the larvae into the cages, the apples were sliced open to make observations on any internal damage. To quantify the location of such damage, the interior of the apple was separated into four

regions. Regions one through three were the base of the stem to the seed cavity, the seed cavity, and the end of the seed cavity to the base of the calyx, respectively. The remainder of the fruit flesh, the outer ring, was designated region four (Figure 10). The percent damage occurring in each region was determined as well as the level of damage to and the number of seeds damaged.

Due to mortality, 15 replicates of oriental fruit moth and 21 replicates of codling moth were performed during the study. Fourteen oriental fruit moth and 16 codling moth replicates were observed for internal damage at termination of the study.

Data Analysis- All statistical analyses were conducted using the SAS program (SAS Institute 2004). Differences among treatment combinations in regards to the presence of subsurface damage, entrance/exit holes, and levels of frass moisture as well as the levels of damage to the fruit flesh and seeds were separated by the Continuity Adjusted Row by Column Chi-square Test. Differences among treatment combinations in regards to the presence of subsurface damage or entrance/exit holes were individually determined by analysis of variance (ANOVA) using generalized linear model (GENMOD) procedures. If the overall treatment Chi-square tests were significant then the treatment means were separated by individual chi-square tests. Differences were considered statistically significant at the level of $P<0.05$ unless otherwise noted.

Figure 1. Wind tunnel housed at the Horticulture Agroforestry Research Center (New Franklin, MO).

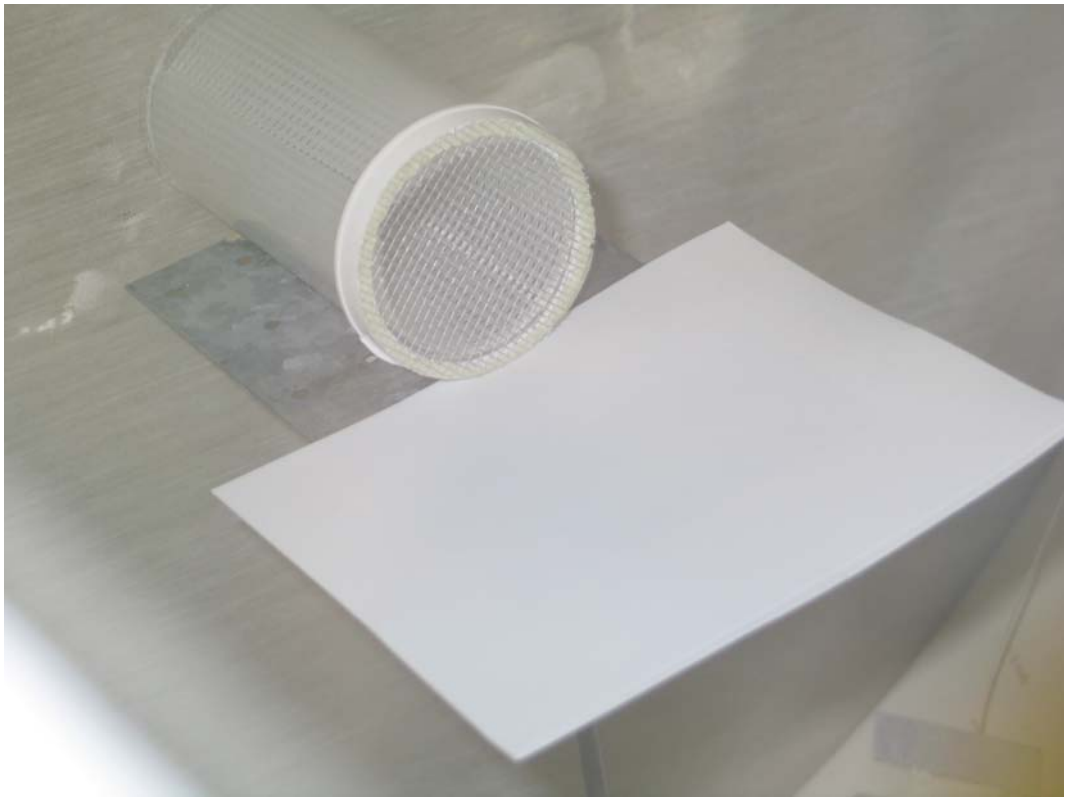

Figure 2. Upwind female exposure/calling cage and male landing platform used in the non-choice flight assay.

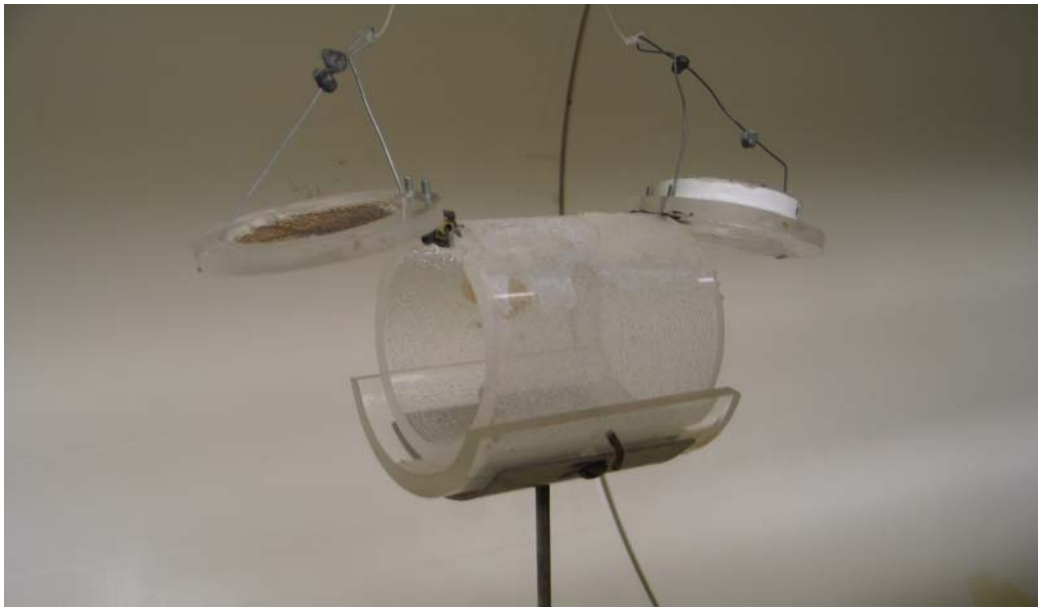

Figure 3. Downwind male release cage used in the non-choice flight assay.

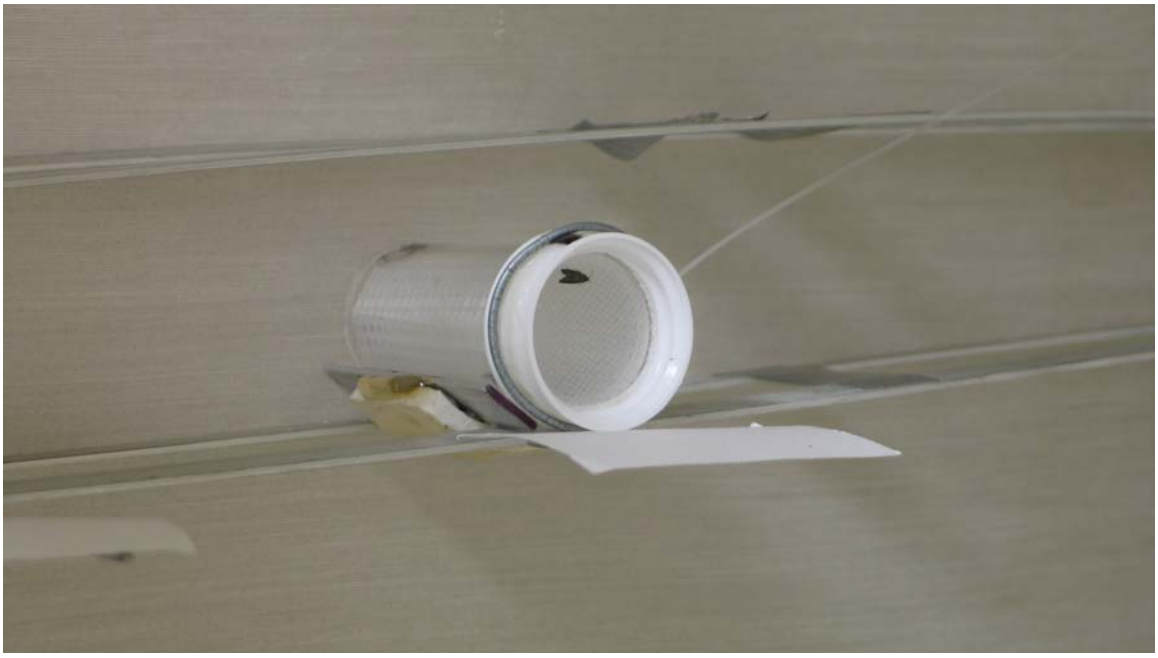

Figure 4. Upwind female exposure/calling cage and male landing platform used in the choice flight assay.

