THE SUBLETHAL EFFECTS OF METHOXYFENOZIDE ON THE FIELD ORIENTATION AND COURTSHIP BEHAVIOR OF *CYDIA POMONELLA* (LINNAEUS) (LEPIDOPTERA: TORTRICIDAE)

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

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CHAPTER I. LITERATURE REVIEW

A. Insect Growth Regulators

Insect growth regulators (IGRs) are essentially the insect hormones that govern an insect's morphogenesis. Studies in insect hormones began as early as 1922 and, by 1936, it was confirmed that substances produced from the brain regulated insect metamorphosis (Vinson and Plapp 1974). Three main hormones regulated insect growth: brain hormone, molting hormone, and juvenile hormone. The proportion of these hormones to each other is a finely orchestrated system that regulates insect molting and development.

When the insect reaches the limits of its exoskeleton, the brain hormone is released, triggering an increase in the titer of steroidal 20-hydroxyecdysone that initiates insect molting (Vinson and Plapp 1974). Molting begins with a process known as apolysis, the separation of the epidermal cells from the old cuticle. The epidermal cells fill the resulting space with molting fluid. Simultaneously, the cuticulin layer is secreted to act as a protective barrier against the molting fluid and eventually becomes the epicuticle of the new exoskeleton (Dhadialla et al. 1998).

In immature insects, the sesquiterpenoid juvenile hormone (JH) is released along with the molting hormone to prevent the development of adult tissue (Dhadialla et al. 1998). In order to undergo a normal pupal molt there must be an absence of JH and a steady increase of ecdysteroid. The only time JH is present in the adult stage is during egg development (Vinson and Plapp 1974). Once the

new cuticular layers have formed, hormone levels decrease and the molting fluid absorbs the endocuticle of the old exoskeleton (Dhadialla et al. 1998). The epidermal cells recycle the digested cuticular layer to begin formation of the new procuticle. As ecdysone levels taper off ecdysis begins. This is a process wherein the insect will begin muscle contractions in order to shed its old exoskeleton.

As illustrated above, insect molting is finely regulated by neurohormones. Any outside influence that alters the natural balance of these hormones or disrupts the synthesis and absorption of the cuticle is detrimental to insect development. Williams was the first to suggest that compounds imitating these hormones could be used as insecticides (Williams 1967a). Hormonal insecticides are promising in that they adhere to the fundamentals of integrated pest management (IPM) principles; low toxicity to beneficial species, highly selective, and few deleterious effects on the environment. Disrupting this molting process was first explored when JH was extracted from the abdomen of *Hyalophora cercopia* (Cherbas) and applied to pupae. This resulted in a pupal molt that produced a deformed insect incapable of survival (Williams 1967a). Although prior to this study many other compounds with JH activity had been identified (Schmialek 1961, Bowers et al. 1965, Bowers et al. 1966), it was not until William's suggestion that the industry began to implement mass research and synthesis of hormone-based insecticides for commercial use (Dhadialla et al. 1998). Currently, there are three basic categories of IGRs differentiated by mode of action: juvenile hormones and their analogs, chitin synthesis inhibitors, and ecydsone agonists.

These IGRs have many great attributes, but as is the case with any insect control, they do have limitations to take into consideration. Certain predators and parasites are negatively affected by either direct exposure or exposure to treated hosts (Zungoli et al. 1983, Reede et al. 1984, Rumpf et al. 1998, Schneider et al. 2003). Although it was originally thought that insects would be unable to develop resistance to insecticides mimicking their own natural hormone, it has become apparent that resistance management must be implemented. There is already evidence of insect resistance and reports of cross-resistance between organophosphates and IGRs (Vinson and Plapp 1974, Moffitt et al. 1988, Perng et al. 1988, Kobayashi et al. 1992, Horowitz and Ishaaya 1994, Sauphanor and Bouvier 1995, Biddinger et al. 1996).

IGRs are slow in nature. The insect can continue feeding after exposure, which can be a problem if crop aesthetics directly affect marketability. Not only are IGRs slow-acting but timing of field applications is critical. In order to maximize their insecticidal potential, the grower must apply them at very specific moments in the insect life cycle. Although there are sublethal effects on both adults and eggs, the most sensitive stages occur in the larval and pupal stages. For example, juvenile hormones and their analogs are best applied late in the last larval instar and the first few days of the pupal stage (Staal 1975). Applications too early in the larval instar can result in supernumerary larval instars that, although may not become adults, can cause an increase in feeding damage. Pupal stages are just as, if not more, sensitive than larval stages but only if applications occur during the first few days of pupal development (Staal 1975). Treatments

made later may require higher doses to have an impact and may still result in a normal pupal molt (Staal 1975).

1. Sublethal Effects of Insect Growth Regulators

IGR's, as illustrated previously, have many attributes that show promise in their potential as addition to many IPM programs. However to have maximum lethality, these compounds must be applied at very specific stages of an insect's development. In order to affect the greatest number of a pest population, a grower must be vigilant in monitoring pest populations and be exact in the timing of applications. Thus the practicality and efficiency of using IGRs in the field can be compromised.

IGR's are designed to alter or hinder the regularity of both the insect's endocrine and nervous system. Therefore, many of these compounds have the potential not only to kill the insect, but also to result in a myriad of physiological and behavioral problems in the insect. These 'problems' are referred to as sublethal effects, effects that do not result in fatality. For example, doses that may not kill a pest but slow feeding would be considered sublethal. If IGRs do result in sublethal effects that effectively minimize pest damage then a grower would not be restricted to making applications in such a small frame of time. Sublethal doses can impact a wide range of an insect's life; for example, mating, courtship, feeding, male and female reproductive systems, oviposition and overall fertility.

2. Chitin Synthesis Inhibitors

Chitin is an amino-sugar polysaccharide, an extremely abundant biopolymer, (Cohen 1987) and is an integral part of an insect's exoskeleton providing protection, support, and structure. Flexible microfibers of chitin are embedded in a matrix of proteins that give the insect exoskeleton its strength and rigidity. Inhibiting chitin synthesis in insects will result in a myriad of problems: inferior exoskeletons, molting problems, loss of fluids and death due to dehydration (Verloop and Ferrell 1977).

Chitin synthesis inhibitors (CSI) belong to a group called benzoylphenyl ureas that were discovered in 1970 to have insecticidal activity on chitin deposition by Philips-Duphar B.V. Company (Weiland et al. 2002). Since then insecticidal benzoylaryl ureas have reached commercial pest status (Cohen 1992). The first CSI released for the market was benzoylphenylurea and diflubenzuron. This CSI was a potent larvicide against *Cydia pomonella* (Miyamoto et al. 1993, Tunaz and Uygun 2004). CSI's are IGRs that act primarily at sites of chitin synthesis in epidermal cells (Cohen 1987) interfering with chitin biosynthesis to prevent molting or to produce a malformed cuticle (Mulder and Gijswijt 1973). In addition, CSIs affect the development by negatively impacting egg hatch and causing hormone imbalances resulting in physiological disturbances such as inhibiting DNA synthesis and altering carbohydrases (Verloop and Ferrell 1977, Retnakaran et al. 1985, Mondal and Parween 2000).

Like other IGRs, CSI's are highly specific and have been regarded as having no impact on beneficial species. However, there have been recent reports to the contrary, showing negative impacts on beneficial and non-target species such as *Diadegma semiclausum* (Helen), *Cotesi plutella* (Kurdjumov), *Chrysoperla curnea* (Stephens), *Bemisia argentifoli* (Bellows & Perring), and *Bombus terrestris* (Linnaeus) (Haseeb et al. 2000, Haseeb and Amano 2002, Liu and Stansley 2004, Medina et al. 2002, Machado et al. 2005, Mommaerts et al. 2006, Schneider et al. 2003). There is also evidence of cross-resistance between benzoylureas and benzoylhydrazines (Sauphanor and Bouvier 1995).

i. Sublethal Effects of Chitin Synthesis Inhibitors

As discussed in the previous section, chitin synthesis is vital to the development and survival of all insects. Exposure to CSI's can be lethal. However, if death does not occur, exposure will often result in a multitude of physiological problems. A majority of the sublethal effects induced by CSI impact overall reproduction at various stages, deform bodies and alter development time.

Three different CSI products were found to affect the hatchability of eggs in the pest termite *Coptotermes formosanus* (Shiraki) and long-term exposure caused mortality of adult reproductives (Rojas and Ramos 2004). In the *Blatella germanica* (Linnaeus), diflubenzuron in combination with other IGRs, induced sterility and noviflumuron caused a significant amount of ovicidal effects (Ross and Cochran 1991, King 2005). Baits laced with diflubenzuron or teflubenzuron

resulted in molting failures and adults with twisted wings in the desert locus, *Schistocerca gregaria* (Forskal) (Wakgari 1997). Treated *Drosophila melanogaster* (Linnaeus) developed deformed wings due to improper cuticle synthesis in addition to suppressed oogenesis and eggs that did not hatch (Wilson and Cryan 1998). Certain species of Lepidoptera displayed reduced fertility and fecundity (Carpenter and Chandler 1994, Perveen 2000). *Cydia pomonella* (Linnaeus) larvae suffered delayed ecdysis when treated with difulbenzuron (Soltani and Soltani-Mazouni 1992). Exposed *Spodoptera litura* (Fabricius) larvae had reduced weight in comparison to non-treated and also displayed reduced egg hatchability (Perveen 2000). Novaluron has been suggested as a valuable tool in controlling *Leptinotarsa decemlineata* (Say) populations due to efficient activity against second-instar larvae and significant reduction in egg production and hatchability (Cutler et al. 2005). *Anthonomus grandis* (Boheman) saw reduction in cuticle hardness, fertility, and flight activity (Haynes and Smith 1994, Villavaso et al. 1995).

It must be taken into consideration, that these sublethal affects can also impact beneficial species and nontarget species. For example, diflubenzuronis is harmful to *Chrysoperla carnea* (Stephens) (Medina et al. 2002) and results in breakable shells in freshwater clams (Machado et al. 2005). Additionally, a beneficial *Delphastus catalinae* (Horn) suffered reduced egg fertility and longevity (Liu and Stansly 2004). Exposure and ingestion to benzoylureas was also toxic to beneficial parasitoids, often adversely affecting parasitism by

increasing development time to the adult stage and high active to other life stages (Haseeb et al. 2000, Liu and Chen 2000, Haseeb and Amano 2002).

3. Juvenile Hormone Analogs

JH is an insect hormone secreted by the corpora allata that regulates the development of insect larvae. In the final stages of the pupal development, there must be an absence of JH in the presence of 20E in order for an insect to undergo transformation to adult. Persistence of JH at this final molt would be extremely disruptive to the insect's development. Wigglesworth was the first to suggest the existence of this hormone (Wigglesworth 1936, Dhadialla et al. 1998). By 1967, it was hypothesized that this hormone could be exploited to disrupt the insect's life cycle (Williams 1967a).

Schmialek shed light on the chemical nature of JH by discovering JH activity as farnesol derivatives in the fecal matter of *Tenebrio molitor* (Linnaeus) (Schmialek 1961, Staal 1975, Wilson 2004). The chemical structure of the first insect JH, referred to as JH I, was elucidated by 1967 (Vinson and Plapp 1974, Staal 1975.) Although what was to become known as JH III was synthesized as early as 1965 (Bowers 1965), it was not until after the elucidation of JH I that both JH II and JH III were identified (Staal 1975). Following the identification of JH 1, mass innovation in the research and synthesis of chemical analogs began.

Although these early creations had chemical structures greatly similar to the natural JH, they were extremely limited in their use due to their lack of stability outside and slow toxic action (Dhadialla et al. 1998). Of all the first

analogs created, methoprene was the most active and commercially successful (Dhadialla et al. 1998, Wilson 2004). Agrochemistry companies developed aromatic non-terpenoidal compounds with JH modes of action (Wilson 2004). These proved to be more suitable for agriculture use, exhibiting greater metabolic and environmental stability (Dhadialla et al. 1998, Wilson 2004). The majority of JH analogs used currently do not have the same chemical structure as the natural JH; however, they are much more effective and still have JH-like effects on insects (Wilson 2004).

The main goal of juvenoids is to disrupt, rather than regulate, insect growth. When applied to an immature insect, JH analogs bind with JH-interacting proteins, affecting gene transcription (Wilson 2004). This results in not only a change in gene products, but also a disruption in the natural development and physiological changes that a maturing insect undergoes. These compounds have been found to act as both JH mimics and antagonists. Exposure to these JH analogs (depending on both dose and timing) will induce an extra, typically lethal, larva-to-larva molt while antagonists inhibit JH, inducing a premature pupa-to-adult molt that often produces sterile adults (Dhadialla et al. 1998). Juvenoids can also cause pupal mortality and mortality in pharate adults (Staal 1973, Dhadialla et al. 1998, Wilson 2004).

Juvenoids offer benefits such as sparing non-target organisms, breaking down rapidly in the environment, and posing little threat to consumer health. However, there is evidence of methoprene impacting vertebrate endocrine responses (Harmon et al. 1995). Despite the low toxicity to non-target species,

juvenile hormones have been found to be toxic to fish and highly toxic to estuarine invertebrates (Glare and O'Callaghan 1999). Although resistance to IGRs is still relatively low-level, there have been reports of cross-resistance to juvenoids in certain insecticide resistant strains of *Tribolium castaneum* (Herbst), *Bemisia tabaci* (Gennadius), and *Musca domestica* (Linnaeus) (Brown and Brown 1974, Cerf and Georghiou 1972, Dyte 1972, Horowitz et al. 1999, Rupes et al. 1976). As is the case with any insecticidal control, resistance is virtually inevitable; therefore, it is key to implement resistance management while reports of resistance are still relatively low-level.

i. Sublethal Effects of Juvenoids

Juvenile hormone analogs exploit the hormone-regulated development of insects resulting in supernumerary and premature molts that are often lethal. Fortunately, there are also a multitude of sublethal affects ranging from behavior to morphology. A common sublethal affect is a significant reduction in fecundity and fertility seen in a variety of insect species such as *Solenopsis invicta* (Buren), *Blatta orientalis* (Linnaeus), *Nilaparvata lugens* (Staal), *Lipaphis erysimi* (Brassica), *Platynota idaeusalis* (Walker), and *Drosophila melanogaster* (Wilson et al. 1983, Glancey and Banks 1988, Evans et al. 1995, Ayoade et al. 1996, Biddinger and Hull 1999, Liu and Chen 2001). In addition, juvenoids have been reported to impact insect egg hatch, reproduction, behavior, and adult diapause (Staal 1973, Dhadialla et al. 1998, Wilson 2004).