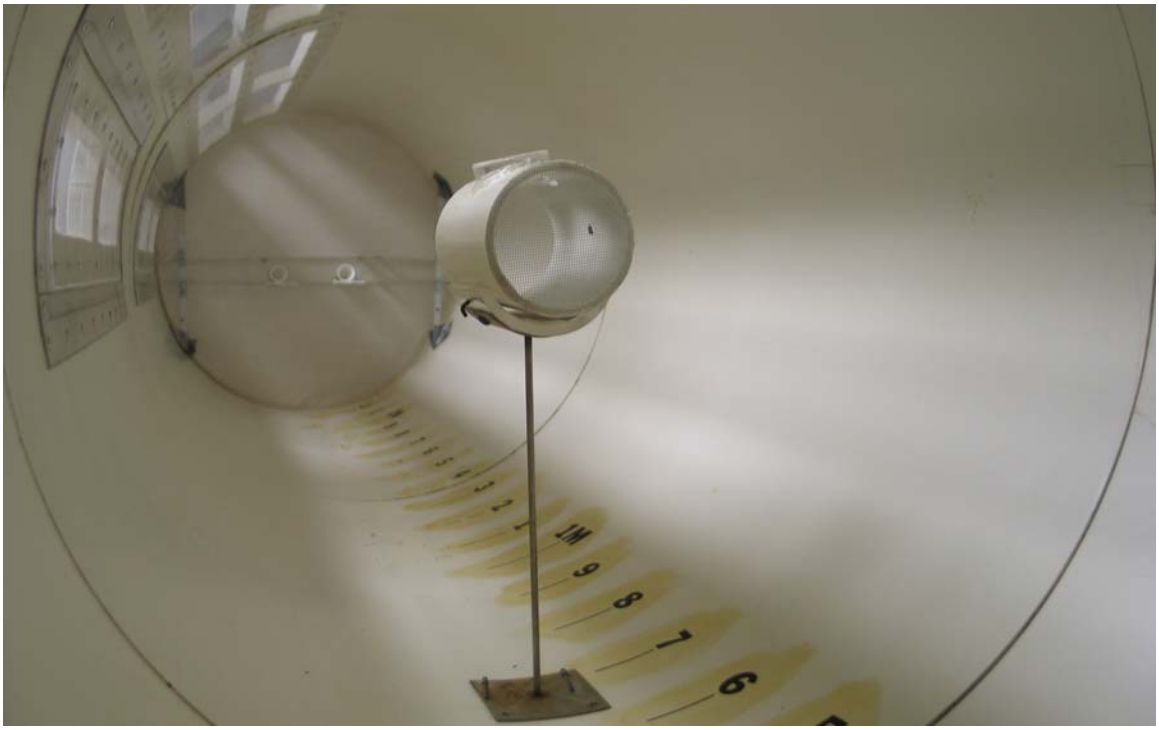

Figure 5. Downwind male release cage (foreground) and two upwind female exposure/calling cages (background) used in the choice flight assay.

Figure 6. Mating and oviposition cage used in the fecundity and fertility assays.

Figure 7. Opaque plastic feeding cage (with lid) and apple supports (12 gauge wire).

Figure 8. Example of visible tunneling damage with frass on Jonathan apple produced by a codling moth larva from the feeding comparison assay.

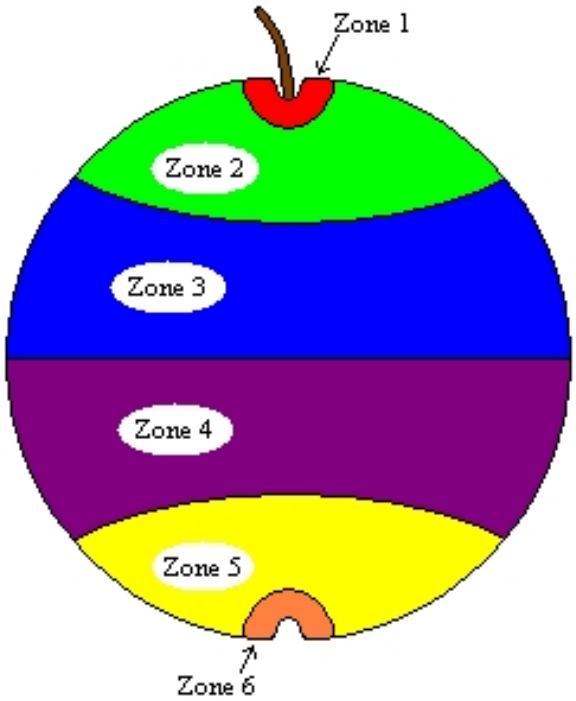

Figure 9. Diagram of apple showing external separation zones.

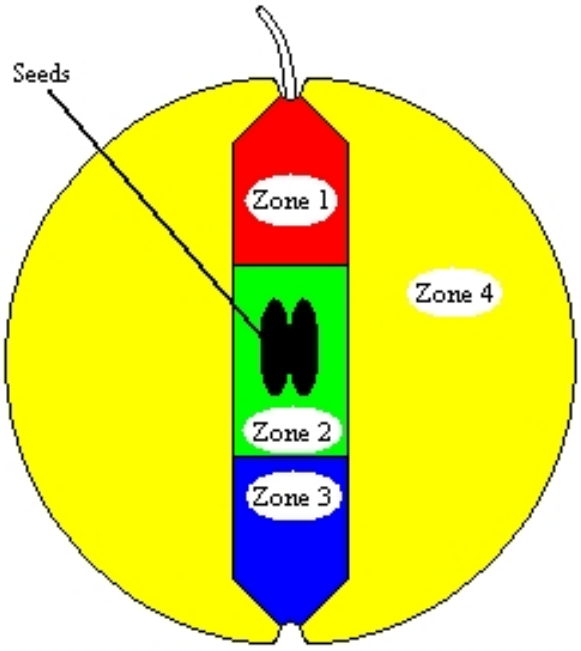

Figure 10. Diagram of apple showing internal separation zones.

Chapter 3: Results

A. Wind Tunnel Assays

1. Non-choice Flight Assay

When both female and male moths were exposed to water-treated surfaces (control) for 48 h over half of the released males, 55.5%, were captured at the female cage (Table 1). When both female and male moths were exposed to Latron-treated surfaces (control), the mean percent capture of released males was 40.1%. These values were not significantly different from each other. When the female was exposed to methoxyfenozide-, and the male exposed to Latron-treated surfaces, there was no significant difference between the 47.4% male capture and the percent recaptures found in the water- and Latron-controls. When the female was exposed to Latron, and the male exposed to methoxyfenozide, there was no significant difference between the mean percent male capture of 32.2% and the Latron-control treatment. However, this was significantly less than the mean capture of the water-control treatment. When males or females were exposed to methoxyfenozide and the other sex exposed to water the mean percent captures of males were 24.7% and 23.8%, respectively (Table 1). These treatments were significantly less than both control means. This was also true when both sexes were exposed to methoxyfenozide (24.3% capture.)

To examine the effect of each female-exposure treatment on overall mean male trap capture, all male treatment data were combined. The pooled mean percent capture of males for females exposed to water-, Latron-, and

methoxyfenozide-treated surfaces were 41.5%, 39.8% and 31.8%, respectively, regardless of male treatment exposure (Table 2). These means were not significantly different at the $P<0.05$ level. At the 90% confidence interval, however, females treated with methoxyfenozide attracted significantly fewer males than females exposed to water-treated surfaces, regardless of male treatment. There were no significant differences between mean male captures for Latron- and methoxyfenozide-treated females.

To examine the effect of each male-exposure treatment on mean male trap captures all female treatment data were combined. The pooled mean percent trap capture of males exposed to water-, Latron- and methoxyfenozide-treated surfaces, regardless of female exposure, were 42.1%, 44.0% and 27.1%, respectively (Table 2). At the 95% confidence interval, methoxyfenozide-treated males were significantly less responsive to females than water- and Latron-treated males, regardless of what the females had been exposed to.

Table 1. Mean percent of treated adult male oriental fruit moth released in a wind tunnel that were captured on an adhesive card in front of caged, treated females.

Treatment Exposures by sex (F / M)[a]	N[b]	Mean (± SE) Percent of Male Captures
Water / Water	10	55.5 ± 7.2 a
Latron / Latron[c]	10	40.1 ± 8.0 ab
Water / Latron	10	44.5 ± 3.0 ab
Latron / Water	10	47.0 ± 5.4 ab
Water / Methoxyfenozide[d]	10	24.7 ± 7.4 c
Methoxyfenozide / Water	10	23.8 ± 6.3 c
Latron / Methoxyfenozide	10	32.2 ± 4.7 b
Methoxyfenozide / Latron	10	47.4 ± 7.3 ab
Methoxyfenozide / Methoxyfenozide	10	24.3 ± 6.5 c

Means followed by the same letter are not significantly different (Fisher's protected LSD, P<0.05). Actual means (not transformed means) are listed.

[a]Female (F) or male (M) moths, 0-24h old, exposed to treated surfaces for 48 h.

[b]Number of replicates per treatment. Each replicate consisted of 4-5 caged females and 5-7 released males. New moths were used for each replicate.

[c]The Latron treatment consisted of a labeled field rate (0.125% vol:vol) of Latron B-1956®, a resin-based nonionic surfactant, mixed with water.

[d]Each methoxyfenozide treatment solution contained a proportionate field rate of Latron B-1956® as per label recommendations.

Table 2. Mean percent of treated adult male oriental fruit moth released in a wind tunnel that were captured on an adhesive card in front of caged, treated females. All female and male treatment data were pooled per treatment exposure.