Juvenoids help suppress pest populations by deleterious affects on reproduction and sexual behavior. For example, the *Anastrepha suspense* (Loew) exhibited earlier sexual signaling, pheromone release and mating when treated with both methoprene and fenoxycarb (Teal et al. 2000). In the male moth *Agrotis ipsilon* (Hufnagel), fluvastatin disrupted normal spermatophore delivery (Duportets et al. 1998). *Cydia pomonella* exposed as pupae saw a reduction in oviposition rates and number of oocytes (Webb et al. 1999). Precocene also reduced mating for the migratory locust and the *Ceratitis capitata* (Wiedemann) (Chang et al. 1984, Shalom and Pener 1986).

Insect growth and development was also significantly impacted due to sublethal doses of juvenoids. Fenoxycarb increased the development time of female *Platynota idaeusalis* (Biddinger and Hull 1999). Exposed *Aphis gossypii* (Glover) had altered population structures and delayed adult emergence resulting in a slower population growth (Satoh et al. 1995). When pyriproxyfen was applied to *Hyadaphis erysimi* (Kaltenbach), the first three nymphal instar had extra molts, increased longevity, and occasionally sterility (Liu and Chen 2001). When treated with pyriproxyfen in the third instar, *Chyrsoperla rufilabris* (Burmeister), a natural enemy, and *Bemisia argentifolii* (Bellows & Perring), a whitefly predator, had a much longer development time to the adult stage (Liu and Stansly 1997, Chen and Liu 2002). Adult emergence can also be greatly suppressed as seen in both *Bemisia tabaci* (Gennadius) and an endoparasite of the whitefly, *Encarsia formosa* (Gahan) (Ishaaya and Horowitz 1992, Liu and Stansly 1997). Crustaceans also are impacted, as seen in the mud crab *Rhithropanopeus harrisii*

(Gould), that saw substantial change in growth of developing larvae when exposed to fenoxycarb (Nates and McKenney 2000).

The *Heliothis virescens* (Fabricius) did not produce viable larva when treated with fenoxycarb (Masner et al. 1987). Pyriproxyfen has ovicidal properties and can reduce egg hatch as evident in *Grapholita molesta* (Busck), *Cydia pomonella*, *Bemisia tabaci* (Gennadius), and the *Chrysoperla rufilabris* (Burmeister) (Yokoyama and Miller 1991, Ishaaya and Horowitz 1992, Chen and Liu 2002). Juvenile hormone analogs, when applied topically, both sexes of *Ips confuses* (LeConte) had highly significant muscle volume decline (Borden and Slater 2004).

4. Ecdysteroids

Ecdysteroids are steroid hormones essential in arthropod molting, metamorphosis, reproduction, and diapause. The first ecdysteroid was isolated in 1954, its structure elucidated in 1965 (Dinan and Lafont 2006). Ecdysteroids can be found in both plants and animals, the most abundant being 20-hydroxyecdysone (20E). 20E is the molting hormone that binds to ecdysteroid receptors to initiate molting in insect larvae.

Ecdysteroids, such as 20E, interact mostly with the ecdysteroid receptor and the ultraspiracle (Dhadialla et al. 1998). Although ecdysone agonists are not structurally similar to 20E, they bind to these same receptors. These compounds then initiate the expression of a group of molt-related genes (Retnakaran et al. 2003). The insect will cease feeding within 4 to 16 hours as the insect prepares to

undergo apolysis (Dhadialla et al. 1998). By 24 hours, the insect will begin to resemble pharate larvae while the head capsule starts to slip in an attempt to ecdyse (Dhadialla et al. 1998, Retnakaran et al. 2003).

The ecdysone agonists will remain permanently bound to the receptors, preventing the release of the eclosion hormone (Dhadialla et al. 1998). The lethality of these compounds on insects is threefold: ecdysis cannot be performed, sclerotization of head capsule and mouthparts cannot take place, and the hindgut is extruded leading to desiccation and death. The overall result is a premature lethal molt. This affect is dependant on both dosage and time of application. Due to the fact that these benzoylhydrazines act directly through an insect receptor complex, they offer insect-specific control with little impact on non-target species (Dhadialla et al. 1998, Retnakaran et al. 2003).

Rohm and Haas Company discovered the first bisacylhydrazine ecdysteroid agonist in 1983 (Dhadialla et al. 1998). Through modification of this early analog came RH-5849, the first bisacylhydrazine to be thoroughly tested in the field. RH-5849 is active against Lepidoptera, Coleoptera, and Diptera pests (Aller and Ramsay 1988, Dhadialla et al 1998). In 1992, Rohm and Haas synthesized tebufenozide, RH-5992, an analog with a similar structure to RH-5849 (Smagghe and Degheele 1994, Dhadialla et al. 1998).

In comparison to RH-5849, tebufenozide has a greater level of selectivity and toxicity towards caterpillars. These attributes make tebufenozide an excellent candidate as a control agent in IPM programs (Smagghe and Degheele 1994, Rodriguez et al. 2001, Trimble and Appleby 2001). Tebufenozide was the first

commercially viable analog. It is sold under the brand names of Mimic®, Romdan®, and Confirm® (Dhadialla et al. 1998). However, there is ample evidence of growing cross-resistance between tebufenozide and standard insecticides such as azinphosmethyl (Sauphanor and Bouvier 1995, Dhadialla et al. 1998, Wearing 1999, Waldstein et al. 1999, Retnakaran et al. 2000, Moulton et al. 2002, Smirle et al. 2002) and low acute toxicity towards certain aquatic organisms (Kreutwesier et al. 1994).

i. Methoxyfenozide

Rohm and Haas Company produced methoxyfenozide, RH-2485, in 1996 (Dhadialla et al. 1996). Like tebufenozide, methoxyfenozide has a high level of specificity and toxicity against a wide range of caterpillars. Unlike earlier analogs, methoxyfenozide exhibits moderate root systemic activity, widening the range of pest species impacted (Carlson et al. 2001). Methoxyfenozide has a higher field efficacy as well, requiring half the rates of application in comparison to tebufenozide (Carlson et al. 2001). As is the case with tebufenozide, resistance management must be implemented in regards to methoxyfenozide due to evidence of possible cross-resistance with standard insecticides (Sauphanor and Bouvier 1995, Dhadialla et al. 1998, Waldstein et al. 1999, Wearing 1999, Retnakaran et al. 2000, Moulton et al. 2002, Smirle et al. 2002, Gore and Adamczyk 2004). Methoxyfenozide is sold under the brand names of Runner®, Intrepid®, and Prodigy® (Carlson et al. 2001).

Methoxyfenozide has been considered a good candidate for IPM programs

because it is has a relatively low level of toxicity towards non-target insects. However there are studies that must be taken into consideration indicating that methoxyfenozide affects natural enemies and nontarget species on a higher level than previously thought. Studies show that methoxyfenozide disrupts natural predation by interrupting the growth of natural parasites (Cordero et al. 2006, Dong-Soon et al. 2006). It is also imperative that more research be done to determine the exact effect on aquatic invertebrates.

ii. Sublethal Effects of Ecdysteroids

Ecdysteroid agonists were designed with the intention of inducing a premature lethal molt. However, it was found that insects surviving to the adult stage still suffered deleterious effects. A frequent side effect to sublethal doses is a significant reduction in fertility and fecundity. Ecdysteroids are not only beneficial in their lethality but in their ability to suppress the overall population growth through a multitude of sublethal effects.

RH-5849 reduced the amount of sperm delivered from male to female in the cluster caterpillar, *Spodoptera litura*, (Fabricius) and negatively impacted mating success (Seth et al. 2004). Ultimately *S. litura* saw significant reduction in fertility and fecundity (Seth et al. 2004). The following lepidopteran pest also saw reduction in fertility and fecundity when exposed to tebufenozide and/or methoxyfenozide: *Spodoptera exigua* (Hubner), *S. littoralis* (Boisduval), *Lobesia botrana* (Denis & Schiffermuller), *Cydia pomonella*, *Argyrotaenia velutinana* (Walker), *Choristoneura rosaceana* (Harris), *Platynota idaeusalis* (Walker)

(Smagghe and Deghele 1994, Sun and Barrett 1999, Sun et al. 2000, Irigaray et al. 2005, Pidea et al. 2007).

Grapholita molesta showed reduction in mate-finding capability and a reduced mean fecundity (Reinke and Barrett 2007). *Cydia pomonella* males exposed to methoxyfenozide had a significantly lowered sexual excitability and were less responsive to calling females (Hoelscher and Barrett 2003). It must be taken in to consideration that these sublethal effects on not limited strictly to pest species. For example, sublethal doses of methoxyfenozide on the general predator, *Deraeocoris brevis* (Knight), showed a slower development in the 4th instar and a 30 percent reduction in fecundity (Dong-Soon et al. 2006).

There are also a range of other sublethal effects negatively impacting insect physiology and behavior. *Spodoptera litura* when exposed to RH-5849 saw a decrease in adult longevity and mating success (Seth et al. 2004). Treatments of tebufenozide for both *Spodoptera exigua* and *S. littoralis* resulted in a reduction in weight gain (Smagghe and Deghelle 1994, Pidea et al. 2007). *Choristoneura fumiferana* (Clemens) and *C. rosaceana* also saw a decrease in weight in addition to: an increase in development time, delay in ovarian maturation, reductions in sperm production, mating success, and males ability to orient to females upwind (Dallaire et al. 2004).

Argyrotaenia velutinana (Walker) and *Choristoneura rosaceana* saw a significant reduction in the mean number of eggs laid and hatched caused by methoxyfenozide and tebufenozide (Sun et al. 2000). In response to tebufenozide, *Platynota idaeusalis* had lowered pupal weights, an increase in deformed adults,

and an overall significant effect on population dynamics (Biddinger et al. 2006). Treating the larvae of the southwest corn borer, *Diatraea grandiosella* (Dyar), retarded larval growth, decreased pupal weight and reduced adult emergence (Trisyono and Chippendale 1998).

B. Experimental Organism

1. Cydia pomonella Biology

Cydia pomonella belongs to a large family of microlepidoptera, known as Tortricidae, that contains a number of economically important pest species in both agriculture and forestry. *Cydia pomonella* originated from Eurasia and the first historical reference, made by Theophrastus, dates back to 371 BC (Tadic 1963). In 1635, Jean Gboedaerdt published the first full description of *Cydia pomonella* (Butt 1975).

The moth was introduced to America in the mid 1750's as European colonists began establishing apple orchards in New England (Johansen 1957). *Cydia pomonella* can now be found in virtually every apple-growing region in the world. A *Cydia pomonella* female can produce up to 30 to 50 eggs in her lifetime (Geest and Evenhuis 1991, Pedigo and Rice 2006). The eggs are deposited individually on branches and leaves near fruit or on the fruit itself. The eggs are white, pancake-shaped with a diameter of 1/25 inch and prior to hatching develop a dark dot in the center (Geest and Evenhuis 1991). Depending on temperature and to some extent on rainfall, it can take eggs 5 to 20 days to hatch (Geest and Evenhuis 1991; Pedigo and Rice 2006).

The larvae are about 0.75 inches long, pale pink with dark sclerotized heads (Geest and Evenhuis 1991). Once hatched, larvae may feed on leaves but within a few hours will hone in on the apple volatiles and make their way to the fruit (Geest and Evenhuis 1991, Landolt et al. 1998, Yang et al. 2004,). The larvae will then begin feeding on the apple, usually beginning at the calyx of the fruit (Geest and Evenhuis 1991). This larval feeding can cause two types of wounds, deep tunneling entries and stings. A sting is a shallow wound on the skin of the apple caused by larvae that are interrupted in their feeding. A deep tunnel entry is caused when larvae are successful in burrowing a tunnel into the fruit interior, usually consisting of both an entry and exit wound.

As the larvae tunnels its way to the apple core it will fill the hole behind it with frass. Once at the core, the larvae may also feed on the apple seeds. The larvae will complete several instars inside the fruit, spending up to 3 or 5 weeks inside the apple (Geest and Evenhuis 1991). Once the larvae is full-grown, it will tunnel its way out of the fruit, seeking shelter in which to pupate. The larvae will construct a silken cocoon and then pupate beneath loose tree bark, in debris beneath the tree, or in packing slates (Geest and Evenhuis 1991, Beers et al. 2003). Pupae are dark brown with an average length of 10mm (Pedigo and Rice 2006).

Recent studies have shown that in spinning their cocoons, the larvae create a cocoon-derived mix of volatiles that help guide other larvae to the pupation site (Jumean et al. 2005). This aggregation also aids in ensuring that the female will mate immediately after eclosion due to a sex pheromone emitted from the female

pupae that arrest the development of males so as to ensure synchronous timing in adult emergence (Duthie et al. 2003). Depending on the climate, it can take anywhere from 14 to 30 days for the adult to emerge (Pedigo and Rice 2006).

Like most tortricids, *Cydia pomonella* adults are small in size, 0.5-0.75 inch wingspan, and have blunt square-tipped forewings (Beers et al. 2003). The *Cydia pomonella* forewings are mottled grey with a coppery dark band at the tip, helping the moth to blend in with the bark as it rests during the day (Pedigo and Rice 2006). Adults then have roughly 14 to 21 days to live and mate (Pedigo and Rice 2006).

Typically in Missouri there are two generations; however, if conditions are favorable there can be a third partial to full generation in late summer. *Cydia pomonella* will overwinter as larvae in thick silken cocoons (Geest and Evenhuis 1991). At this time, although weather hardy, larvae can be killed by a drop in temperature to -25^{0}F (Geest and Evenhuis 1991). The over-wintering generation will often emerge shortly after petal fall in spring (Greene et al. 2003). This ensures that the larvae produced by this first generation in the spring, known as the first flight, will emerge at the same time the tree has developing fruit to feed on (Ferree and Warrington 2003).