Treatment Exposure	N[a]	Mean (± SE) Percent of Male Captures[b]	
		Female Treatments Pooled	Male Treatments Pooled
Water	30	42.1 ± 4.2 a	41.5 ± 4.3 a y
Latron[c]	30	44.0 ± 3.6 a	39.8 ± 3.7 a yz
Methoxyfenozide[d]	30	27.1 ± 4.3 b	31.8 ± 3.6 a z

Means followed by the same letter are not significantly different (Fisher's protected LSD). Actual means (not transformed means) are listed.

[a]Number of replicates per treatment. Each replicate consisted of 4-5 caged females and 5-7 released males. New moths were used for each replicate.

[b]The first column of letters (a, b, c) recognize significant difference at $P<0.05$. The second column of letters (x, y, z) recognize significant difference at $P<0.10$.

[c]The Latron treatment consisted of a labeled field rate (0.125% vol:vol) of Latron B-1956®, a resin-based nonionic surfactant, mixed with water.

[d]Each methoxyfenozide treatment solution contained a proportionate field rate of Latron B-1956® as per label recommendations.

2. Treatment Choice Assay

All mean percent male captures for each male exposure treatment and female exposure treatment combination are given in Table 3. The highest mean level of incidence where males were observed within the pheromone plume (60%) was found with Latron-treated males exposed to the methoxyfenozide- and water-treated female combination. This level of response also occurred with Latron-treated males in response to a septum lure when paired with a water-treated female.

The female-treatment combinations that attracted 50% of the males, regardless of male exposure, were the female exposure combinations of water / water and septum lure / water for Latron-treated males, and the water / water combination for water-treated males (Table 3). The female- and male-treatment combinations that failed to attract any males in female plumes were with methoxyfenozide-treated males exposed to methoxyfenozide-treated females. This also occurred with methoxyfenozide-treated males exposed to Latron- / water- and Latron- / Latron- treated females.

The largest difference between mean occurrences of males in female plumes occurred when 50% of water-treated males found the plume of water-treated females and only 10% of the water-treated males occurred in the plume of methoxyfenozide-treated females (Table 3). This, however, was still not significant for this study, only showing significance at a 64% confidence interval.

Similar patterns were observed for males making contact with female cages. For example, 60% of Latron-treated males made cage contact with water-

treated females whereas 50% of males made cage contact with methoxyfenozide-treated females (Table 3). Other treatment combinations where 50% of the released males made female cage contact were Latron-treated males on water-treated females that were paired against water-treated females and 1mg septum lure, and water-treated males to water- and Latron-treated females paired against water- and methoxyfenozide-treated females, respectively (Table 3). The only treatments with no males coming into contact with a female cage were all within the methoxyfenozide-treated males.

In order to examine the overall attractiveness of female treatment combinations, data on male responses to each female treatment pairing were combined. The female water / water combination had 50% of the water-treated males orient within their plumes (Table 4). The female methoxyfenozide / methoxyfenozide combination attracted the lowest percent of water-treated males, 15%. This was the only female treatment combination that was significantly less from the female water / water treatment. Interestingly, the highest observance of Latron-treated males that oriented within the female plumes (60%) was with the female methoxyfenozide / water combination. This was followed closely by 55% and 50% of Latron-treated males finding the pheromone plumes from the septum / water and water / water female combinations, respectively. The lowest observance of Latron-male plume orientation, and the only female treatment combination significantly different from the methoxyfenozide / water female treatment, was the Latron / water female treatment, at 15%. All female treatments exposed to methoxyfenozide-treated

males resulted in 0% to 35% of the males orienting within the female plumes. These were not significantly different at the 95% confidence interval, but at the 90% confidence interval, the 35% of methoxyfenozide-treated males reaching the plume in the female water / water treatment combination was significantly different from all other female treatment combinations within the methoxyfenozide-treated male grouping (Table 4).

When all male treatment data were pooled together, 45% of males oriented within the pheromone plumes in the female water / water treatment combination (Table 4). This was significantly greater than all other female treatments save the methoxyfenozide / water treatment, at 31.7% of males attracted. The methoxyfenozide / methoxyfenozide female treatment combination attracted only 15% of the males.

In terms of males contacting female cages, 40% of water-treated males came into contact with at least one of the cages in the female water / water treatment (Table 4). This was the highest response among the water-treated males. The lowest number of water-treated males contacting female cages was 15% in the methoxyfenozide / methoxyfenozide and septum / water female treatments. These were not significantly different at the 95% confidence interval, but were significantly different at the 90% confidence interval. The female methoxyfenozide / water combination had the highest percent of latron-treated males contacting the female cages, 55% (Table 4). The only latron-treated male responses with significantly lower rates of cage contact were the methoxyfenozide / methoxyfenozide and latron / water female treatment

combinations, at 20% and 10%, respectively. The actual numbers of methoxyfenozide-treated males contacting female cages were so few that no analysis could be performed. However, the highest mean percentage among these males that made cage contact was 25% in the water / water female treatment combination. The 0% male response to the female methoxyfenozide / methoxyfenozide treatment was the lowest among methoxyfenozide-treated males (Table 4).

When all male treatment data were pooled, 35% of males made cage contact with the female water / water combination (Table 4). The only female treatment combinations that were significantly less in percent male cage contact were the methoxyfenozide / methoxyfenozide treatment, at 11.7%, and the Latron / water treatment, at 18.3%.

To consider the effect of each female treatment, regardless of the female treatment it was paired with on male responses, all identical female treatment data were pooled. Of water-treated males, 40% oriented to the pheromone plumes of water-treated females, but only 12.5% of such males oriented within the plumes of methoxyfenozide-treated female plumes (Table 5). Regarding Latron-treated males, there was no significant difference among the percentages of males oriented within the plumes of the water-treated (44%), Latron-treated (27.5%), and methoxyfenozide-treated (40%) females. The highest percent of methoxyfenozide-treated males that made pheromone plume orientation was 22%, with the female water-treated combination. Latron- and methoxyfenozide-

treated males were both at 5% towards the Latron- and methoxyfenozide-treated females, respectively (Table 5).

When male data were pooled, water-treated females had 35.3% of the males finding their pheromone plume than both Latron- and methoxyfenozide-treated females, at 21.7% and 19.2%, respectively (Table 5).

The mean percent of water-treated males that contacted the methoxyfenozide-treated female cages was 12.5% (Table 5). This was significantly less than both the water- and Latron-treated females, at 30.0% and 32.5%, respectively. Regarding Latron-treated males, 40% contacted the cages of water-treated females, 27.5% contacted the cages of methoxyfenozide-treated females, and 25% contacted cages of Latron-treated females. These values were not significantly different. There was also no difference among the methoxyfenozide-treated males contacting female cages. The males contacted Latron-treated female cages and methoxyfenozide-treated female cages 5% of the time. Methoxyfenozide-treated males contacted Water-treated female cages 14% of the time (Table 5).

When all male data were pooled, males came in contact with cages of water-treated females 28.0% of the time (Table 5). This was significantly more than the 15% cage contact with the methoxyfenozide-treated females. Males made cage contact with Latron-treated females 20.8% of the time. This was significantly less than the water-treated females at the 90% confidence interval.

To examine the effect of male treatment on plume orientation and female cage contact, all female treatment data were combined. Only 11.4% of

methoxyfenozide-treated males oriented to a pheromone plume, which was significantly less than the 28.6% and 39.3% of plume orientation from water- and Latron-treated males, respectively (Table 6). The water-treated males oriented to a pheromone plume significantly less than Latron-treated males, at the 90% confidence interval. Water-treated and Latron-treated males contacted female cages 26.4% and 33.6% of the time, respectively. These percentages were both significantly higher than the 8.6% cage contact from methoxyfenozide-treated males (Table 6).

Table 3. Percent of treated adult male oriental fruit moth released in a wind tunnel that were found to be orienting within each treatment pheromone plume or making treatment cage contact of two upwind treatment sources (choice assay).

Female Treatment Exposures[a] (Treatment A/Treatment B)	Water Treated Males			Latron Treated Males			Methoxyfenozide[c] Treated Males			Pooled Male Treatments		
	N[b]	Left	Right	N	Left	Right	N	Left	Right	N	Left	Right
Plume												
Water / Water	10	50.0	50.0	10	50.0	50.0	10	30.0	40.0	30	43.3	46.7
Latron / Water	10	30.0	40.0	10	20.0	10.0	10	0.0	20.0	30	16.7	23.3
Latron / Latron[c]	10	30.0	20.0	10	30.0	30.0	10	10.0	0.0	30	23.3	16.7
Methoxyfenozide[d] / Water	10	10.0	40.0	10	60.0	60.0	10	10.0	10.0	30	26.7	36.7
Methoxyfenozide / Latron	10	10.0	50.0	10	40.0	30.0	10	10.0	10.0	30	20.0	30.0
Methoxyfenozide / Methoxyfenozide	10	10.0	20.0	10	30.0	30.0	10	0.0	0.0	30	13.3	16.7
Septum Lure / Water	10	20.0	20.0	10	60.0	50.0	10	10.0	10.0	30	30.0	26.7
Cage												
Water / Water	10	30.0	50.0	10	30.0	50.0	10	30.0	20.0	30	30.0	40.0
Latron / Water	10	30.0	40.0	10	10.0	10.0	10	0.0	20.0	30	13.3	23.3
Latron / Latron	10	30.0	20.0	10	30.0	30.0	10	10.0	0.0	30	23.3	16.7
Methoxyfenozide / Water	10	10.0	40.0	10	50.0	60.0	10	10.0	0.0	30	23.3	33.3
Methoxyfenozide / Latron	10	10.0	50.0	10	40.0	30.0	10	10.0	10.0	30	20.0	30.0
Methoxyfenozide / Methoxyfenozide	10	10.0	20.0	10	20.0	20.0	10	0.0	0.0	30	10.0	13.3
Septum Lure / Water	10	10.0	20.0	10	40.0	50.0	10	10.0	0.0	30	20.0	23.3

Actual means (not transformed means) are listed. No significant differences exist for any treatment (Generalized Linear Model-odds ratio=1, P<0.10)

[a]Moths, 0-24h old, exposed to treated surfaces for 48h.