2. Mating Behavior

A majority of adult activity takes place in the upper part of the tree canopy (Borden 1931). Generally, although there is some debate, most adult activity occurs 1 to 2 hours before and after sunset with some reports of activity at sunrise

(Castrovillo and Carde 1979, Knight et al. 1994). Adult activity is greatly controlled by temperature, preferring temperatures 13^{o}C to 27^{o}C, thus summers with high temperatures can result in a large highly destructive moth population (Castrovillo and Carde 1979).

Female calling and male response are stimulated by environmental cues such as lighting and temperature. Apple volatiles not only aid adults in host-finding but will stimulate female pheromone release and oviposition (Yan et al. 1999, Reed and Landolt 2002). The female will begin calling, releasing the sex pheromone, with her wings and abdomen raised. The male moth will then cue in on the pheromone, flying towards the vicinity of the female. Once in close proximity, the male will land and approach the female while fanning his wings. Once the male antenna is in contact with the female, he will immediately bend his abdomen towards the female while opening and closing his claspers. He will then orient his posterior to that of the female genitalia so that they will be end-to-end as they mate (Castrovillo and Carde 1980).

Depending on both the success of the male to clasp the female and the willingness of the female, this mating process can be repeated numerous times. Once the male successfully claps on to the female genitalia, all wing fanning stops and lateral abdominal bending ceases. The moths will mate end to end, laying with each other in a linear fashion. After females are mated they no longer attract males (Howell et al. 1978) and may emit compounds that repel males (Rothschild 1982). Once mated, the female will then, as mentioned above, cue in on apple volatiles to lay her eggs near the fruit (Yan et al. 1999).

3. Mating Disruption as a Control Tactic

Communication between male and female moths is generally achieved by the emission of sex pheromones. Sex pheromones enable long-distance mate location and can also work as close-up chemical stimulation facilitating courtship (Carde et al. 2005). It is often the female who 'calls' the male by releasing her pheromone. The male will then cue in on her pheromone plume, flying upwind until he locates the females. Once in proximity of the female, courtship is aided by visual, tactile, and auditory cues (Baker and Carde 1979, Castrovillo and Carde 1980, and Howell et al. 1978).

In the early 1960's, many begin to hypothesize that if continuously high levels of pheromone could be maintained in the environment that mate location could be suppressed, thereby, disrupting mating of certain pest species (Beroza 1960, Babson 1963, Wright 1964). In 1967, the main component of *Trichoplusia ni* (Hubner) pheromone was evaporated into an area to determine the impact it would have on the sexual orientation. The experiment was the first confirmation that continuous presence of pheromone interrupted the males' ability to locate females (Gaston et al. 1967). In 1974, hexalure, a synthetic attractant, was continuously evaporated into cotton fields throughout the cotton production season (Shorey 1973). The permeation of pheromone in the environment disrupted the sexual communication to such an extent that *Helicoverpa zea* population suffered reductions comparable to those caused by conventional insecticides (Shorey 1973). These early studies proved the feasibility of mating disruption for population management and since then this technique has been

found effective against a multitude of pest species.

Bartell (1982) provided a review describing the proposed mechanisms behind mating disruption that was later updated by Carde and Minks (1995). The continuous presence of pheromone will cause a lack of response or a higher response threshold within the male nervous system. This is achieved either by habituation of central processing or adaptation of the antennae's peripheral receptors (Carde and Minks 1995). The strength of synthetic pheromones renders the male unable to discern the weaker pheromone plume of the female, essentially camouflage calling females.

Males may also respond to the man-made pheromone well before females began calling, resulting in a diminished male attraction to virgin females (Carde and Minks 1995). Interference plays a part as well since the longer a male orients to artificial point sources the less time it will have to orient to calling females. Another factor is the influence that plume structure has on moth flight patterns. It is possible that the synthetic pheromone could disarrange natural plume patterns in such a manner as to alter the male ability to locate a mate (Carde and Minks 1995). In addition certain species, such as the oriental fruit moth and the *Keiferia lycopersicella* (Walshingham), are not attracted to areas with high pheromone concentration and will actually stop normal mating behavior (Carde and Minks 1995). It may be safe to assume that in these species mating disruption would also elicit a similar behavioral response.

The efficacy of this method has been demonstrated repeatedly. The pheromone for the pink bollworm, *Pectinophora gossypiella* (Saunders), was the

first pheromone product provided for an agriculture pest (Carde and Minks 1995). In California and Arizona, an area wide program targeting the pink bollworm was a success, demonstrating an improved yield and significantly less crop damage in comparison to field treated conventionally (Carde and Minks 1995). Another management program using mating disruption against the *Pectinophora gossypiella* was incredibly successful, showing an initial 23 percent larval infestation to no larvae in the sample area three years later (Carde and Minks 1995). South Africa, suffering from severe infestations of *Grapholita molesta*, reported no fruit infestation after initiation of a year long mating disruption program (Carde and Minks 1995).

An IPM program for the Mexican tomato market implemented mating disruptive techniques and found that the IPM programs were often more profitable, yielding a much higher net profit in comparison to a conventional approach (Trumble and Alvarado-Rodriquez 1993). No males of the pea moth, *Cydia nigricana* (Fabricius), were caught in pheromone traps in a disruptant-treated field, indicating that these moths actually avoid a pheromone-permeated field (Bengtsson et al. 1993). Mating disruption is also effective against the highly noxious *Lymantria dispar* (Linnaeus), resulting in a decreased male trap catch, mating success, and population density (Webb et al. 1990, Leonhardt et al. 1996).

Despite these successes, there are many constraints that must be taken into consideration in order to successfully implement a mating disruption program. Migration of mated females from areas outside the treated plots is a perhaps the

largest weakness in mating disruption programs. Area wide management would help to eliminate population fluctuations due to migration. Population density is another factor to consider when deciding when to employ mating disruption. In general, mating disruption has not been effective in orchards with high-density pest populations (Carde 1990, Pree et al. 1994, Barclay and Judd 1995, Friedrich and Schirra 2001). This is due to the fact that in high density populations, mates are much closer to each other, therefore, easier to find and mate tracking can be done via short-range cues such as touch or sound (Carde and Mink 1995).

One such consideration is finding the right pheromone formulation that provides the most effective and disruptive impact to pest mating behavior. There are many pheromone products on the market and variation in ingredients can effect how well the product works in the field and it's ability to disrupt mating (Carde and Minks 1995). Naturally, the pheromone formulation that most closely resembles the natural pheromone would be the most effective.

Another contingency to consider is that certain orchards deal with a variety of pest moths and it would be impractical to release specific pheromones for each individual species. It has been suggested that release of a formulation with common pheromone components that all targeted species share would be a more practical and effective approach (Minks and Carde 1988, Carde and Minks 1995). However, this approach has met with mixed results. European studies have had success in controlling the complex of leafrollers, reporting economically acceptable fruit damage (Carde and Minks 1995) whereas studies in Virginia and New York where unable to implement a blanket control over all pests involved

(Reissig et al. 1978, Pfeiffer et al. 1993, Carde and Minks 1995).

As illustrated above, the pest species involved and their behavior are naturally a huge consideration in considering what pheromone product to use, how to dispense the disruptant, and whether mating disruption is even a viable means of control. The efficacy of mating disruption programs can be diminished by species-specific behaviors such as the calling strategy of females or activity patterns of males. It is vital to have a thorough understanding of the ecology and overall behavior of the pest species when implementing mating disruption. There are rare conditions in which the male will also participate in mate recruitment by attracting the female (Willis and Birch 1982, Landolt and Heath 1990, Heath et al. 1992, Landolt 1995) This would essentially provide the moths a loophole, allowing the moths to overcome mating disruption that replicates only the female pheromone emissions.

Research into mating disruption as a viable means of controlling *Cydia pomonella* began as early as the 1970's (Carde and Minks 1995). Experiments were conducted around the world, and early reports offered promising results in regards to the control offered by pheromone use (Carde et al. 1977, Rothschild 1982, Moffitt and Westigard 1984, Carde and Minks 1995, Knight 1996, Gut and Brunner 1998). By the 1990s commercial products were available for use by growers in the United States (Brunner et al. 2002). A study conducted in small plots showed a reduction of fruit injury as much as 90 percent in comparison to untreated plots (Howell et al. 1992). Even in organic orchards, mating disruption was able to effectively suppress *Cydia pomonella* populations when used in

tandem with cultural control methods (Judd et al. 2004).

Although there have been many experiments exploring different pheromone dispensers under a variety of climates, there are constraints when using mating disruption that are virtually unanimous (Brunner et al. 2002, Carde and Minks 1995). Many studies have yielded good control in orchards with low *Cydia pomonella* populations, however, mating disruption is ineffective against high-density populations and is not recommended as a stand-alone tactic (Carde and Minks 1995). Another issue to consider is that the release of pheromone does not provide uniform control within an orchard block, as indicated by the much higher rate of fruit injuries in border rows compared to trees in the orchard interior (Brunner et al. 2002).

In order to achieve adequate control through mating disruption, it is recommended that this technique be used in highly isolated area with low *Cydia pomonella* populations. These conditions are rare in fruit-growing areas but despite these limitations the use of mating disruption has grown in popularity among fruit-growers. From 1990 to 2000, Washington state alone went from 0 hectares to 35,000 hectares using mating disruption (Brunner et al. 2002). Area-wide use of pheromone techniques could help alleviate some of the limitations inherent in mating disruption programs. In 1994, a *Cydia pomonella* Areawide Management Project (CAMP) was proposed with the main goals of implementing mating disruption over large areas in hopes of reducing broad-spectrum insecticides by 80 percent over five years and to enhance the control of secondary pests by preserving natural enemies in the area (Brunner et al. 2002).

For the most part CAMP sites saw a significant reduction in *Cydia pomonella,* so much so that they were able to reduce supplementary insecticidal applications as well as pheromone applications (Brunner et al. 2002). Although the projected goal of a 80 percent reduction in broad spectrum insecticides was not achieved, CAMP orchards were applying 75 percent fewer broad-spectrum insecticides in comparison to orchards not involved in CAMP (Brunner et al. 2002). Due to this reduction, there were no outbreaks of secondary pests and leafroller densities were kept at a manageable level. However, area-wide programs can be compromised if even one grower does not participate or uses bad control practices. For example, one grower who did not participate and also did not provide adequate control accounted for 80 percent of the damage seen by fruit growers in one CAMP site (Brunner et al. 2007).

CAMP has been very beneficial to the adoption of mating disruption for *Cydia pomonella* control by demonstrating and educating growers on the benefits of pheromone use. However there are still weaknesses that must be overcome. Mating disruption is still a relatively high cost control method for growers who at times see low profits from their product (Brunner et al. 2007). Mating disruption can be greatly enhance by proper monitoring of moth populations and utilizing more selective insecticides as supplemental controls preserving biological agents (Brunner et al. 2007). Therefore, it is pivotal to continue research on mating disruption and education for growers so as to constantly improve the benefits awarded by mating disruption for *Cydia pomonella* control.

C. Lepidoptera Behavioral Research

Manipulation of Lepidopteran pheromone communication as a form of pest control has shown great potential (Carde et al. 1977, Rothschild 1982, Moffitt and Westigard 1984, Carde and Minks 1995, Knight 1996, Gut and Brunner 1998). In making this form of pest control a reality, studies on insect mating behavior were essential. An early behavioral study on *Cydia pomonella* mating surmised that males are attracted to flying females and the visual stimuli of their wing fluttering (Borden 1931). However, later studies found that not only are males attracted to caged females, but they are also much more attracted to virgin rather than mated females(Proverbs 1965, Howell and Thorp 1972). The evidence of pheromone attraction was further supported when males exposed to extracts from the females attempted sex with other males and empty pupal cases (Butt and Hathaway 1966).

The sexual sequence of most moth species begins with the release of sex pheromones from female glands that interact with the antennal receptor of sexually responsive males (Foster and Harris 1997, Baker and Carde 1979, and Castrovillo and Carde 1980). For most species the sex pheromone is located in the intersegmental membrane of the eighth and ninth abdominal segments (Gotz 1951). At rest the female pheromone gland is often invaginated and when she is ready to mate she will extrude the glandular area, exposing the gland to the open air. Females often 'code' their pheromone plume by releasing their pheromone in varying pulses of high and low concentration. This is achieved either by varying wing beat frequencies or by alternately retracting and exposing the pheromone gland (Gotz 1951, Kettlewell 1960, Doane 1968). Once the males come into

contact with the female pheromone a sequence of behavior is elicited.

Prior to contact with sex pheromone the male moth is in the 'resting' position, defined as the moth holding wings roof-like over the body with the antennae held back alongside the wing (Castrovillo and Carde 1980). Once the sex pheromone comes into contact with the antennal receptor, the male arousal is indicated with the antenna coming forward while the wings are extended and vibrating (Baker and Carde 1979, Castrovillo and Carde 1980). Antennal receptors are pivotal in mating, males that had their antennae completely removed prevented the mating of moths held in close proximity to each other (Fluri et al. 1974). The excited wing flapping is thought to be a means of warming the thorax temperature to the required level for flight (Heath and Adams 1967). A bioassay on *Grapholita molesta* found that this wing fanning was highly correlated with the male ability to hone in on the pheromone source (Baker and Carde 1979). Once the male is ready for flight he will fly upwind, orientating on the pheromone plume to find the female.

How exactly the male follows the pheromone plume to orient toward the female has been a matter of some debate. Locating females via pheromone is a form of chemotaxis that requires the insect be able to detect chemical gradients. Essentially an insect using this type of taxis follows the direction of higher concentration to find the source. It has been found that in a number of species male moths are able to orient to pheromone sources solely by airborne pheromone molecules and the directions of air movement (Shorey et al. 1968). However, it may be extremely difficult for insects to detect the direction of the pheromone

gradients once it has drifted more than a few centimeters from the source (Dethier 1957, Wright 1958).