[b]Number of replicates per treatment. Each replicate consisted of one male moth and two cages each with 2 females moths. New moths were used for each replicate.

[c]The Latron treatment consisted of a labeled field rate (0.125% vol:vol) of Latron B-1956®, a resin-based nonionic surfactant, mixed with water.

[d]Each methoxyfenozide treatment solution contained a proportionate field rate of Latron B-1956® as per label recommendations.

Table 4. Percent of treated adult male oriental fruit moth released in a wind tunnel that were found to be orienting within a treatment combination pheromone plume or making treatment cage contact of one of two upwind treatment sources (choice assay).

Female Treatment Exposures[a] (Treatment A/Treatment B)	Water Treated Males[c]		Latron Treated Males[c]		Methoxyfenozide Treated Males[c]		Pooled Male Treatments[c]	
	N[b]	%	N	%	N	%	N	%
Plume								
Water / Water	20	50.0 a y	20	50.0 a	20	35.0 a y	60	45.0 a x
Latron / Water	20	35.0 ab yz	20	15.0 b	20	10.0 a z	60	20.0 bc yz
Latron / Latron[d]	20	25.0 ab yz	20	30.0 ab	20	5.0 a z	60	20.0 bc yz
Methoxyfenozide[e] / Water	20	25.0 ab yz	20	60.0 a	20	10.0 a z	60	31.7 ab xy
Methoxyfenozide / Latron	20	30.0 ab yz	20	35.0 ab	20	10.0 a z	60	25.0 bc yz
Methoxyfenozide / Methoxyfenozide	20	15.0 b z	20	30.0 ab	20	0.0 a z	60	15.0 c z
Septum Lure / Water	20	20.0 ab z	20	55.0 a	20	10.0 a z	60	28.3 bc y
Cage								
Water / Water	20	40.0 a y	20	40.0 a xy	20	25.0 *	60	35.0 a x
Latron / Water	20	35.0 a yz	20	10.0 c z	20	10.0 *	60	18.3 b yz
Latron / Latron	20	25.0 a yz	20	30.0 abc xyz	20	5.0 *	60	20.0 ab yz
Methoxyfenozide / Water	20	25.0 a yz	20	55.0 a x	20	5.0 *	60	28.3 ab xy
Methoxyfenozide / Latron	20	30.0 a yz	20	35.0 abc xy	20	10.0 *	60	25.0 ab xy
Methoxyfenozide / Methoxyfenozide	20	15.0 a z	20	20.0 bc yz	20	0.0 *	60	11.7 b z
Septum Lure / Water	20	15.0 a z	20	45.0 ab x	20	5.0 *	60	21.7 ab yz

Means followed by the same letter are not significantly different (Generalized Linear Model-odds ratio=1). Actual means (not transformed means) are listed.

[a]Moths, 0-24h old, exposed to treated surfaces for 48h.

[b]Number of replicates per treatment. Each replicate consisted of one male moth and two cages each with 2 females moths. New moths were used for each replicate.

[c]The first column of letters recognize significant difference as P<0.05. The second column of letters recognize significant difference as P<0.10.

[d]The Latron treatment consisted of a labeled field rate (0.125% vol:vol) of Latron B-1956®, a resin-based nonionic surfactant, mixed with water.

[e]Each methoxyfenozide treatment solution contained a proportionate field rate of Latron B-1956® as per label recommendations.

*Number of males coming into contact with female cages was not high enough to compare for significant differences.

Table 5. Percent of treated adult male oriental fruit moth released in a wind tunnel that were found to be orienting within a treatment combination pheromone plume or making treatment cage contact of one of two upwind treatment sources (choice assay). The female treatment data were pooled per male treatment.

Treatment Exposures of Females[a]	Water Treated Males		Latron Treated Males		Methoxyfenozide[e] Treated Males		Pooled Male Treatments[c]	
	N[b]	%	N	%	N	%	N	%
Plume								
Water	50	40.0 a	50	44.0 a	50	22.0 a	150	35.3 a
Latron[d]	40	32.5 a	40	27.5 a	40	5.0 a	120	21.7 b
Methoxyfenozide[e]	40	12.5 b	40	40.0 a	40	5.0 a	120	19.2 b
Cage								
Water	50	30.0 a	50	40.0 a	50	14.0 a	150	28.0 a y
Latron	40	32.5 a	40	25.0 a	40	5.0 a	120	20.8 ab z
Methoxyfenozide	40	12.5 b	40	27.5 a	40	5.0 a	120	15.0 b z

Means followed by the same letter are not significantly different (Generalized Linear Model-odds ratio=1). Actual means (not transformed means) are listed.

[a]Moths, 0-24h old, exposed to treated surfaces for 48h.

[b]Number of replicates per treatment. Each replicate consisted of one male moth and two cages each with 2 females moths. New moths were used for each replicate.

[c]The first column of letters recognize significant difference as P<0.05. The second column of letters recognize significant difference as P<0.10.

[d]The Latron treatment consisted of a labeled field rate (0.125% vol:vol) of Latron B-1956®, a resin-based nonionic surfactant, mixed with water.

[e]Each methoxyfenozide treatment solution contained a proportionate field rate of Latron B-1956® as per label recommendations.

Table 6. Percent of treated adult male oriental fruit moth released in a wind tunnel that were found to be orienting within a treatment combination pheromone plume or making contact with cages of treated females (choice assay). The female treatment data were pooled per male treatment.

Treatment Exposures of Males[a]	N[b]	Mean Percent Individuals Orienting in Pheromone Plume[c]	Mean Percent Individuals Contacting Female Cage
Water	140	28.6 a y	26.4 a
Latron[d]	140	39.3 a x	33.6 a
Methoxyfenozide[e]	140	11.4 b z	8.6 b

Means followed by the same letter are not significantly different (Generalized Linear Model-odds ratio=1). Actual means (not transformed means) are listed.
[a]Moths, 0-24h old, exposed to treated surfaces for 48h.
[b]Number of replicates per treatment. Each replicate consisted of one male moth and two cages each with 2 females moths. New moths were used for each replicate.
[c]The first column of letters recognize significant difference as $P<0.05$. The second column of letters recognize significant difference as $P<0.10$.
[d]The Latron treatment consisted of a labeled field rate (0.125% vol:vol) of Latron B-1956®, a resin-based nonionic surfactant, mixed with water.
[e]Each methoxyfenozide treatment solution contained a proportionate field rate of Latron B-1956® as per label recommendations.

B. Fecundity and Fertility Assays

1. Continuous exposure: treated pairings

Continuous exposure to surfaces treated with tebufenozide and methoxyfenozide negatively impacted the mean number of eggs laid (Table 7). For example, the mean fecundity of moth pairings exposed to surfaces treated with water and Latron were 11.3 and 1.1 eggs/female, respectively. These two means were not significantly different. However, the mean number of eggs laid from moths exposed to tebufenozide- and methoxyfenozide-treated surfaces, 0.1 and 0.0 eggs/female, respectively, were significantly less than the water-control treatment mean (Table 7).

The mean percent fertility from water-, Latron-, and tebufenozide-treated pairings were 74%, 31%, and 100%, respectively, none of which were significantly different from one another (Table 7). The mean fertility from the methoxyfenozide-treated pairings could not be determined, because there were no eggs laid in any replicates.

Exposure to tebufenozide and methoxyfenozide also negatively impacted the longevity of adult females. The median lifespan of females in the water- and Latron-controls were 8.0 and 7.5 days (Table 7). The females in methoxyfenozide-treated pairings had a median shorter lifespan than both controls, at 6.5 days. At 7.0 days, median longevity of tebufenozide-treated females was not significantly different from any other treatment.

When considering the mean number of females that laid eggs throughout the study, no more than 10% of the females oviposited by day 3 (Table 8). By

day 5, however, 7 of 10 females in the water-control and 5 of 10 females in the Latron-control had laid eggs. These were both significantly more than the methoxyfenozide treatment, which had no ovipositing females. The tebufenozide treatment had 20% of females lay eggs, which was significantly lower than the water-control. In all treatments, no additional females laid eggs between day 5 and day 10 (Table 8).

By 8 days after the start of the study, 50% of the water-control replicates had eggs that hatched (Table 8). This was significantly greater than the methoxyfenozide treatment that had no eggs hatch. By day 10, 60% of replicates in the water-control had hatched eggs. This was still significantly higher than the methoxyfenozide treatment. At the 90% confidence interval there was a significant reduction in replicates with hatched eggs in the tebufenozide study when compared to the water-control. There were also significantly more replicates with eggs that hatched in the Latron-control then the methoxyfenozide treatment. In all treatments, no additional replicates had eggs hatch between days 10 and 14 (Table 8).

Table 7. Mean fecundity, percent fertility and median longevity of oriental fruit moth continuously exposed to surfaces treated with methoxyfenozide, tebufenozide or Latron.