Determining exactly how male orient to pheromone has prompted many studies in the upwind orientation of moths to a pheromone source (Schwinck 1958, Traynier 1968, Willis and Baker 1994). A classic study using silkworm males found that males who encountered the pheromone would orient to the air stream, even after the female had been removed (Schwink 1958). It has since been hypothesized that the moths will actually orient to the direction of the air stream rather than the pheromone source (Schwinck 1958). A study conducted on the *Ephestia kuehniella* (Zeller), found that the male moths would instinctively perform the same turn when encountering a pheromone-laden airstream and another flight maneuver specific to encounters with less favorable airstreams (Traynier 1968, Willis and Baker 1994). Essentially the male moth is programmed to orient and maneuver its flight path in a specific way upon encountering areas of different pheromone concentrations that would eventually help the male locate the female.

Once the male is in close proximity to the female, upwind orientation to pheromone ceases and short-range orientation begins. Tactile and visual stimuli aid in short-range orientation, inducing courtship behavior in many male moths (Doane 1968, Traynier 1968). Visual stimulus at close range has been proven to play a part in *Cydia pomonella* mating (Hutt and White 1977). Moths orient to dead females in their immediate vicinity with a greater frequency than to the nearby pheromone sources (Castrovillo and Carde 1980). Although visual

stimulus is important it has been found to impact only the frequency and direction of mating attempts with females and not the perseverance in any certain direction (Castrovillo and Carde 1980).

Once in close proximity, sexual behavior leading to mating can vary and is often species-specific. For example, males of the Indian meal moth, *Plodia interpunctella* (Hobner), release a scent that causes a female to remain submissive while turning her abdomen as the male positions himself for mating (Grant and Brady 1975). Males of *Grapholita molesta* will not touch females upon approach but will extrude their hairpencils resulting in the female triggering male copulation behavior by touching his abdomen with her head (Baker and Carde 1979). *C. pomonella* mating is largely dependent on male behavior triggering female behavior (Castrovillo and Carde 1980). One initial releaser is needed to trigger the behavioral sequence in male *C. pomonella* (Castrovillo and Carde 1980). Typically the male moth touches the female abdomen and the glandular area with his antennae. Once in contact, male bend their abdomens to clasp onto the female. If not successful the male will repeat the behavioral sequence.

Any step in Lepidoptera courtship behavior can be manipulated by applying a variety of different stimuli. Studies have shown that behavioral responses based on chemical stimuli can either be inhibited or exaggerated by adding different compounds into the environment. Males of the redbanded leafroller, *Argyrotaenia velutinana* (Walker), when exposed to different components of the female pheromone, displayed increased wing fanning and orientation in flight (Baker et al. 1976). Exposure to insect growth regulators such as

methoxyfenozide and tebufenozide, reduced the attractiveness and responsiveness of males in both *C. pomonella* and oriental fruit moths (Hoelscher and Barrett 2003, Reinke and Barrett 2007). Trifluoromethyl ketones have also been found to disrupt the flight of male moths (Bau et al. 1999). Behavioral studies such as these have greatly enhanced our ability to manipulate the pheromone-mediated communication of moths and expand our fundamental grasp of their sexual behavior.

D. Research Objectives

In order to determine the extent to which the sublethal effects of methoxyfenozide (or other ecdysone agonists) might impact moth pest populations, working alone or in combination with mating disruption tactics, field and laboratory assessments were needed. The two main objectives of this research project are: 1) determine the sublethal effects of field applications of methoxyfenozide with and without mating disruption on the responsiveness and orientation of wild male *C. pomonella* in a small orchard block; and 2) determine the effect of adult exposure to methoxyfenozide-treated surfaces on the close-range courtship behavior of *C. pomonella*.

CHAPTER II. MATERIALS AND METHODS

A. Insects

All *Cydia pomonella* used in both field and laboratory studies came from a laboratory colony maintained at the University of Missouri, Horticulture and Agroforestry Center (HARC), New Franklin, MO, or were obtained as mature larvae shipped from the USDA-ARS facility at Wapato, WA. Both the laboratory colony and shipped specimens were maintained in environmental chambers set at 24^{o}C with a photoperiod of 16L:8D.

B. Field Study

1. Chemicals and Trap Treatments

Twenty-five large plastic delta traps (Scentry, Billings, MT) containing disposable liners with a sticky coating were used to monitor moth densities. Because green colored traps were reported to be efficient in capturing *Cydia pomonella* over the standard white traps (Knight and Miliczky 2003), the exterior of all traps were painted green (No. 2327 Spring Grass; Krylon, Cleveland, OH).

Traps were baited with the following five lure treatments:

Treatment	Lure Type
1	2 virgin females
2	2 virgin females exposed to Intrepid-treated surfaces
3	1 mg codlemone lure
4	10 mg codlemone lure
5	Pear ester DA lure

For traps baited with female moths (Treatments 1 and 2), mature pupae were pulled from the colony and separated by gender. Newly emerged adults were then promptly removed to ensure the age of all moths. To ensure maximal sexual activity, all moths used were $\leq$ 48 hours old. Prior to female moths being placed inside treatment traps 1 and 2 they were first exposed for 24 h to plastic-mesh surface treated with either water or methoxyfenozide, respectively.

Each cage used to expose females to treated surfaces consisted of a 10 cm long section of polyvinyl chloride (PVC) pipe, 9 cm wide, with two end lids. Each cage was lined with removable plastic-mesh screening (2 x 2 mm). The end lids consisted of thin PVC rings covered with the same plastic-mesh screening. The mesh cage liners and end lids were immersed in a treatment solution (agitated) for 10 minutes and allowed to air dry. To avoid contamination, after each use, the exposure cages and liners were washed thoroughly with hot soapy water and dried before they were retreated with same treatment solution and reused.

Females for Treatment 2 were exposed to methoxyfenozide-treated surfaces. Formulated methoxyfenozide (Intrepid® 2F, Dow AgroSciences, Indianapolis, IN) was prepared in water (500 ml) at a concentration that corresponded to the recommended field rate, 0.30 g (AI)/liter water (0.27 kg (AI)/ha). Because field applications of methoxyfenozide are recommended to include a surfactant, such as Latron B-1956® (UAP-Loveland Products, Greeley, CO) or a similar spreader-sticker, a proportionate field rate of Latron (0.125% vol:vol) was added to the methoxyfenozide treatment solutions (hereafter referred to as the methoxyfenozide treatment).

Female moths used in Treatment 1 and 2 traps that were exposed to the treated plastic-mesh surfaces (methoxyfenozide and control, respectively) for 24 h were removed from exposure cages and placed inside 4 x 4.5 cm cages made of either plastic-mesh (2 x 2 mm) or aluminum-wire screening (1.5 x 1.5 mm) (1 female per cage). These nontreated mesh holding cages were hung from the inside of the top of the plastic delta-style traps mentioned previously.

Lures used in Treatment 3 traps consisted of a red rubber septum containing 1 mg of codlemone, the *Cydia pomonella* sex pheromone, (*E,E*)-8, 10-dodecadien-1-ol (E8,E10-12:OH) (CM Standard Lure, Trécé Inc., Adair, OK).

This lure load is considered the industry standard for monitoring *Cydia pomonella* populations in conventional, insecticide-treated orchards. Lures in Treatment 4 traps consisted of a rubber septum treated with 10 mg of codlemone (CM 10X Lure, Trécé Inc., Adair, OK). The 10 mg codlemone lure is 'supercharged' and is recommended for monitoring *Cydia pomonella* populations

in orchards under a mating disruption program. Lures used in Treatment 5 traps were Pherocon® CM-DA Combo™ lure. This lure contains both codlemone (10 mg) and ethyl (2E, 4Z)-2, decadienoate, a volatile chemical released from ripening pears (referred to as a pear ester kairomone) (Trécé Inc., Adair, OK). This lure is attractive to both male and female *Cydia pomonella*.

2. Experiment Design

The field bioassay was conducted in a research apple block (H) located at the University of Missouri, Horticulture and Agroforestry Research Center (HARC), New Franklin, MO. This block was 2 ha in size and consisted of alternating 'Jonathan', 'Golden Delicious', and 'Red Delicious' cultivars. Trees were planted 5.5 m apart within rows, and the distance between rows was 8 m. The trees were 20 years old and approximately 5 to 6 m tall.

There are three flights of *Cydia pomonella* during the summer, usually occurring May/June, June/July, and August/September. During the first flight, a control phase was established in which the orchard block was kept free of insecticide treatment. Prior to the second flight, the orchard block was treated with methoxyfenozide only. For the final flight, both an application of methoxyfenozide and mating disruption dispensers, Isomate® M-100 pheromone twist-ties, were applied to the orchard block. The Intrepid was applied every two weeks, with Latron, at labeled field rates of 16 oz. per acre.

All twenty-five traps were placed in the upper-third of the canopy in selected trees (via a rope and pulley system mounted on a 4 m aluminum pole)

following a Latin-square design. During the control phase, there where five repetitions of the Latin-square design involving all five trap treatments as well as 2 repetitions involving female-baited traps only. During the methoxyfenozide-treated only phase, there were two repetitions of the Latin-square design involving all five trap treatments and 3 repetitions involving female-baited traps only. For the methoxyfenozide and mating disruption treatment of the orchard block, there were 3 repetitions of the Latin-square design involving all five trap treatments. Trapping periods with female-baited traps only consisted of ten traps placed in the orchard block with the same placement as other trapping periods in accordance with the Latin-square design.

Trap lures were hung from the inside of the top of a plastic delta-style trap. Each baited tree had a trap-free tree between it and the next baited tree. In addition, tree rows with traps had a buffer consisting of a tree row with no traps between the next baited tree row.

The Latin-square design is as shown below:

Tree Row	Trap Treatments				
1	1	2	3	4	5
2	2	3	4	5	1
3	3	4	5	1	2
4	4	5	1	2	3
5	5	1	2	3	4

Each individual trapping period lasted for three days. From the onset of trap placement, a count of the males caught (both male and female counts were taken from traps baited with the pear ester DA lure) at 24 hours, 48 hours, and 72 hours. Trap liners were cleaned periodically so as to prevent pheromone contamination. Female lures were checked to ensure the survival of female moths throughout the trapping period.

3. Data Analysis

All statistical analyses were analyzed using the PROC MIXED procedures in SAS (SAS Institute 2004). Differences in the mean number of wild male *C. pomonella* caught among the different treatment combinations was analyzed as a latin-square design. Differences were considered statistically significant at the level of $P<0.05$ unless otherwise noted.

C. Laboratory Bioassay

1. Chemicals and Treatments

All behavioral bioassay observations were conducted in a laboratory of the Agricultural Building, University of Missouri, Columbia, MO.

The cages used for exposing moths to treated surfaces in this bioassay consisted of 9.2 cm long, 4.5 cm wide, and 5.7 cm long, 2.5 cm wide, respectively, clear plastic vials. The bottom of the vial as well as the center portion of the vial's pliable plastic snap-cap lid were removed and replaced with

wire mesh (1.5 x 1.5 mm) screening. The cages were lined with removable plastic mesh (2 x 2 mm) screening.

For treatments, the plastic-mesh cage liners and screened cage lids were immersed in either a solution of methoxyfenozide or surfactant for 60 second and allowed to air-dry. This consisted of a total 10 treated exposure cages with 5 repetitions for both the surfactant and methoxyfenozide treatments for each of the different orchard block treatments. Liners and lids for control cages followed the same procedures but were dipped in water. To avoid contamination, after each use, the exposure cages and liners were washed thoroughly with hot soapy water and dried before they were retreated and reused.

Formulated methoxyfenozide (Intrepid® 2F, Dow AgroSciences, Indianapolis, IN) was prepared in water (250 ml) at a concentration corresponding to the recommended field rate, 0.30 g (AI)/liter water (0.27 kg (AI)/ha). Because field applications of methoxyfenozide are recommended to include a surfactant, such as Latron B-1956® (UAP-Loveland Products, Greeley, CO) or a similar spreader-sticker, a proportionate field rate of Latron (0.125% vol:vol) was added to methoxyfenozide treatment solutions (hereafter referred to as the methoxyfenozide treatment). Solutions of surfactant (mixed with water) and water only (control) were also prepared. Treatment solutions were refrigerated in glass flasks wrapped in aluminum foil. Fresh solutions were prepared weekly to guarantee maximum effect.

Female and male moths were each treated with one of the three different treatment solutions (methoxyfenozide, surfactant and water). The following are the seven treatment combinations:

Treatments	Female	Male
1	H_2O	H_2O
2	H_2O	methoxyfenozide
3	methoxyfenozide	H_2O
4	methoxyfenozide	methoxyfenozide
5	H_2O	surfactant
6	surfactant	H_2O
7	surfactant	surfactant

Once the plastic mesh was treated it was allowed to air dry before being placed in glass vials. The treated plastic mesh was placed in glass vials such that no horizontal surface area was left unexposed. Males and females were kept separate in the growth chamber to ensure the virginity of subjects used. All adults were 48 hours old or less. Moths were then placed in exposure cage vials and returned to the growth chamber to undergo the 24-hour exposure time.

C. pomonella adults become active a few hours before and after twilight (Howell et al. 1978). Therefore, moths were not introduced into the arena until the onset of the growth chamber's dark cycle and only took place during the first 4 hours of scotophase. Both females and males were then removed from the growth chamber in a manner that resulted in the least amount of disturbance

possible. During this transfer, no moth was exposed to any light other than red light.

2. Video Setup and Mating Arena

Recordings of *C. pomonella* mating behavior were taken with a RECO 204 DVR system and 2 B/W Panasonic digital cameras with the ability to take recordings at light levels as low as .08 lux. The RECO DVR uses a MPEG2 compression that creates digital data storage as it records. Once all recordings were taken, the DVR hard drive was removed and placed in a computer. MPEG Streamclip 1.7© was used to both convert the MPEG files and to play the video footage. The MPEG Streamclip 1.7© enhanced the accuracy of behavioral observations by allowing for slow motion playback frame by frame, frame length being .033 seconds long.

Petri dishes, 9.5 cm in diameter, were used as mating arenas. Plastic mesh was attached to all petri dishes to constrict the moths to the field of vision, creating a rectangular mating area 9.5 cm long and 6 cm wide. Foam board was used to establish a fixed placement for all mating arenas. Arenas were situated prior to moth introduction to help reduce disturbance. After each use, the arenas were either discarded or washed with warm soapy water to prevent pheromone contamination. During recordings, the light was no higher then 10 lux and only came from a headlamp with a red light and two lamps with red bulbs. All recordings were taken at room temperature.

3. Experimental Design and Mating Behaviors

The experimental design consisted of an incomplete block design that was repeated twice. Each recording had a duration of 20 minutes. This resulted in 56 recordings with a total of 18.6 hours. The design was as follows:

Block No.	Treatments
1	3 5 6 7
2	1 4 6 7
3	1 2 5 7
4	1 2 3 6
5	2 3 4 7
6	1 3 4 5
7	2 4 5 6

Males were placed next to the mating arena to minimize disturbance. Once females had been chilled, cameras were turned on. Moths were gently introduced into the arena with male and females consistently being introduced on opposite sides. Once moths were in place, the top was placed over the area.

When making behavioral observations, each recording was analyzed thoroughly, often watched at a rate slower than 1 second per minute. Both the duration and frequency of all behaviors were recorded. Also observed were the start of sexual behavior for both sexes and the preceding 4 behaviors.

Below is a list of the *Cydia pomonella* behaviors observed:

Cydia pomonella Courtship Behavior
movement towards partner
movement away from partner
wing fanning
antennal contact with partner's abdomen
raised wings
antennal stroking
walking in circles
genital contact
lateral abdominal bending

4. Data Analysis

All statistical analyses were analyzed using the mixed procedure (PROC MIXED) in SAS (SAS Institute 2004). Differences among treatment combinations in regards to mating behavior were analyzed as a incomplete block design. Differences were considered statistically significant at the level of $P<0.05$ unless otherwise noted.

CHAPTER III. RESULTS

A. Field Study

1. Trap Catches – Trees Not Treated

Within the first 24 hours of the May 15-18 trapping period, traps baited with the DA and 10 mg septa lures captured significantly more male *C. pomonella* than traps baited with the 1 mg septa lure and treated or non-treated female moths (Table 1). However, there were no significant differences in mean moth captures between the DA and 10 mg traps. In addition, the 1 mg and female-baited traps were not significantly different from each other. 10 mg lures attracted the greatest mean number of moths with 10 males per trap, while non-treated female-baited traps captured a mean of 0.2 males per trap (Table 1). Interestingly, during the 48- and 72-hour periods, 1 mg septa traps caught significantly greater mean numbers of males, 10 and 4.2 males per trap, respectively. There were no significant differences in mean moth counts between remaining trap types during these latter two trapping periods.

When trap data are pooled across the 72-hour period, trap types with the greatest mean numbers of males captured were the 10 mg, 1 mg and DA septa-baited traps with 16.4, 15.4 and 10.0 males per trap, respectively (Table 1). These three means were not significantly different from each other. Both the 1 and 10 mg lures caught significantly more males than traps baited with female lures.

The second control trapping period began on May 19. As was done prior to each trapping period, all female-baited traps were baited with new treated and non-treated females. Septa lures that had been used in the previous trapping

period (May 15-18) were used again (up to this point these rubber septa lures had a field exposure of 4 days). Twenty-four hours after deployment, traps with 1 mg and 10 mg lures had the highest mean number of males captured both with means of 4.0 males per trap (Table 2). There were no significant differences between these means and they were not significantly greater than means from the DA lure trap of 1.4 males. Although all codlemone-baited traps were significantly higher ($P < 0.07$) than treated and untreated female traps, both resulting in a mean of 0.4 males per trap, the DA lure was only significantly greater than untreated female traps.

After 48 hours in the field there were no significant differences in mean male captures among all of the trap types (Table 2). After 72 hours, the 1 mg baited trap had the highest mean capture of 4.8 males per trap. Although not significantly different from the 10 mg and DA lures, the 1 mg lure was the only pheromone-baited trap that had a significantly higher mean male catch than both the treated and untreated female baited traps.

After pooling the trap data across the 72-hour trapping period, the 1 mg baited traps captured a mean of 10.8 males per trap. This mean was significantly higher ($P < 0.07$) than that found with in female-baited traps, but not significantly different from the 10 mg and DA septa traps (Table 2).

The third control trapping period was initiated on May 23. Septa lures that had been used in the previous two trapping periods were used again during this period (with a field exposure of 8 days). After the first 24 hours, traps with 1 mg septa lures had a mean capture of 3.6 males per trap, which was greater but not

significantly different from traps baited with 10 mg and DA septa lures (Table 3). However, traps with the 1 mg septa lures were the only traps with mean male captures significantly higher than that found in treated and non-treated female-baited traps. After 48 hours, 1 mg septa traps again had the highest mean male capture, 2.4 males per trap, which was significantly greater than all other trap types. After 72 hours there were no significant differences among trap types in the mean number of male moth captures.

When the data are pooled the highest mean number of males captured occurred in traps baited with the 1 mg septa, and this mean was significantly greater than the means from all the other trap types. The mean values for the remaining four lure types were not significantly different from one another (Table 3).

The fourth control trapping period was initiated on May 30. All pheromone-based traps received new septa lures. None of the trap types showed significant differences in mean male moth captures after 24, 48, and 72 hours from deployment (Table 4). Similarly, the pooled data revealed no significant differences between trap types. However, traps with the 1 mg septa lures did have the highest mean of 11 males per trap, while the traps baited with non-treated females resulted in the lowest mean of 5 males per trap.

The fifth control trapping period was initiated on June 2. Septa lures that had been used in the previous trapping period were used again during this period (with a field exposure of 3 days). Twenty-four hours after trap deployment, traps baited with 1 mg septa lures had captured a mean of 3 males per trap, which was

significantly greater than the mean captures from the other trap types (Table 5). There were no significant differences between the remaining four trap types. Interestingly, after 48 hours traps with non-treated females captured the greatest mean number of males, 3.8 males per trap, and this mean was significantly higher than the remaining trap captures. None of the remaining trap types showed any significant differences from one another. After 72 hours of trap deployment, traps with the 1 mg and 10 mg septa lures had the highest mean male catch of 2.2 and 1.6 males per trap, respectively (Table 5). Although these means were not significantly different from one another they were significantly greater than trap captures in the female-baited traps.

When the data are pooled, the 1 mg septa and untreated female moth baited had significantly greater mean male captures than all other treatment types (6.0 and 4.6 males per trap respectively). These values were not significantly different from each other.

On June 6, a sixth control trapping period was conducted that evaluated wild male moth responsiveness to traps baited with caged females only. During the entire period, traps with non-treated females consistently caught the highest mean number of males. There were no significant differences between the two female-baited traps 24 and 48 hours after trap deployment (Table 6). After 72 hours, the mean number of males captured in the non-treated female traps (4.6 males) was significantly greater than the mean from the treated female trap (0.8 males). Similarly, when the data is pooled for the entire 72-hour period, the mean male capture in the non-treated female-baited traps (11.4 males) was also

significantly greater than traps baited with that from the treated female trap (3.0 males) (Table 6).

On June 24, a seventh control trapping period was conducted that repeated the examination of the attractiveness of traps containing only nontreated and treated females. There were no significant differences in mean male captures between them 24, 48, and 72 hours after trap deployment (Table 7). Means from pooled data revealed traps baited with non-treated females captured 6.8 males per trap, and treated females captured 6.2 males per trap.

Similarly, another female-baited trap comparison beginning on July 1 revealed no significant differences in mean male captures between two trap types throughout the 72-hour period. Analysis of the pooled data showed means of 7.4 and 7.6 males per trap occurring in the non-treated and treated female baited traps, respectively (Table 8).

Table 1. The mean number of male *Cydia pomonella* captured, beginning May 15, during a 72 h period (at 24, 48 and 72 hours) in traps with different lures placed in the control block (no insecticide applications).

	Mean (±SE) No. of Males Captured			
Lure Type	24 h	48 h	72 h	Total
1. Female moths (nontreated)	0.2±0.20 a	0.8±0.49 a	0.4±0.40 a	1.4±0.74 a
2. Female moths (treated)	1.2±0.80 a	1.6±0.87 a	0.6±0.60 a	3.4±2.20 ab
3. 1 mg septum	1.2±0.80 a	10.0±2.09 b	4.2±1.02 b	15.4±3.17 c
4. 10 mg septum	10.0±1.97 b	4.4±1.96 a	2.0±0.44 a	16.4±2.97 c
5. DA septum	6.4±1.80 b	2.6±0.60 a	1.0±0.77 a	10.0±2.56 bc

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$).

Table 2. The mean number of male *Cydia pomonella* captured, beginning May 19, during a 72 h period (at 24, 48 and 72 hours) in traps with different lures placed in the control block (no insecticide applications).

	Mean (±SE) No. of Males Captured			
Lure Type	24 h	48 h	72 h	Total
1. Female moths (nontreated)	0.4±0.24 a	1.0±0.44 a	1.4±0.51 ab	2.8±0.97 a
2. Female moths (treated)	0.4±0.24 a	0.2±0.20 a	0.6±0.60 a	1.2±0.58 a
3. 1 mg septum	4.0±0.89 b	2.0±0.44 a	4.8±1.20 c	10.8±1.28 b
4. 10 mg septum	4.0±1.81 b	0.8±0.37 a	1.8±0.58 abc	6.6±1.93 ab
5. DA septum	1.4±0.92 ab	2.2±1.74 a	4.0±2.02 bc	7.6±4.62 ab

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.07$).

Table 3. The mean number of male *Cydia pomonella* captured, beginning May 23, during a 72 h period (at 24, 48 and 72 hours) in traps with different lures placed in the control block (no insecticide applications).

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	0.2±0.20 a	0.0±0.00 a	0.8±0.58 a	1.0±0.54 a
2. Female moths (treated)	0.8±0.80 a	0.4±0.24 a	0.0±0.00 a	1.2±0.97 a
3. 1 mg septum	3.6±1.16 b	2.4±0.92 b	2.4±1.24 a	8.4±3.01 b
4. 10 mg septum	1.6±0.67 ab	0.4±0.40 a	1.2±0.37 a	3.2±0.80 a
5. DA septum	1.6±0.51 ab	0.2±0.20 a	1.2±0.58 a	3.0±1.14 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$).

Table 4. The mean number of male *Cydia pomonella* captured, beginning May 30, during a 72 h period (at 24, 48 and 72 hours) in traps with different lures placed in the control block (no insecticide applications).

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	0.8±0.37 a	2.4±1.12 a	1.8±0.80 a	5.0±2.23 a
2. Female moths (treated)	0.4±0.40 a	3.8±2.22 a	1.4±0.74 a	5.6±3.18 a
3. 1 mg septum	3.2±1.39 a	4.6±1.74 a	3.2±1.46 a	11.0±4.00 a
4. 10 mg septum	0.0±0.00 a	2.8±0.66 a	4.6±1.36 a	7.4±1.86 a
5. DA septum	1.8±1.56 a	3.6±1.74 a	1.6±0.92 a	7.0±3.22 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$).

Table 5. The mean number of male *Cydia pomonella* captured, beginning June 2, during a 72 h period (at 24, 48 and 72 hours) in traps with different lures placed in the control block (no insecticide applications).

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	0.2±0.20 a	3.8±0.58 b	0.6±0.40 a	4.6±0.98 bc
2. Female moths (treated)	0.8±0.80 a	0.2±0.20 a	0.2±0.20 a	1.2±0.97 a
3. 1 mg septum	3.0±1.04 b	0.8±0.58 a	2.2±0.73 b	6.0±1.58 c
4. 10 mg septum	0.4±0.24 a	0.2±0.20 a	1.6±0.51 ab	2.2±0.37 ab
5. DA septum	0.8±0.49 a	0.0±0.00 a	0.4±0.40 a	1.2±0.80 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$).

Table 6. The mean number of male *Cydia pomonella* captured, beginning June 6, during a 72 h period (at 24, 48 and 72 hours) in female moth-baited traps placed in the control block (no insecticide applications).

	Mean (±SE) No. of Males Captured			
Lure Type	24 h	48 h	72 h	Total
1. Female moths (nontreated)	2.4±1.12 a	4.4±2.37 a	4.6±1.16 b	11.4±3.18 b
2. Female moths (treated)	0.6±0.24 a	1.6±1.03 a	0.8±0.49 a	3.0±1.09 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$).

Table 7. The mean number of male *Cydia pomonella* captured, beginning June 24, during a 72 h period (at 24, 48 and 72 hours) in female moth-baited traps placed in the control block (no insecticide applications).

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	1.8±0.91 a	1.8±0.73 a	3.2±0.91 a	6.8±1.82 a
2. Female moths (treated)	2.0±0.63 a	2.6±1.24 a	1.6±0.81 a	6.2±2.45 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$).

Table 8. The mean number of male *Cydia pomonella* captured, beginning July 1, during a 72 h period (at 24, 48 and 72 hours) in female moth-baited traps placed in the control block (no insecticide applications).

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	3.4±0.67 a	2.0±0.54 a	2.0±1.04 a	7.4±0.74 a
2. Female moths (treated)	3.4±1.60 a	3.4±0.92 a	0.8±0.20 a	7.6±2.54 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$).

2. Trap Catches – Trees Treated With Methoxyfenozide

On July 13, all five trap types were placed in a block just after it had been treated with the ecdysone agonist methoxyfenozide (Intrepid 2F). Septa lures that had been used in the previous two trapping periods were used during this trial as well (with a total field exposure of 8 days). As done previously during the control trials, traps were checked at 24, 48 and 72 hours after deployment.

After 24 hours, the greatest mean number of male *C. pomonella* captures occurred within the 10 mg septa traps (3.0 males). However, this mean was not significantly greater from other trap means which ranged from 0.2 to 2.6 males per trap (Table 9). Similarly there were no significant differences in mean male moth captures among the five trap types at the 48 and 72 hour counts, although the greatest means during these two periods (4.8 and 2.6 males respectively) were found in the 1 mg traps (Table 9). When the trap data are pooled, there are no significant differences in mean male moth captures among all trap types, but the highest mean (10.0 males) still occurred within the 1 mg septa traps.