Treatment Exposures[a]	N[b]	Mean (± SE) Fecundity	N[b]	Mean Percent (± SE) Fertility	N[b]	Median (± SE) Longevity (days)
Water	8	11.3 ± 14.4 a x	6	73.5 ± 16.8 a	8	8.0 ± 0.55 a
Latron[c]	8	1.2 ± 1.56 ab y	4	31.3 ± 20.6 a	8	7.5 ± 0.40 a
Tebufenozide[d]	8	0.1 ± 0.35 bc z	1	100.0 ± 41.2 a	8	7.0 ± 0.37 ab
Methoxyfenozide[d]	8	0.0 ± 0.0 c z			8	6.5 ± 0.52 b

Means followed by the same letter are not significantly different (Fisher's Protected LSD, P<0.05). Actual means (not transformed means) are listed.

[a]Moths, 0-24h old, exposed to treated surfaces for the duration of the study (10 days).

[b]Number of replicates per treatment. Each replicate consisted of two male moths and one female moth. New moths were used for each replicate.

[c]The Latron treatment consisted of a labeled field rate (0.125% vol:vol) of Latron B-1956®, a resin-based nonionic surfactant, mixed with water.

[d]Each methoxyfenozide and tebufenozide treatment solution contained a proportionate field rate of Latron B-1956® as per label recommendations.

Table 8. Mean percent of female oriental fruit moth, per treatment, continuously exposed to surfaces treated with methoxyfenozide, tebufenozide or Latron that laid eggs, and the mean percent of replicates with eggs that hatched over time.

Treatment Exposures[a]	N[b]	Mean Percent of Females that Laid Eggs by:			Mean Percent of Replications with Eggs hatched by:		
		day 3	day 5	day 10	day 8	day 10[c]	day 14[c]
Water	10	10 a	70 a	70 a	50 a	60 a x	60 a x
Latron[d]	10	0.0 a	50 ab	50 ab	20 ab	30 ab xy	30 ab xy
Tebufenozide[e]	10	10 a	20 bc	20 bc	20 ab	20 ab yz	20 ab yz
Methoxyfenozide[e]	10	0.0 a	0.0 c	0.0 c	0.0 b	0.0 b z	0.0 b z

Means followed by the same letter are not significantly different (Row by Column Chi Squared). Actual means (not transformed means) are listed.

[a]Moths, 0-24h old, exposed to treated surfaces for the duration of the study (10 days).

[b]Number of replicates per treatment. Each replicate consisted of two male moths and one female moth. New moths were used for each replicate.

[c]The first column of letters (a, b, c) recognize significant difference at $P<0.05$. The second column of letters (x, y, z) recognize significant difference at $P<0.10$.

[d]The Latron treatment consisted of a labeled field rate (0.125% vol:vol) of Latron B-1956®, a resin-based nonionic surfactant, mixed with water.

[e]Each methoxyfenozide and tebufenozide treatment solution contained a proportionate field rate of Latron B-1956® as per label recommendations.

2. 24 hour Exposure: treated/non treated pairings

When male moths were exposed for 24 h to surfaces treated with water, Latron, tebufenozide, and methoxyfenozide, then paired with a non treated female, the mean number of eggs laid per female were 26.8, 25.0, 17.9, and 26.8 eggs/ female, respectively (Table 9). There were no significant differences among these treatment means. When female moths were exposed for 24 h to surfaces treated with water, Latron, tebufenozide, and methoxyfenozide, then paired with a non treated male, the mean number of eggs laid were 23.5, 44.2, 11.3, and 2.1 eggs/female, respectively. The largest mean fecundity, 44.2 eggs/female, occurred with Latron-treated females. This mean was significantly higher than all other treatment means regardless of the treated sex. The lowest mean fecundities, 11.3 and 2.1 eggs/female, occurred with the tebufenozide and methoxyfenozide treatment exposures, respectively. These two treatments were significantly lower than all other treatment means regardless of the sex treated (Table 9).

When male moths were exposed for 24 h to surfaces treated with water, Latron, tebufenozide, and methoxyfenozide, then paired with a non treated female, the mean percent of egg hatch per replication were 84.1%, 81.5%, 76.3% and 75.0%, respectively (Table 9). When female moths were exposed for 24 h to surfaces treated with water, Latron, tebufenozide, and methoxyfenozide, then paired with a non treated male, the mean percent of egg hatch per replication were 84.8%, 94.4%, 91.4%, and 77.3%, respectively. The mean percent egg hatch for replicates where males were treated with methoxyfenozide

was significantly lower than replicates where males were exposed to Latron, females exposed to Latron and females exposed to water treated surfaces. The mean percent egg hatch for replicates where males were exposed to Latron was significantly lower than replicates where females were exposed to Latron (Table 9).

The median longevity of females paired with males exposed to water and methoxyfenozide for 24 h was 9.0 days, and when paired with males exposed to Latron and tebufenozide was 6.0 days (Table 9). The females paired with tebufenozide-treated males had a significantly lower mean fecundity than females paired with both water- and methoxyfenozide-treated males.

The median longevity of females treated with water and Latron then paired with untreated males was 10.0 days (Table 9). The median longevity of females treated with tebufenozide and methoxyfenozide then paired with untreated males was 9.5 and 5.5 days, respectively. Methoxyfenozide-treated females had a median longevity that was significantly lower than the water- and Latron-treated females.

By Day 3 of the study, the mean percent of females that laid eggs after being paired with males exposed to water-, Latron-, tebufenozide-, and methoxyfenozide-treated surfaces for 24 h were 80%, 60%, 90% and 90%, respectively (Table 10). There were no significant differences between these treatment means, nor did the treatment means change on Days 5 and 10.

When females were exposed to water-, Latron-, tebufenozide-, and methoxyfenozide-treated surfaces for 24 h then paired with nontreated males,

the mean percent that laid eggs by Day 3 of the study were 70%, 80%, 50%, and 40%, respectively (Table 10). The only significant differences that occurred between these means were at the 90% confidence interval between methoxyfenozide and Latron treatments. By Day 5, 80% of water-treated females had laid eggs. This was also significantly higher than methoxyfenozide-treated females, at the 90% confidence interval. There were no changes in the treatment means by Day 10.

When considering both male-exposure and female-exposure treatments for Day 3, females exposed to methoxyfenozide had significantly fewer replicates laying eggs than replicates with males exposed to methoxyfenozide and tebufenozide (Table 10). Females exposed to tebufenozide had significantly fewer replicates laying eggs than replicates with males exposed to methoxyfenozide and tebufenozide, at the 90% confidence interval.

In regards to the mean percent of replicates that had egg hatch, virtually all replicates that had egg laying on Days 3, 5 and 10 also had egg hatch on Days 8, 10 and 14 (Table10). There were no significant differences among male-exposure and female-exposure treatments in mean percent of replicates with egg hatch through Days 8, 10 and 14. However, methoxyfenozide treated females had a significantly lower number of replicates with egg hatch than several other treatments including both water-treated female and male controls, at the 90% confidence interval.

Table 9. Mean fecundity, percent fertility and median longevity of oriental fruit moth exposed, by sex, to surfaces treated with methoxyfenozide, tebufenozide or Latron for 24 h, then paired with a non-exposed partner (opposite sex) in a non-treated cage.

Treatment Exposures[a]	N[b]	Mean (± SE) Fecundity	Mean Percent (± SE) Fertility[c]	Median (± SE) Female Longevity (days)[d]
Water – M	10	26.8 ± 6.9 b	84.1 ± 9.1 abc yz	9.0 ± 0.78 A
Latron[e] – M	10	25.0 ± 9.7 b	81.5 ± 10.5 b z	6.0 ± 0.79 AB
Tebufenozide[f] – M	10	17.9 ± 4.5 b	76.3 ± 8.6 abc yz	6.0 ± 0.18 B
Methoxyfenozide[f] – M	10	26.8 ± 6.8 b	75.0 ± 8.6 c z	9.0 ± 0.47 A
Water – F	10	23.4 ± 7.2 b	84.8 ± 9.1 ab y	10.0 ± 0.47 a
Latron – F	10	44.2 ± 13.4 a	94.4 ± 9.1 a y	10.0 ± 0.43 a
Tebufenozide – F	10	11.3 ± 5.0 c	91.4 ± 11.5 abc yz	9.5 ± 0.97 ab
Methoxyfenozide – F	10	2.1 ± 1.1 c	77.3 ± 12.9 abc yz	5.5 ± 0.81 b

Means followed by the same letter or symbol are not significantly different (Fisher's Protected LSD). Actual means (not transformed means) are listed.

[a]Moths, 0-24h old, exposed to treated surfaces for 24h.

[b]Number of replicates per treatment. Each replicate consisted of two male moths and one female moth. New moths were used for each replicate.

[c]The first column of letters (a, b, c) recognize significant difference at P<0.05. The second column of letters (x, y, z) recognize significant difference at P<0.10.

[d]Longevity of the water-control treatments were significantly different. As such, male treatments and female treatments were analyzed separately.

[e]The Latron treatment consisted of a labeled field rate (0.125% vol:vol) of Latron B-1956®, a resin-based nonionic surfactant, mixed with water.

[f]Each methoxyfenozide and tebufenozide treatment solution contained a proportionate field rate of Latron B-1956® as per label recommendations.

Table 10. Mean percent fecundity and fertility, over time, of oriental fruit moth exposed, by sex, to surfaces treated with methoxyfenozide, tebufenozide or Latron for 24 h, and then paired with a non-exposed partner (opposite sex) in a non-treated cage.