The second trapping period conducted in the methoxyfenozide-treated block was initiated on July 16; 3 days after the ecdysone agonist had been applied to the trees. New treated and non-treated females were place in traps, as was done before each trapping period. Septa lures that had been used in the previous trapping periods were used again (with a field exposure of 9 days). There were no significant differences in mean male captures among all five lure types at 24, 48, and 72 hour assessments (Table 10). However, traps with the treated female moths consistently had the lowest mean male captures of all trap types throughout

the 3-day trapping period. Although no significant differences among trap types were found when the data were pooled, the 10 mg lure had the highest mean male catch (11.6 males per trap) while the treated-female traps had the lowest mean (1.2 males per trap).

Twelve days after the first application of methoxyfenozide, July 25, two types of female-baited lure traps were placed in the block. Mean male captures after 24, 48, 72 hours revealed no significant differences between the two trap types (Table 11). When the data are pooled there was no significant difference. Non-treated female traps captured a mean of 21.6 males per trap and treated females traps captured a mean of 16.2 males (Table 11).

On July 28, a second application of methoxyfenozide was made to the trees. After a 2-3 hour of drying period, female-baited traps were placed within the block. As with the previous trial there were no significant differences in mean male captures between the two trap types after the 24, 48, and 72 hour periods (Table 12). After the initial 24 hours, non-treated female traps had a higher male capture at 48 and 72 hours, but again none of these values were significantly higher than treated female traps. When the data are pooled, traps with the non-treated females captured a mean of 32.8 males per trap, while traps with treated females had a mean of 28.6 males per trap. These two means were not significantly different from each other.

On Aug. 8, 11 days after the second methoxyfenozide application, the two female-baited trap types were again placed in the block. After 24 hours, non-treated female traps captured a mean of 28.6 males per trap; however, it was not

significantly greater than the mean of 5.8 males captured from treated female traps (Table 13). After 48 hours, non-treated female traps had the greatest, but not significantly greater, mean number of male caught (10.6 and 7.2 male respectively) (Table 13). After 72 hours, nontreated female traps caught 7.2 males per trap which was significantly higher than treated female traps mean of 0.4 males per trap. When the data are pooled the non-treated female traps had a significantly greater mean catch of 46.4 males, whereas the treated female traps had a mean catch of 8.2 males per trap (Table 13).

Table 9. The mean number of male *Cydia pomonella* captured, beginning July 13, during a 72 h period (at 24, 48 and 72 hours) in traps with different lures placed in the methoxyfenozide-treated block.

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	1.2±0.49 a	2.2±1.56 a	2.0±1.76 a	5.4±3.70 a
2. Female moths (treated)	0.2±0.20 a	1.4±0.74 a	1.0±0.54 a	2.6±0.92 a
3. 1 mg septum	2.6±0.98 a	4.8±1.35 a	2.6±0.92 a	10.0±1.87 a
4. 10 mg septum	1.4±0.40 a	0.4±0.24 a	1.2±0.49 a	3.0±0.70 a
5. DA septum	3.0±2.53 a	2.0±2.00 a	0.8±0.58 a	5.8±4.65 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$).

Table 10. The mean number of male *Cydia pomonella* captured, beginning July 16, during a 72 h period (at 24, 48 and 72 hours) in traps with different lures placed in the methoxyfenozide-treated block.

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	1.0±1.00 a	3.4±2.27 a	3.0±2.00 a	7.4±4.51 a
2. Female moths (treated)	0.0±0.00 a	0.6±0.60 a	0.60±0.40 a	1.2±0.58 a
3. 1 mg septum	2.8±0.37 a	4.0±1.51 a	1.6±0.67 a	8.4±1.53 a
4. 10 mg septum	2.2±0.37 a	5.4±2.78 a	4.0±0.63 a	11.6±2.94 a
5. DA septum	1.8±1.20 a	2.2±0.97 a	2.4±1.69 a	6.4±3.31 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$). Methoxyfenozide applied to trees on July 13

Table 11. The mean number of male *Cydia pomonella* captured, beginning July 25, during a 72 h period (at 24, 48 and 72 hours) in female moth-baited traps placed in the methoxyfenozide-treated block.

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	3.0±2.14 a	10.6±7.15 a	8.0±4.33 a	21.6±12.75 a
2. Female moths (treated)	1.8±1.35 a	11.6±5.87 a	2.8±1.59 a	16.2±5.25 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$). Methoxyfenozide applied to trees on July 13.

Table 12. The mean number of male *Cydia pomonella* captured, beginning July 28, during a 72 h period (at 24, 48 and 72 hours) in female moth-baited traps placed in the methoxyfenozide-treated block.

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	10.4±3.84 a	11.6±2.96 a	10.8±2.95 a	32.8±6.65 a
2. Female moths (treated)	14.6±0.92 a	9.0±2.49 a	5.0±1.00 a	28.6±3.26 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$). Methoxyfenozide applied to trees on July 28.

Table 13. The mean number of male *Cydia pomonella* captured, beginning August 8, during a 72 h period (at 24, 48 and 72 hours) in female moth-baited traps placed in the methoxyfenozide-treated block.

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	28.6±10.03 a	10.6±5.68 a	7.2±1.82 b	46.4±14.13 b
2. Female moths (treated)	5.8±2.35 a	2.0±0.54 a	0.4±0.24 a	8.2±2.87 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$). Methoxyfenozide applied to trees on July 28.

3. Trap Catches – Trees Treated With Methoxyfenozide and Mating Disruption

On August 24, a third application of methoxyfenozide was applied to the block, nearly a month since the second application. Previously, placement of mating disruption dispensers (Isomate C-Plus) in the same trees occurred on August 15. On August 26, all lure types (pheromone- and female moth-based) were placed in the treated block. After the first 24 hours, the 1 mg and 10 mg septa lures had the highest mean capture of males (12.4 and 7.4 males per trap respectively) (Table 14). Although these values were not significantly different from one another, mean male capture from 1 mg septa traps was significantly higher than all the remaining three trap types. Female-baited and DA lure traps caught the lowest number of males after the first 24 hours, less than 0.5 males per trap (Table 14).

After 48 hours, the 1 mg septa trap again captured a significantly greater mean number of males than the other trap types (22.4 males per trap) (Table 14). There were no significant differences between the remaining trap types. At the 72-hour period, the 10 mg lure captured the greatest number of males (22.8 males per trap). When trap data are pooled, female (non-treated and treated) and DA septa-baited traps captured the lowest mean number of males (1.4, 0.8, and 6.0 males per trap respectively) (Table 14). Conversely, 1 mg and 10 mg traps captured significantly greater mean number of males, 46 and 38.4 males per trap respectively. There was no significant difference between these two means.

On September 8, 10 days after the last methoxyfenozide application and about a month (24 days) after mating disruption dispensers had been applied, the

five trap types were again placed throughout the block. The septa-baited traps received new lures.

Twenty-four hours after trap deployment, the two trap types with the significantly highest mean number of male moths (11.8 and 13.6 males per trap respectively) were found in the 10 mg and 1 mg septa-baited traps (Table 15). There was no significant difference between these means. This pattern was repeated the following 24 hours, with 1 mg and 10 mg codlemone septa-baited traps having the highest mean male moth captures (13.6 and 9.4 males per trap respectively) (Table 15). The 1 mg septa trap mean capture was significantly greater than the 10 mg septa trap capture.

At the 72-hour assessment, 10 mg septa lures had a significantly higher mean number of males captured (10.2 males per trap). Remaining trap captures were not significantly different from each other. After pooling the data, mean numbers of males captured in the 1 and 10 mg septa traps (31.6 and 31.4 males respectively) were significantly greater than all other trap types.

On Sept. 12, another application of methoxyfenozide was applied to the trees (at this time mating disruption dispensers had a field exposure of 32 days). After a short drying period the five trap types were placed throughout the block. Septa lures used in the previous trapping period were used for this trapping period as well.

After 24 hours, the 10 mg septa traps had the significantly greatest mean number of males caught than all other trap types (10.8 per trap) (Table 16). The other trap types showed no significant differences between each other, however, non-treated and treated female traps had the lowest mean catch (0.2 and 0 males per trap respectively) (Table 16). This same pattern of mean

male captures was repeated 48 hours after trap deployment. After pooling the data, traps with 10 mg septa lures captured a significantly greater mean number of males (25) than the other four trap types.

Table 14. The mean number of male *Cydia pomonella* captured, beginning August 26, during a 72 h period (at 24, 48 and 72 hours) in traps with different lures placed in the methoxyfenozide- and mating disruption-treated block.

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	0.4±0.24 ab	1.0±0.63 a	0.0 ±0.00 a	1.4±0.67 a
2. Female moths (treated)	0.0±0.00 a	0.2±0.20 a	0.6±0.40 a	0.8 ±0.37 a
3. 1 mg septum	12.4±3.77 c	22.4±9.80 b	11.2±2.95 b	46.0±9.42 b
4. 10 mg septum	7.4±3.94 bc	8.2±2.08 a	22.8±7.31 c	38.4±13.11 b
5. DA septum	0.4±0.24 ab	2.8±2.77 a	2.8±0.80 ab	6.0±0.89 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$). Methoxyfenozide applied to trees on August 24. and mating disruption applied on August 15.

Table 15. The mean number of male *Cydia pomonella* captured, beginning September 8, during a 72 h period (at 24, 48 and 72 hours) in traps with different lures placed in the methoxyfenozide- and mating disruption-treated block.

Lure Type	Mean (±SE) No. of Males Captured			
	24 h	48 h	72 h	Total
1. Female moths (nontreated)	0.2±0.20 a	0.2±0.20 a	0.6±0.40 a	1.0±0.54 a
2. Female moths (treated)	0.0±0.00 a	0.0±0.00 a	0.0 ±0.00 a	0.0 ±0.00 a
3. 1 mg septum	13.6±3.37 b	13.6±1.88 c	4.4±1.03 a	31.6±4.53 b
4. 10 mg septum	11.8±2.13 b	9.4±2.15 b	10.2±2.92 b	31.4±4.89 b
5. DA septum	2.0±0.94 a	2.2±1.06 a	3.0±1.22 a	7.2±2.49 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$). Methoxyfenozide applied to trees on August 24. and mating disruption applied on August 15.

Table 16. The mean number of male *Cydia pomonella* captured, beginning September 12, during a 72 h period (at 24, 48 and 72 hours) in traps with different lures placed in the methoxyfenozide- and mating disruption-treated block.

	Mean (±SE) No. of Males Captured			
Lure Type	24 h	48 h	72 h	Total
1. Female moths (nontreated)	0.2±0.20 a	0.0±0.00 a	0.0±0.00 a	0.2±0.20 a
2. Female moths (treated)	0.0±0.00 a	0.0±0.00 a	0.0 ±0.00 a	0.0 ±0.00 a
3. 1 mg septum	2.2±0.58 a	1.0±0.54 a	2.2±1.02 ab	5.4±1.36 a
4. 10 mg septum	10.8±3.56 b	4.8±2.78 b	9.4±6.75 b	25.0±10.31 b
5. DA septum	4.4±1.56 a	0.2±0.20 a	0.0±0.00 a	4.6±1.60 a

Means followed by the same letter are not significantly different (Fisher's protected LSD; $P<0.05$).
Methoxyfenozide applied to trees on August 24. and mating disruption applied on August 15.

4. *Trap Catches-Summary*

The mean number of males caught per lure type during the 72-hour trapping periods conducted in the control block (no insecticide applications) were calculated. Traps baited with methoxyfenozide-treated females had the lowest mean number of males caught among all lure types (3.13 males per trap) (Table 17). Although this mean was not significantly different from non-treated female lure mean catch, 3.84 males per trap, it was significantly lower than all remaining lure types. The 1 mg septum lure had a significantly greater mean number of males caught (7.04) than all lure types except for the 10 mg septum lure (5.75 males per trap) ($P<0.05$). No other significant differences were found (Table 17).

The total mean number of males caught per lure type for the 72-hour trapping periods conducted in the methoxyfenozide-treated block were also calculated. No significant differences were found between lure types (Table 18). The total mean number of males captured ranged from 4.29 to 5.88 males per trap among lure types, with methoxyfenozide-treated females having the lower mean catch and the 1 mg septum lure having the highest.

The total mean number of males caught per lure type for the 72-hour trapping periods conducted in the methoxyfenozide and mating disruption-treated block were calculated as well. Methoxyfenozide-treated females caught a significantly lower total mean number (0.68 males per trap) in comparison to all t other lures ($P<0.05$) (Table 19). There were no significant differences found between the 1mg and 10 mg septum lure (9.02 and 9.59 respectively), however both were significantly higher than all remaining lure types.

Table 17. The total mean number of male *Cydia pomonella* captured for each different lure type in the control block (data pooled).

Lure Type	Total Mean No. of Males Captured
1. Female moths (nontreated)	3.8449 cd
2. Female moths (treated)	3.1303 d
3. 1 mg septum	7.0439 a
4. 10 mg septum	5.7582 ab
5. DA septum	5.1338 cb

Means followed by the same letter are not significantly different. (Fisher's protected LSD; $P<0.05$).

Table 18. The total mean number of male *Cydia pomonella* captured for each different lure type in the methoxyfenozide-treated block (data pooled).

Lure Type	Total Mean No. of Males Captured
1. Female moths (nontreated)	4.393 a
2. Female moths (treated)	4.299a
3. 1 mg septum	5.886 a
4. 10 mg septum	5.614 a
5. DA septum	4.359 a

Means followed by the same letter are not significantly different. (Fisher's protected LSD; $P<0.05$).

Table 19. The total mean number of male *Cydia pomonella* captured for each different lure type in the methoxyfenozide-treated and mating disruption-treated block (data pooled).

Lure Type	Total Mean No. of Males Captured
1. Female moths (nontreated)	1.3727 c
2. Female moths (treated)	0.6828 c
3. 1 mg septum	9.0253a
4. 10 mg septum	9.5978 a
5. DA septum	4.1197 b

Means followed by the same letter are not significantly different. (Fisher's protected LSD; $P<0.05$).