Treatment Exposures[a]	N[b]	Mean Percent of Females that Laid Eggs by:[c]			Mean Percent of Replications with Eggs hatched by:[c]		
		day 3	day 5	day 10	day 8	day 10	day 14
Water – M	10	80 ab xy	80 ab xy	80 ab xy	80 a y	80 a y	80 a y
Latron[d] – M	10	60 ab xyz	60 ab xyz	60 ab xyz	60 a yz	60 a yz	60 a yz
Tebufenozide[e] – M	10	90 a x	90 a x	90 a x	80 a y	80 a y	80 a y
Methoxyfenozide[e] – M	10	90 a x	90 a x	90 a x	80 a y	80 a y	80 a y
Water – F	10	70 ab xyz	80 ab xy	80 ab xy	70 a y	70 a y	70 a y
Latron – F	10	80 ab xy	80 ab xy	80 ab xy	80 a y	80 a y	80 a y
Tebufenozide – F	10	50 ab yz	50 ab yz	50 ab yz	50 a yz	50 a yz	50 a yz
Methoxyfenozide – F	10	40 b z	40 b z	40 b z	40 a z	40 a z	40 a z

Means followed by the same letter are not significantly different (Row by Column Chi Squared). Actual means (not transformed means) are listed.
[a]Moths, 0-24h old, exposed to treated surfaces for 24h.
[b]Number of replicates per treatment. Each replicate consisted of two male moths and one female moth. New moths were used for each replicate.
[c]The first column of letters (a, b, c) recognize significant difference at $P<0.05$. The second column of letters (x, y, z) recognize significant difference at $P<0.10$.
[d]The Latron treatment consisted of a labeled field rate (0.125% vol:vol) of Latron B-1956®, a resin-based nonionic surfactant, mixed with water.
[e]Each methoxyfenozide and tebufenozide treatment solution contained a proportionate field rate of Latron B-1956® as per label recommendations.

C. Codling Moth and Oriental Fruit Moth Feeding Comparison Assay

Four days after placing 1st instar codling moth (CM) or oriental fruit moth (OFM) on individual apples, 86.2% of the CM replicates showed levels of tunneling damage (Table 11). This level of damage was significantly higher than the 18.2% in the OFM replicates. By Day 7, 93.1% of CM replications had visual tunneling damage. This was still significantly higher than the OFM replications, at 36.4%. By Day 10, a large increase occurred in the percent of apples with OFM tunneling damage, 77.3%. This was still significantly less than the 96.6% of apples with CM damage at the 90% confidence interval. By Day 14, 95.5% of the apples had OFM-induced damage, which was not significantly different from the 96.6% CM damage (Table 11). The CM damage at Day 14 was not significantly higher than any of the previous days for CM. The damage pattern for OFM showed significant increases from Days 4 and to Days 10 and 14.

To determine if there was any preference displayed by CM and OFM for apple site entry, the apples were separated into upper and lower zones. Of the 44.4% of the CM larvae that produced visible tunneling damage, 34.9% entered the upper half of the apples, while 9.5% entered the lower half of the apples (Table 12). These levels were not significantly different. OFM larvae showed the same trend, preferring the upper half of the applse over the lower half, at 31.1% and 13.3%, respectively. These were also not significantly different. There was a statistically higher percent incidence of CM damage in the upper half of the apples than OFM damage in the same portion.

To determine the spatial distribution of sites containing frass, the apple exterior was further divided into several zones (Figure 9). CM showed a preference for the upper portion of the apple (Zone 2), with 52.4% of the replicates exhibiting frass (Table 13). This was closely followed by Zone 3, the mid-upper part of the apple, with 33.3%. The lower portion of the apple (Zone 5) and the calyx end (Zone 6) had significantly fewer replicates with observable frass, both at 4.8%. Conversely, OFM larvae showed a preference for the portion of the apple immediately surrounding the stem (Zone 1), at 80.0%. This was significantly higher than all other apple zones, save the calyx, at 33.3%. OFM preferred the calyx end of the apple significantly more so than CM. There was no significant difference in frass sites between the two species when considering the stem end. CM had significantly higher incidences of damage of all other zones, when compared to OFM. When the zones were combined together, both CM and OFM showed preferences for the upper half versus the lower of the apples, 34.9% and 8.0% and 31.1% and 11.1%, respectively (Table 13). Only CM damage was significant.

The progression of visible frass occurrence was compared between the two species. On Day 4, 82.8% of the CM replicates already showed frass (Table 14). This was significantly higher than the 18.2% exhibited by OFM. This trend continued on Day 7, with 89.7% and 36.4% of CM and OFM replicates showing frass, respectively. The number of OFM replicates showing frass almost doubled by Day 10 (68.2%). This was still significantly lower than the 93.1% of CM replicates with frass. By Day 14, there was no increase in frass occurrence for

CM, but the number of OFM replicates with frass increased to 86.4%, and the two species were not significantly different at this time (Table 14). Within species, there was no significant difference over time on the percent of CM replicates displaying frass. Conversely, on Days 4 and 7, OFM replicates showed significantly fewer incidences of frass than Days 10 and 14.

The volume of frass produced by CM and OFM during the study was also compared. On Day 4, CM and OFM frass volumes produced were not significantly different, at 2.8 mm^3 and 1.0 mm^3, respectively (Table 15). This trend continued, with the mean volume of frass increasing for both species, until Day 14 when CM frass volume had increased to 17.7 mm^3 and the OFM frass volume was 64.9 mm^3. There was a significant difference between the two species on Day 14. Within species, there was a significant difference for OFM frass production across time, Day 14 being significantly greater than any of the previous days. There was no significant increase in frass volume over time for CM.

At the termination of the study the apples were cut open to determine the spatial distribution of damage. To determine the spatial distribution of internal feeding, the apple interior was divided into several zones (Figure 10). For CM, 25% of the larvae had attacked the core of the apple under the stem (Zone 1) (Table 16). Significantly more (71.4%) OFM damaged this area. None of the CM replicates showed damage to the core around the seeds (Zone 2). For OFM, 35.7% of the replicates showed damage to this area, and to the core of the apple just above the calyx (Zone 3). CM showed significantly less damage (6.3%) in

this area. For Zone 4 (the peripheral flesh surrounding the core), 62.5% of the CM replicates had damaged the area. This was significantly more than OFM whose damage was at 14.3%. Significantly more seeds were damaged by CM larvae than by OFM larvae, at 68.8% and 7.1%, respectively (Table 16). CM preferred the peripheral flesh of the apple and the seeds significantly more than the core flesh of the apple. OFM preferred the core, with significantly more replications showing damage to the upper portion of the core than to the peripheral flesh and seeds.

Table 11. Mean percent of apples exposed to codling moth or oriental fruit moth larvae that had tunneling damage over time.

		Mean Percent Tunneling Damage[a c]			
Species	N[b]	Day 4	Day 7	Day 10[d]	Day 14
Codling Moth	21	86.2 a *	93.1 a *	96.6 a x *	96.6 a *
Oriental Fruit Moth	15	18.2 b **	36.4 b **	77.3 a y *	95.5 a *

Means followed by the same letter and symbol are not significantly different (Row by Column Chi Squared). Actual means (not transformed means) are listed.

[a]Tunneling damage included lateral feeding immediately below the surface of the apple and entrance and exit holes protruding through the skin into the meat of the apple.

[b]Number of replicates per treatment. Each replicate consisted of two 1st instar larvae of the same species less than 48 hours old placed on the upper portion of the apple (Zone 2).

[c]Significant difference designated by letters are determined vertically. Significant differences designated by asterisks are determined laterally.

[d]The first column of letters (a, b, c) recognize significant difference at $P<0.05$. The second column of letters (x, y, z) recognize significant difference at $P<0.10$.

Table 12. Mean percent of apples exposed to codling moth or oriental fruit moth larvae that had tunneling damage on the fruit's upper or lower zones at the end of the study.

	Mean Percent Tunneling Damage[a c]	
Fruit Surface Zones	Codling Moth n=21[b]	Oriental Fruit Moth n=15[b]
Zone 1 (upper half)	34.9 a *	31.1 a **
Zone 2 (lower half)	9.5 a *	13.3 a *

Means followed by the same letter and symbol are not significantly different (Generalized Linear Model-odds ratio=1, P<0.05). Actual means (not transformed means) are listed.
[a]Tunneling damage included lateral feeding immediately below the surface of the apple and entrance and exit holes protruding through the skin into the meat of the apple.
[b]Number of replicates per treatment. Each replicate consisted of two 1st instar larvae of the same species less than 48 hours old placed on the upper portion of the apple (Zone 2).
[c]Significant difference designated by letters are determined vertically. Significant differences designated by asterisks are determined laterally.

Table 13. Mean percent of apples exposed to codling moth and oriental fruit moth larvae that produced sites with frass exuding from entrance/exit holes[a] located on the different surface areas (Zones) of the apple by the end of the study.

	Mean Percent with Presence of Frass[c e]	
Fruit Surface Zones	Codling Moth n=21[b]	Oriental Fruit Moth n=15[b]
Zone 1 (stem)	19.0 a x *	80.0 a w *
Zone 2 (upper)	52.4 a x *	6.7 ab xy **
Zone 3 (mid-upper)	33.3 a x*	6.7 b y **
Zone 4(mid-lower)	14.3 ab xy *	0.0 c z **
Zone 5 (lower)	4.8 b y *	0.0 c z **
Zone 6 (calyx)	4.8 c z **	33.3 a wx *
Zone A (upper half)[d]	34.9 a *	31.1 a **
Zone B (lower half)[d]	8.0 b *	11.1 a *

Means followed by the same letter and symbol are not significantly different (Generalized Linear Model-odds ratio=1, P<0.05). Actual means (not transformed means) are listed.

[a]Entrance and exit holes consisted of small, distinctly round holes that continued through the apple skin.

[b]Number of replicates per treatment. Each replicate consisted of two 1st instar larvae of the same species less than 48 hours old placed on the upper portion of the apple (Zone 2).

[c]Significant difference designated by letters are determined vertically. Significant differences designated by asterisks are determined laterally.

[d]Zone 'A' consists of an average of Zones 1, 2 and 3. Zone 'B' consists of an average of Zones 4, 5 and 6.

[e]The first column of letters (a, b, c) recognize significant difference at P<0.05. The second column of letters (x, y, z) recognize significant difference at P<0.10.

Table 14. Mean percent of apples exposed to codling moth and oriental fruit moth larvae that produced sites with frass exuding from entrance/exit holes of the apple[a].

		Mean Percent with Presence of Frass[c]			
Moth Species	N[b]	Day 4	Day 7	Day 10	Day 14
Codling Moth	21	82.8 a *	89.7 a *	93.1 a *	93.1a *
Oriental Fruit Moth	15	18.2 b **	36.4 b **	68.2 b *	86.4 a *

Means followed by the same letter and symbol are not significantly different (Row by Column Chi Squared, $P<0.05$). Actual means (not transformed means) are listed.

[a]Entrance and exit holes consisted of small, distinctly round holes that continued through the apple skin.

[b]Number of replicates per treatment. Each replicate consisted of two 1st instar larvae of the same species less than 48 hours old placed on the upper portion of the apple (Zone 2).

[c]Significant difference designated by letters are determined vertically. Significant differences designated by asterisks are determined laterally

Table 15. Mean volume ($mm^{3)}$ of frass exuding from entrance/exit holes of apples damaged by codling moth or oriental fruit moth larvae over time[a].

		Mean (± SE) Volume of Frass (mm^3)[c]			
Moth Species	N[b]	Day 4	Day 7	Day 10	Day 14
Codling Moth	21	2.8 ± 0.9 a *	5.9 ± 1.6 a *	15.4 ± 5.2 a *	17.7 ± 6.2 a *
Oriental Fruit Moth	15	1.0 ± 0.9 a *	1.0 ± 0.9 a *	27.5 ± 20.7 a *	64.9 ± 32.7 b **

Means followed by the same letter and symbol are not significantly different (Row by Column Chi Squared, $P<0.05$). Actual means (not transformed means) are listed.

[a]Entrance and exit holes consisted of small, distinctly round holes that continued through the apple skin.

[b]Number of replicates per treatment. Each replicate consisted of two 1st instar larvae of the same species less than 48 hours old placed on the upper portion of the apple (Zone 2).

[c]Significant difference designated by letters are determined vertically. Significant differences designated by asterisks are determined laterally

Table 16. Mean percent of apples exposed to codling moth or oriental fruit moth larvae with damage occurring at different internal fruit zones.

	Mean Percent Internal Apple Zones with Larval Feeding Damage[a]	
Fruit surface zones	Codling Moth n=16[b]	Oriental Fruit Moth n=14[b c]
Zone 1 (above core to stem)	25.0 b **	71.4 a x *
Zone 2 (core excluding seed cavity)	0.0 b **	35.7 ab y *
Zone 3 (below core to calyx)	6.3 b **	35.7 ab y *
Zone 4 (peripheral flesh around core)	62.5 a *	14.3 b yz **
Seeds	68.8 a *	7.1 b z **

Means followed by the same letter and symbol are not significantly different (Generalized Linear Model-odds ratio=1). Actual means (not transformed means) are listed.

[a]Significant difference designated by letters are determined vertically. Significant differences designated by asterisks are determined laterally.

[b]Number of replicates per treatment. Each replicate consisted of two 1st instar larvae of the same species less than 48 hours old placed on the upper portion of the apple (Zone 2).

[c]The first column of letters (a, b, c) recognize significant difference at $P<0.05$. The second column of letters (x, y, z) recognize significant difference at $P<0.10$.

Chapter 4: Discussion

A. Wind Tunnel Assays

Few studies have examined the sublethal effects of methoxyfenozide on male moth orientation. Hoelscher and Barrett (2003 a,b) observed that adult male exposure to methoxyfenozide-treated surfaces decreased the ability of redbanded leafroller, *Argyrotaenia velutinana* (Walker), obliquebanded leafroller, *Choristoneura rosaceana* (Harris), and codling moth, *Cydia pomonella* (L.), (Lepidoptera: Tortricidae) males to orient towards females. In both of these studies, there was no observable effect on female attractiveness when they were treated with methoxyfenozide.

Other studies have also reported negative effects of chemical insecticide exposure on the mate-finding abilities of other male Lepidoptera. Henneberry et al. (1966) found that feeding the chemosterilant tepa (an aziridine compound) to adult male cabbage looper, *Trichoplusia ni* (Hübner), made them less responsive to the female sex pheromone. Permethrin, a synthetic pyrethroid, has also been shown to affect male response. For example, flight initiation and upwind orientation of male pink bollworm, *Pectinophora gossypiella* (Saunders), were reduced when they were exposed to sublethal doses of permethrin via liquid topical application (Haynes and Baker 1985). Moore (1988) found similar results using similar methods with the bollworm, *Helicoverpa zea* (Boddie), and reported that females treated with sublethal levels of permethrin attracted fewer males than untreated females.

Linn and Roelofs (1984) reported that permethrin, as well as carbaryl (a carbamate insecticide) and chlordimeform (a formamidine insecticide), reduced the ability of male oriental fruit moth, *Grapholita molesta* (Busck) (OFM), to orient to untreated females when treated topically with the chemical. This was substantiated by Evenden et al. (2005) when they showed that contact with an OFM attracticide containing permethrin reduced the subsequent responses of male OFM to the pheromone source for 24 h post-treatment.

All of the wind tunnel assays in the current study have demonstrated that exposing adult OFM, regardless of sex, to methoxyfenozide has an effect on the mate-finding ability of the male. The non-choice study showed that, in most cases, when either sex was treated with methoxyfenozide a smaller percentage of males were captured on the sticky card in front of the female cage (Tables 1 and 2). This suggests that either treated males were not as responsive to the female pheromone as untreated males or that treated females were not as attractive as untreated females.

These effects were substantiated by the wind tunnel choice study. We found a lower percent of males, when treated with water as well as when all treatment data were pooled together, orienting to the pheromone plumes of the methoxyfenozide / methoxyfenozide female treatment combination than to the female water control (Table 4). This trend continued for the percent of males that made contact with the female cages. Similarly, methoxyfenozide-treated males consistently had the lowest percent of individuals orienting to the female pheromone plumes. In fact, exposure to methoxyfenozide-treated surfaces had

such an effect on the males' ability to reach the female cages that the percent numbers were too few to analyze.

There were no significant differences within the Latron- and methoxyfenozide-treated males for the mean percent of males found orienting to the pheromone plume and contacting cages of water, Latron-, or methoxyfenozide-treated females, but the water-treated males did respond to a treatment effect, being less responsive to methoxyfenozide-treated females than to water- and Latron-treated females (Table 5). Methoxyfenozide also reduced the male moths' ability to orient to a pheromone plume and to make contact with the female cages, regardless of the female treatment (Table 6).

It is unclear what modes of action are occurring in the moths when they are exposed to surfaces treated with methoxyfenozide. Exposure could have a physical effect upon the pheromone release of the female and/or the chemosensors of the male, although unpublished electroantennagram data (Bruce Barrett, University of Missouri; Kenneth Haynes, University of Kentucky) suggests that treated male redbanded leafroller are still sensitive to the main components of the pheromone. Further work is pending. Tebufenozide was reported to delay the mean onset of calling by female spruce budworms, *Choristoneura fumiferana* (Clem) (Lepidoptera: Tortricidae), by one h (Dallaire et al. 2004). Methoxyfenozide could have a similar effect on the calling of OFM. It is also possible the ecdysone agonist is capable of modifying the chemical makeup of the female pheromone, or physiologically inhibiting the female from releasing the pheromone. Trimble et al. (2004) reported that female obliquebanded

leafroller exposed to azinphosmethyl-treated surfaces released less pheromone as well as delaying the onset and incidence of calling. Future physiological research is needed in order to determine the mechanisms involved in the sublethal effects of methoxyfenozide on the attractiveness and responsiveness of OFM.

B. Fecundity / Fertility Assays

Several studies have reported that continuous exposure (and in some cases exposure for only 24 h) of adult moths to both methoxyfenozide- and tebufenozide-treated surfaces reduced the mean fecundity and percent fertility of both redbanded leafroller, *Argyrotaenia velutinana* (Walker), obliquebanded leafroller, *Choristoneura rosaceana* (Harris), and codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Sun and Barrett 1999, Sun et al. 2000, Smagghe et al. 2004). Knight (2000) substantiated these results with tebufenozide, stating that only a 1 h exposure to treated surfaces is required to negatively affect the fecundity and fertility of codling moth. However, Sun et al.(2004) reported that a 6 h exposure period to surfaces treated with the ecdysone agonists did not significantly reduce mean fecundity and fertility of adult codling moth from the control treatment.