B. Laboratory Bioassay

1. *Displays of Movement Towards Moth Partner*

When the data were pooled by sex, females and males exposed to water (Treatment 1) displayed the highest mean number of individual movements towards one another (48.8) per 20-minute observation period (Table 20). Conversely, pairs where both males and females had been exposed to methoxyfenozide (Treatment 4) averaged only 23.6 movements towards their partner. For the remaining treatment combinations, the mean number of movements towards a partner per observation period ranged from 28.1 to 38.6 times. Data analysis revealed no significant differences between treatments regarding this behavior ($P = 0.234$) (Table 20).

Pooling data by treatment revealed no significant differences between males and females in the mean number of movements towards a partner (Table 21). There were also no significant differences between sexes for movement towards a partner within individual treatments (Table 22). However, significant differences were found for this behavior when all treatment types were compared per sex per treatment combination (Tables 22-24). For example, water-treated females from Treatment 1 moved to their water-treated partner an average of 45.2 times, which was significantly higher than what the methoxyfenozide-treated females in Treatment 4 who moved 17.1 times towards their methoxyfenozide-treated partner ($P<0.04$) (Table 23). There was also a close significant difference between water-treated females in Treatment 6, 42.2 times, and methoxyfenozide-treated females of Treatment 4 ($P<0.06$).

Significant differences were found when comparing the mean number of movements-towards-partner for male moths amongst treatment types. Water-treated males from Treatment 1, moved toward water-treated females significantly more (52.3) than water-treated moved toward surfactant-treated females in Treatment 5 (24.3 times) ($P<0.05$) (Table 24). Males from Treatment 1 displayed a marginal significantly lower number of toward movements when compared to surfactant-treated males paired with water-treated females in Treatment 7 (26.8 toward movements) ($P<0.06$).

Table 20. The mean number of times paired male and female *Cydia pomonella* displayed movements towards partner of opposite sex during courtship by treatment (sex data pooled).

Treatment No.	Treatment Exposure By Sex		Mean No. Times (±SE) Movement Towards Partner
	Males	Females	
1	water	water	48.8±7.22
2	water	methoxy	34.5±7.22
3	methoxy	water	28.1±6.42
4	methoxy	methoxy	23.6±6.69
5	water	surfact	38.6±7.22
6	surfact	water	38.6±7.22
7	surfact	surfact	28.7±6.78
P-value			0.2343

Table 21. The mean number of times paired male and female *Cydia pomonella* displayed movements towards partner of opposite sex during courtship (treatment data pooled).

Sex	Mean No. Times (±SE) Movement Towards Partner
Female	30.6±3.57
Male	35.0±3.56
P-value	0.3561

Table 22. Mean number times *Cydia pomonella*, by sex, displayed movement towards partner of opposite sex during courtship and the within-treatment P-value (Nonprotected LSD).

Sex	1	2	3	4	5	6	7
Female	45.2 (water)	24.6 (methoxy)	24.3 (water)	17.1 (methoxy)	30.5 (surfact)	42.2 (water)	30.5 (surfact)
Male	52.3 (water)	44.4 (water)	32 (methoxy)	30 (methoxy)	24.3 (water)	35 (surfact)	26.8 (surfact)
Pvalue	0.5764	0.122	0.4953	0.2615	0.654	0.5764	0.7573

Each methoxyfenozide treatment solution also contained a field rate of the surfactant Latron B-1956 (as per label recommendations). The surfactant treatment consisted of a labeled field rate (0.125%, vol:vol) of Latron B-1956 mixed with water.

Table 23. P-values of treatment comparisons of female *Cydia pomonella* regarding the mean number of times they displayed movement towards male partner using a Nonprotected LSD test.

Sex: Female				Treatment			
Treatment	1	2	3	4	5	6	7
1	---	0.1332	0.1062	0.0321*	0.3127	0.8256	0.2691
2	---	---	0.9763	0.5632	0.9783	0.1996	0.6561
3	---	---	---	0.5597	0.6498	0.1661	0.6150
4	---	---	---	---	0.3354	0.0553*	0.2870
5	---	---	---	---	---	0.4221	0.9997
6	---	---	---	---	---	---	0.3795
7	---	---	---	---	---	---	---

Table 24. P-values of treatment comparisons of male *Cydia pomonella* regarding the mean number of times they displayed movement towards female partner using a Nonprotected LSD test.

Sex: Male				Treatment			
Treatment	1	2	3	4	5	6	7
1	---	0.5638	0.1166	0.0913	0.0497*	0.2064	0.0553*
2	---	---	0.3355	0.2741	0.1571	0.4912	0.1843
3	---	---	---	0.8748	0.5673	0.8132	0.6789
4	---	---	---	---	0.6737	0.7037	0.8004
5	---	---	---	---	---	0.4486	0.8524
6	---	---	---	---	---	---	0.5349
7	---	---	---	---	---	---	---

2. *Displays of Movements Away from Partner*

When data were pooled by sex, water-treated females and males of the control (Treatment 1) had the highest mean number of movements away from their partner during the 20-minute observation period, 42.3 times (Table 27). Methoxyfenozide-treated females and males (Treatment 4) had a much lower mean number (22.5 times). Remaining treatments had means ranging from 27.2 to 39.7 times (Table 27). Interestingly, these results were similar to results found for the behavior of towards movements, with the control having the higher mean number than that of the methoxyfenozide-treated moths. However, when considering all treatment types, the data analysis revealed no significant differences between treatments for this particular courtship behavior ($p>0.22$) (Table 27).

Pooling these data by treatment type revealed no significant differences between sexes in the mean number of times they moved away from their partner ($p>0.9$) (Table 28). Comparisons of mean away movements by sex within each treatment also found no significant differences between female and male moths (Table 29).

Significant differences were found when comparing the mean number of times female moths moved away from their male partners amongst all the treatment types (Table 30). For example, water-treated females (Treatment 1) moved away from water-treated males an average of 52.3 times, which was significantly greater than methoxyfenozide-treated females of Treatment 4 moved towards their methoxyfenozide-treated males (16.7 times) ($P<0.01$) (Table 30).

Females from Treatment 4 also had a significantly lower mean than water-treated females of Treatment 6 ($P<0.05$) (Table 30). There were no significant differences found when comparing males of all treatment types for this behavior (Table 31).

Table 25. The mean number of times paired male and female *Cydia pomonella* displayed movements away partner of opposite sex during courtship by treatment (sex data pooled).

Treatment No.	Treatment Exposure By Sex		Mean No. Times(±SE) Movement Away From Partner
	Males	Females	
1	water	water	47.3±7.27
2	water	methoxy	35.5±7.27
3	methoxy	water	28.0±6.47
4	methoxy	methoxy	22.5±6.78
5	water	surfact	27.2±8.08
6	surfact	water	39.7±7.27
7	surfact	surfact	28.2±6.83
P-value			0.22463

Table 26. The mean number of times paired male and female *Cydia pomonella* displayed movements away from partner of opposite sex during courtship (treatment data pooled).

Sex	Mean No. Times (±SE) Movement Away From Partner
Female	33.0±3.55
Male	32.5±3.54
P-value	0.9047

Table 27. Mean number times *Cydia pomonella*, by sex, displayed movement away from partner of opposite sex during courtship and the within-treatment P-value (Nonprotected LSD).

Sex	1	2	3	4	5	6	7
Female	52.3 (water)	27.6 (methoxy)	27.3 (water)	16.7 (methoxy)	30.0 (surfact)	45.0 (water)	32.3 (surfact)
Male	42.3 (water)	43.3 (water)	30.6 (methoxy)	28.2 (methoxy)	24.4 (water)	34.4 (surfact)	24.1 (surfact)
Pvalue	0.0701	0.2077	0.7682	0.3085	0.6856	0.1468	0.4820

Each methoxyfenozide treatment solution also contained a field rate of the surfactant Latron B-1956 (as per label recommendations). The surfactant treatment consisted of a labeled field rate (0.125%, vol:vol) of Latron B-1956 mixed with water.

Table 28. P-values of treatment comparisons of female *Cydia pomonella* regarding the mean number of times they displayed movement away from male partner using a Nonprotected LSD test.

Sex: Female	Treatment						
Treatment	1	2	3	4	5	6	7
1	---	0.0701	0.0532	0.0066**	0.1226	0.5923	0.1302
2	---	---	0.9833	0.4020	0.8714	0.2003	0.7209
3	---	---	---	0.3852	0.8497	0.1695	0.6887
4	---	---	---	---	0.3411	0.0302*	0.2152
5	---	---	---	---	---	0.2966	0.8662
6	---	---	---	---	---	---	0.3348
7	---	---	---	---	---	---	---

Table 29. P-values of treatment comparisons of male *Cydia pomonella* regarding the mean number of times they displayed movement away from female partner using a Nonprotected LSD test.

Sex: Male	Treatment						
Treatment	1	2	3	4	5	6	7
1	---	0.9411	0.3608	0.2800	0.2063	0.5588	0.1660
2	---	---	0.3214	0.2474	0.1822	0.5103	0.1440
3	---	---	---	0.8445	0.6455	0.7667	0.5986
4	---	---	---	---	0.7828	0.6343	0.7453
5	---	---	---	---	---	0.4802	0.9806
6	---	---	---	---	---	---	0.4323
7	---	---	---	---	---	---	---

3. *Wing Fanning Behavior*

Wing fanning was displayed the highest number of times (48.08) when both sexes were treated with water (Treatment 1). The methoxyfenozide-treated pair (Treatment 4) had the lowest mean (23.5 times) (Table 30). The mean number of times wings were fanned per 20-minute observation period for all remaining treatment types ranged from 27.7 to 41.5. There were no significant differences between treatment types for this behavior ($p>0.30$) (Table 30).

Pooling treatment data also revealed no significant differences between female and male moths in regards to mean number of times wing fanning was displayed ($p>0.80$) (Table 31). In addition, there were no significant differences in mean wing fanning between sexes within each treatment (Table 32).

When comparing females among treatments, water-treated females paired with water-treated males (Treatment 1) fanned their wings a mean of 50 times per observation period, while methoxyfenozide-treated females paired with methoxyfenozide-treated males (Treatment 4) fanned their wings significantly less (19.6 times) ($P<0.05$) (Tables 32-33). There were no significant differences for wing fanning found among males between treatments (Table 34).

Table 30. The mean number of times paired male and female *Cydia pomonella* displayed wing fanning during courtship by treatment (sex data pooled).

Treatment No.	Treatment Exposure By Sex		Mean No. Times (±SE) Wing Fanning
	Males	Females	
1	water	water	48.08±8.21
2	water	methoxy	33.0±8.21
3	methoxy	water	30.40±7.31
4	methoxy	methoxy	23.5±7.74
5	water	surfact	27.7±9.17
6	surfact	water	41.5±8.21
7	surfact	surfact	29.7±7.71
P-value			0.3964

Table 31. The mean number of times paired male and female *Cydia pomonella* displayed wing fanning during courtship (treatment data pooled).

Sex	Mean No. Times (±SE) Wing Fanning
Female	33.9±3.91
Male	32.9±3.89
P-value	0.8262

Table 32. Mean number times *Cydia pomonella*, by sex, displayed wing fanning during courtship and the within-treatment P-value (Nonprotected LSD).

Sex	1	2	3	4	5	6	7
Female	50.0 (water)	28.8 (methoxy)	26.4 (water)	19.6 (methoxy)	35.0 (surfact)	45.2 (water)	32.5 (surfact)
Male	46.1 (water)	37.2 (water)	34.4 (methoxy)	27.3 (methoxy)	20.4 (water)	37.8 (surfact)	27.0 (surfact)
P-value	0.7683	0.5256	0.4957	0.5181	0.3117	0.5730	0.6538

Each methoxyfenozide treatment solution also contained a field rate of the surfactant Latron B-1956 (as per label recommendations). The surfactant treatment consisted of a labeled field rate (0.125%, vol:vol) of Latron B-1956 mixed with water.

Table 33. P-values of treatment comparisons of female *Cydia pomonella* regarding the mean number of times they displayed wing fanning using a Nonprotected LSD test.

Sex: Female				Treatment			
Treatment	1	2	3	4	5	6	7
1	---	0.1571	0.9600	0.0349*	0.3439	0.7484	0.2270
2	---	---	0.8637	0.5197	0.7001	0.2730	0.8009
3	---	---	---	0.6142	0.5726	0.1840	0.6563
4	---	---	---	---	0.3173	0.0750	0.3532
5	---	---	---	---	---	0.5183	0.8732
6	---	---	---	---	---	---	0.3794
7	---	---	---	---	---	---	---

Table 34. P-values of treatment comparisons of male *Cydia pomonella* regarding the mean number of times they displayed wing fanning using a Nonprotected LSD test.

Sex: Male	Treatment						
Treatment	1	2	3	4	5	6	7
1	---	0.5493	0.4058	0.1926	0.1000	0.5769	0.1859
2	---	---	0.8422	0.4925	0.2815	0.9674	0.4792
3	---	---	---	0.6019	0.3454	0.8086	0.5860
4	---	---	---	---	0.6474	0.4664	0.9811
5	---	---	---	---	---	0.2645	0.6638
6	---	---	---	---	---	---	0.4535
7	---	---	---	---	---	---	---

4. *Antennal Stroking Behavior*

When the data are pooled by sex, there were no significant differences between treatment types in to the mean number of displays of antennal stroking ($p>0.07$) (Table 35). Means ranged from 0.3 to 0.9 times per 20-minute observation period. Pooling the treatment data revealed no significant differences in displays of antennal stroking between males and females, 0.5 and 0.6 times respectively (Table 36). In addition, comparisons of displays of antennal stroking revealed no significant differences between female and male moths within each individual treatment (Table 38).

When comparing only female displays of antennal stroking among the treatments, significant differences were found (Table 38). The methoxyfenozide-treated female paired with the water-treated male (Treatment 2) resulted in the highest mean of antennal stroking displayed, 1.2 times (Table 38). This mean was significantly higher than the water-treated females of Treatment 3 which displayed a mean of 0.4 times antennal stroking ($P<0.02$) (Tables 37-38). The surfactant-treated females paired with the water-treated males (Treatment 5) also had a significantly lower mean, 0.2 times, than the methoxyfenozide-exposed females of Treatment 2 ($P<0.01$) (Tables 37-38).