Several mechanisms have been suggested for the reductions in fecundity and fertility associated with exposure to methoxyfenozide and tebufenozide. Carpenter and Chandler (1994) noted that larvae fed a diet containing RH-5992 (a precursor to methoxyfenozide and tebufenozide) not only reduced the mean

fecundity in corn earworm adults, *Helicoverpa zea* (Boddie), but also resulted in a reduction in the number of adult males that were able to transfer sperm. Dallaire et al. (2004) reported similar results, showing a reduction in weight of the spermatophore and a lower number of eupyrenes when male obliquebanded leafroller were fed, as larvae, sublethal doses of tebufenozide. The ecdysone hormone produced in the adult testes has a role in stimulating spermatogenesis (Hagedorn 1985), and exposure to ecdysone agonists might negatively impact this spermatogenic cycle

Other studies have suggested that reductions in fecundity and fertility are due to the impacts on the reproductive capacities of females. Some suggest tebufenozide has a chemosterilizing effect on oogenesis, either by inhibiting new oocyte formation or by degeneration and resorption of existing ovarioles (Wing et al. 1988, Smagghe and Degheele 1994a, Smagghe et al. 1996).

Borchert et al.(2005) reported that methoxyfenozide has no deleterious effects on the fecundity of oriental fruit moth, *Grapholita molesta* (Busck) (OFM), when exposed to methoxyfenozide-treated surfaces. Results from the current studies contradict that fact. Here it is reported that methoxyfenozide, as well as tebufenozide, has been shown to be capable of affecting the fecundity and, in some cases, fertility of OFM. Female OFM longevity can also be affected by methoxyfenozide and tebufenozide.

When both sexes were continuously exposed to treated surfaces, the methoxyfenozide and tebufenozide treatments had a negative impact on mean fecundity. In fact, moths exposed to methoxyfenozide produced no eggs. As

such, no statistical analysis on mean percent fertility could be made. However, there was no difference in mean percent fertility between tebufenozide and the controls. Similarly, Saenz-de-Cabezon Irigaray et al.(2005) reported that, when the grape berry moth, *Lobesia botrana* Dennis & Schiffermuller (Lepidoptera: Tortricidae), was fed methoxyfenozide orally via a water trough there was a reduction in fecundity, but no effect on fertility. Several studies have documented the increased effects of methoxyfenozide over tebufenozide (Sun and Barrett 1999, Sun et al. 2000). One explanation for this could be the longer residual acitivity of methoxyfenozide over tebufenozide. This, however, is questionable, as tebufenozide, and methoxyfenozide have been shown to require up to one month to decline by 80% in the field (Borchert et al. 2004b). Residual differences should not be a factor for a two day laboratory experiment under controlled conditions. Female median longevity was lower for moths exposed to both methoxyfenozide and tebufenozide than for the water control. This was interesting since Saenz-de-Cabezon Irigaray et al. (2005) reported that the grape berry moth showed no change in longevity when fed methoxyfenozide orally via a water trough throughout the study.

Egg oviposition was slow the first couple of days of the study, with only 10% of moths laying eggs in the highest treatments. This increased by Day 5, with significantly higher numbers of moths laying eggs in the water control than in either the methoxyfenozide or tebufenozide treatments. Again, there were no eggs laid throughout the study by methoxyfenozide exposed females. This trend continued for fertility with a lower percentage of eggs from methoxyfenozide

exposed moths hatching than in the water control by Day 8 of the study. By Day 10 tebufenozide also had a significantly lower number of replicates with egg hatch than the water control.

The sexes were separated for the next study with only one sex being exposed to treated surfaces. This study shows that methoxyfenozide and tebufenozide affected female OFM more than males. For example, there was no difference in mean fecundity between any of the male exposure treatments, all of which were equal to the water control female exposure treatment. However, females exposed to methoxyfenozide- and tebufenozide-treated surfaces had a lower mean fecundity than all other treatments. Only when males were exposed to methoxyfenozide-treated surfaces did mean percent fertility drop below the controls. One of the only instances where tebufenozide seemed to have a stronger effect than methoxyfenozide was in female longevity when males were exposed to treated surfaces. Only when males were exposed to tebufenozide-treated surfaces did a reduction in median longevity occur over the control. The trend reversed for females exposed to the chemicals, with methoxyfenozide negatively affecting female median longevity.

Oviposition occurred sooner in this study than the previous study where both sexes were continuously exposed to treated surfaces. This may be due to the fact that the female moths were 24 h younger when introduced to males in the continuous study. Oriental fruit moths have been shown to have a longer pre-ovipositional period when introduced to a mate on their day of emergence than when introduced two or more days after emergence (Fraser and Trimble 2001).

The number of replicates with egg lay among females paired with treated males were equal, while females exposed to methoxyfenozide-treated surfaces had a lower number of replicates that produced eggs. This trend continued for replicates with egg hatch.

Previous studies have reported negative effects of the Latron surfactant on the percent fertility of codling moth (Sun and Barrett 1999, Knight, 2000). At no point in these studies did Latron have a negative impact on fecundity, percent fertility, or median longevity of OFM. All effects were due to the chemical nature of the ecdysone agonists modifying the males' ability to transfer viable sperm, and/or the females' ability to develop mature, viable eggs. These results, in combination with the results from the wind tunnel assays, show promising possibilities for the use of methoxyfenozide, and possibly tebufenozide, in controlling OFM populations more effectively than by simply targeting only the 1st instar larval stage. Data such as these need to be taken into account when developing more effective spray schedules for control of OFM.

C. Feeding Comparison Assay

Identification of a pest presence as well as the pest species are critical components of an effective control program. Fruit packers must be able to identify pest damage in order to cull damaged fruit. Importers and exporters must have positive identification of infestation species due to quarantine issues (Barcenas et al. 2005). It is extremely difficult to separate the larvae of codling moth, *Cydia pomonella* (L.) (CM), and oriental fruit moth, *Grapholita molesta*

(Busck) (OFM), with the naked eye. Even with the aid of 10-20x magnification, early larval instars are difficult, if not impossible, to separate. Thus it has been important to develop another method of identifying and separating the two species.

One method of damage identification was proposed by Schatzki et al.(1997) when they attempted to use X-ray imaging to detect apples infested with CM. The results were inconclusive, but showed promise. Barcenas et al. (2005) showed that DNA diagnostics could be developed to identify species of internal apple feeding Tortricidae for use in pest quarantine and stored product conditions.

This study examined and characterized the visual feeding damage produced by CM and OFM and demonstrated the visual characteristics of damage between CM and OFM. The percentage of CM that produced visual tunneling damage (shallow sub-surface feeding that produces a discoloration of the skin) was much higher than OFM early in the study (Table 11). There was little development of tunneling damage throughout the study for CM, while OFM damage increased significantly. This could be due to a couple of reasons. First, OFM have a shorter development time, with several instances of mature larvae leaving the apple in search of a pupating site before the end of the two week study period. Second, OFM entered the apple much more often in the stem and calyx ends than CM. Due to the structure of the apple, damage at the stem or calyx is much more difficult to see. Glen and Clark (1985) observed that damage on the sides of apples produced by 1st instar CM was visible, but damage

produced by CM in the calyx of the apples was not observable until larvae reached 3rd instar. The same results were observed for the presence of frass.

Jackson (1982) reported that CM, when placed directly on an apple, take approximately 17 min to bite the apples, and 45 min to completely enter the apples. This leaves ample time for the larvae to traverse to any desirable portion of the fruit. The majority of OFM entered the apples via the stem or calyx. Thus the majority of frass observed for OFM was also in these zones (1 and 6) (Table 13, Figure 1). Frass was most often observed on Zones 2 and 3 of CM replicates. This difference could be due to the smaller size of OFM and its propensity for the flesh at the core of the apple. It has been suggested that the initial bite into the fruit takes considerable energy. Thus, larvae may attempt to gain easier access to the flesh of the fruit through the calyx, or at cuts, stings, protuberances, lesions, or other irregularities of the fruit surface (Putman 1963). Both species showed an affinity for the upper half of the apple over the lower half. This, however, was likely an artifact of the experimental procedure. All larvae were placed in Zone 2 (upper third) of each apple. This was done because several publications had reported the majority of CM and OFM eggs are laid on leaves and stems within easy access to fruit instead of fruit itself (Jackson 1979, Yokoyama and Miller 1988). We tried to place the larvae on the apple close to where they would typically first come into contact with the fruit.

The mean volume of frass produced also showed a trend. While not significant early in the study, CM frass volume was consistently higher than that of OFM (Table 15). The trend reversed for the last half of the study, with OFM

frass volume higher than that of CM, significantly so by Day 14. This was due to the increase in exit holes and presence of frass associated with mature larvae creating exit holes and clearing a path out of the apple to pupate.

The internal damage observed at the completion of the study also showed marked differences between the two species. CM showed a much higher affinity toward the seeds as confirmed by Beers et al. (1993), as well as the peripheral flesh (Table 16). OFM had an attraction towards the flesh associated with the core of the apple, while ignoring the seeds.

All observed characters showed distinct separation of the two species. The use of frass volume and percent presence as well as placement and percent presence of tunneling damage are all possible variables in field determinations of which species is present. They are, however, dependent on life stage. This would mean the amount of time post-egg hatch would be required to accurately utilize these parameters. The differences in internal damage were among the most distinct of the study. The apparent differences in the external and internal damage preferences of CM and OFM in this study may play an important role in the development of a field-based procedure to accurately separate CM and OFM damage.

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