Significant differences were also found when comparing mean male antennal stroking among the treatments (Table 38). The water-treated males of the control (Treatment 1) displayed significantly higher mean number of antennal stroking, 0.8 times, than the methoxyfenozide-treated males (Treatment 3), 0.4 times ($P<0.05$) (Tables 37-38). The males of Treatment 1 also had a significantly greater mean number of antennal stroking displays than the surfactant-treated males of Treatment 7 ($P<0.05$) (Tables 37-38).

Table 35. The mean number of times paired male and female *Cydia pomonella* displayed antennal stroking during courtship by treatment (sex data pooled).

Treatment No.	Treatment Exposure By Sex		Mean No. Times (±SE) Antennal Stroking
	Males	Females	
1	water	water	0.8±0.17
2	water	methoxy	0.9±0.17
3	methoxy	water	0.3±0.15
4	methoxy	methoxy	0.5±0.15
5	water	surfact	0.5±0.18
6	surfact	water	0.5±0.17
7	surfact	surfact	0.3±0.16
P-value			0.0765

Table 36. The mean number of times paired male and female *Cydia pomonella* displayed antennal stroking during courtship (treatment data pooled).

Sex	Mean No. Times (±SE) Antennal Stroking
Female	0.6±0.08
Male	0.5±0.08
P-value	0.3740

Table 37. Mean number times *Cydia pomonella*, by sex, displayed antennal stroking during courtship and the within-treatment P-value (Nonprotected LSD).

Sex	1	2	3	4	5	6	7
Female	0.8 (water)	1.2 (methoxy)	0.4 (water)	0.5 (methoxy)	0.2 (surfact)	0.6 (water)	0.5 (surfact)
Male	0.8 (water)	0.7 (water)	0.1 (methoxy)	0.5 (methoxy)	0.7 (water)	0.5 (surfact)	0.1 (surfact)
P-value	1	0.115	0.3012	0.9397	0.1687	0.846	0.2744

Each methoxyfenozide treatment solution also contained a field rate of the surfactant Latron B-1956 (as per label recommendations). The surfactant treatment consisted of a labeled field rate (0.125%, vol:vol) of Latron B-1956 mixed with water.

Table 38. P-values of treatment comparisons of female *Cydia pomonella* regarding the mean number of times they displayed antennal stroking using a Nonprotected LSD test.

Sex: Female	Treatment						
Treatment	1	2	3	4	5	6	7
1	---	0.2447	0.2271	0.3760	0.0915	0.4376	0.3118
2	---	---	0.0154*	0.0352	0.0057**	0.0533	0.278
3	---	---	---	0.7300	0.5190	0.6973	0.8591
4	---	---	---	---	0.3432	0.9483	0.8746
5	---	---	---	---	---	0.3370	0.4309
6	---	---	---	---	---	---	0.8321
7	---	---	---	---	---	---	---

5. ***Displays of Raised Wings***

Water-treated males and females of the control treatment (Treatment 1) raised their wings the highest mean number of times (1.5) per observation period (Table 39). When considering all treatment types, the data analysis revealed a significant difference in mean number of times wings were raised between the control and remaining treatments ($P<0.05$) (Tables 39, 43).

When the data are pooled by treatment, males raised their wings significantly more times than females ($P<0.001$) (Table 40). When comparing this behavior by sex within each treatment, males treated with water raised their wings significantly more times than their female partners in 2 out of the 7 treatments (Table 41).

There were no significant differences found when comparing female-only means for displays of raised wings among treatments (Table 42). Water-treated males paired with water-treated females (Treatment 1) raised their wings significantly more times (2.8) than males from all the other treatments ($P<0.001$)(Tables 4).

Table 39. The mean number of times paired male and female *Cydia pomonella* displayed raised wings during courtship by treatment (sex data pooled).

Treatment No.	Treatment Exposure By Sex		Mean No. Times(±SE) Raised Wings
	Males	Females	
1	water	water	1.5±0.27
2	water	methoxy	0.1±0.27
3	methoxy	water	0.3±0.24
4	methoxy	methoxy	0.4±0.24
5	water	surfact	0.3±0.30
6	surfact	water	0.4±0.27
7	surfact	surfact	0.0±0.25
P-value			0.0246

Table 40. The mean number of times male and female *Cydia pomonella* displayed raised wings during courtship (treatment data pooled).

Sex	Mean No. Times (±SE) Raised Wings
Female	0.0±0.14
Male	0.8±0.14
P-value	0.0001

Table 41. Mean number times *Cydia pomonella*, by sex, displayed raised wings during courtship and the within-treatment P-value (Nonprotected LSD).

Sex	1	2	3	4	5	6	7
Female	1.3 (water)	0.0 (methoxy)	0.0 (water)	0.0 (methoxy)	0.01 (surfact)	0.0 (water)	0.0 (surfact)
Male	2.8 (water)	0.2 (water)	0.6 (methoxy)	0.8 (methoxy)	0.4 (water)	0.8 (surfact)	0.1 (surfact)
Pvalue	<.0001	<.0001	0.2362	0.0735	0.6243	0.1462	0.9092

Each methoxyfenozide treatment solution also contained a field rate of the surfactant Latron B-1956 (as per label recommendations). The surfactant treatment consisted of a labeled field rate (0.125%, vol:vol) of Latron B-1956 mixed with water.

Table 42. P-values of treatment comparisons of female *Cydia pomonella* regarding the mean number of times they displayed raised wings using a Nonprotected LSD test.

Sex: Female				Treatment			
Treatment	1	2	3	4	5	6	7
1	---	0.8081	0.8765	0.7974	0.9544	0.8081	0.8888
2	---	---	0.9193	1.0000	0.7748	1.0000	0.9121
3	---	---	---	0.9141	0.8371	0.9193	0.9902
4	---	---	---	---	0.7637	1.0000	0.9067
5	---	---	---	---	---	0.7748	0.8491
6	---	---	---	---	---	---	0.9121
7	---	---	---	---	---	---	---

Table 43. P-values of treatment comparisons of male *Cydia pomonella* regarding the mean number of times they displayed raised wings using a Nonprotected LSD test.

Sex: Male				Treatment			
Treatment	1	2	3	4	5	6	7
1	---	<0.0001**	<0.0001**	0.0002**	<0.0001**	0.0002**	<0.0001**
2	---	---	0.4826	0.2374	0.7324	0.3320	0.7797
3	---	---	---	0.6030	0.7534	0.7458	0.3066
4	---	---	---	---	0.4353	0.1038	0.1305
5	---	---	---	---	---	0.5527	0.5350
6	---	---	---	---	---	---	0.2012
7	---	---	---	---	---	---	---

6. Male-only Behaviors

a. Antennal Contact

The water-treated males from the control (Treatment 1) displayed significantly more mean antennal contacts with their female partners (6.7) than the males from all other treatments. Aside from the control, there were no significant differences in mean antennal contact among males between the remaining treatments (Tables 44, 45).

b. Lateral Abdominal Bending

The water-treated males from the control (Treatment 1) displayed the highest mean number of abdominal bendings (2.0 times) among all treatments (Table 46). However, when each treatment is compared with other treatments, males from the control (Treatment 1), displayed significantly more abdominal bending than all other treatments. Aside from the control, there were no significant differences in mean abdominal bending among males between remaining treatments (Table 47).

Table 44. The mean number of times paired male *Cydia pomonella* displayed antennal contact during courtship by treatment.

Treatment No.	Treatment Exposure By Sex		Mean No. Times (±SE) Antennal Contact
	Males	Females	
1	water	water	6.7±0.93
2	water	methoxy	1.8±0.93
3	methoxy	water	3.3±0.83
4	methoxy	methoxy	3.3±0.85
5	water	surfact	1.3±1.00
6	surfact	water	2.4±0.93
7	surfact	surfact	1.41±0.87
P-value			0.0774

Table 45. P-values of treatment comparisons of male *Cydia pomonella* regarding the mean number of times they displayed antennal contact towards female partner using a Nonprotected LSD test.

Sex: Male	Treatment						
Treatment	1	2	3	4	5	6	7
1	---	0.0003**	0.0069**	0.0079**	0.0001**	0.0012**	<0.0001**
2	---	---	0.2271	0.2273	0.7202	0.6505	0.7625
3	---	---	---	0.9883	0.1251	0.4650	0.1169
4	---	---	---	---	0.1260	0.4619	0.1183
5	---	---	---	---	---	0.4270	0.9379
6	---	---	---	---	---	---	0.4420
7	---	---	---	---	---	---	---

Table 46. The mean number of times male *Cydia pomonella* displayed lateral abdominal bending during courtship by treatment (sex data pooled).

Treatment No.	Treatment Exposure By Sex		Mean No. Times (±SE) Abdominal Bent Laterally
	Males	Females	
1	water	water	2.0±0.40
2	water	methoxy	0.3±0.40
3	methoxy	water	0.5±0.36
4	methoxy	methoxy	0.6±0.36
5	water	surfact	0.3±0.44
6	surfact	water	0.4±0.40
7	surfact	surfact	0.4±0.38
P-value			0.068

Table 47. P-values of treatment comparisons of male *Cydia pomonella* regarding the mean number of times they displayed antennal contact towards female partner using a Nonprotected LSD test.

Sex: Male	Treatment						
Treatment	1	2	3	4	5	6	7
1	---	0.0003**	0.0069**	0.0079**	0.0001**	0.0012**	<0.0001**
2	---	---	0.2271	0.2273	0.7202	0.6505	0.7625
3	---	---	---	0.9883	0.1251	0.4650	0.1169
4	---	---	---	---	0.1260	0.4619	0.1183
5	---	---	---	---	---	0.4270	0.9379
6	---	---	---	---	---	---	0.4420
7	---	---	---	---	---	---	---

CHAPTER V. DISCUSSION

A. Field Study

Many studies have found that exposure to insecticides and insect growth regulators have a negative impact on a moth's ability to orient to pheromone signals. For example, sublethal doses of a pyrethroid known as permethrin have been found to reduce adult male pink bollworms, *Pectinophora gossypiella* (Saunders), responsiveness to synthetic pheromone and decrease their upwind orientation (Floyd and Crowder 1981, Haynes and Baker 1985). In addition permethrin rendered female corn earworms, *Helicoverpa zea* (Boddie), less attractive to males in comparison to the nontreated females (Moore 1988). Similar results were found when the oriental fruit moth, *Grapholita molesta* (Busk), was exposed to neuroactive compounds, greatly suppressing the males ability to orient to pheromone sources (Linn and Roelofs 1985, Evenden et al. 2005).

Exposure to methoxyfenozide-treated surfaces reduced the sexual excitability, orientation and response to pheromone for not only male *C. pomonella* but the males of the redbanded leafroller, *Argyrotaenia velutinana* (Walker), and the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Hoelscher and Barrett (2003). Oriental fruit moths exposed to methoxyfenozide also displayed a reduction in their ability to orient to calling female moths in a wind tunnel (Reinke and Barrett 2007). A field study in small orchard blocks showed that adult moth exposure to methoxyfenozide treated surfaces significantly dampens male *C. pomonella* response to both calling females and synthetic pheromone lures (Barrett 2008).

In my field study, although the treated females consistently had a lower mean trap catch than that of the nontreated female lures, we found no significant differences between the female-baited traps. This was not too surprising due to the fact that prior studies have shown exposure to methoxyfenozide had less of a negative impact on the female's ability to attract males in comparison to the impact on male's responsiveness (Hoelscher and Barrett 2003). This was further supported by our field study when comparing the trap data from the control treatment of the orchard block to the treatment involving an application of methoxyfenozide. It was not until the orchard was treated with methoxyfenozide, exposing the wild males to treated surfaces, did we see a difference in trap catch among the five different lure types.

Typically, the 1 mg codlemone lure is the standard lure for monitoring *C. pomonella* populations. The 10 mg 'supercharged' lure is recommended in areas of mating disruption as the higher dosage overcomes sensory adaptation in the males (McBrien et al. 1998). Higher trap catches have also been found when using traps baits with higher doses in both orchard block with and without mating disruption (McBrien et al. 1998). Therefore, as expected, both the 1 mg and 10 mg codlemone lures were significantly more attractive to the males than either of the female-baited lures during our control treatment of the orchard block (see Table 17). Whereas, when the orchard block was treated with an application of methoxyfenozide, the results revealed no significant differences between the mean trap catch of the pheromone lures versus the female lures (see Table 18). This would indicate that exposing the male moths in the orchard to

methoxyfenozide reduced their responsiveness to pheromone lures that are normally considered to be greatly attractive.

When mating disruption and methoxyfenozide were both applied to the orchard block, the female-baited lures had the significantly lowest mean number of males per trap while the codlemone pheromone lures had the highest mean trap catch. It is surprising, however, that there were no significant differences between the 1 mg lure and the 10 mg codlemone lures. Several studies have found mating disruption to effectively suppress moth populations as well as reducing male response to pheromone and calling females (Barnes et al. 1992, Lawson et al. 1996). Therefore, the 10 mg codlemone lure is recommended in monitoring moth populations in areas undergoing mating disruption (Barrett 1995), because this 'supercharged' lure is more attractive to the males in areas that are saturated with female pheromone (Thompson et al. 2001).

Our data suggests that the application of methoxyfenozide diminished the male response to pheromone sources in such a way that the stronger 10 mg lure, typically more attractive under mating disruption, was no more attractive than the 1 mg lure. If this is the case, then an application of methoxyfenozide in tandem with mating disruption could further aid in disrupting the males ability to find the females. Conceivably, this would help suppress *C. pomonella* populations. Further investigations would be greatly benefited by including fruit infestations surveys and field studies involving multiple growing seasons to better assess the impact on *C. pomonella* populations. Methoxyfenozide diminishing the effectiveness of the 10 mg lure, however, is a hindrance to proper monitoring of

the population during mating disruption. This must be taken in consideration when using methoxyfenozide with mating disruptions as it is pivotal to timing treatment applications to achieve the highest amount of control.

More research on the physiological effects of methoxyfenozide on moths is needed to determine exactly how the orientation and response to pheromone is being suppressed. Many studies have found that insect growth regulators decrease moth fecundity and fertility (Sun and Barrett 1999, Knight 2000, Smagghe et al. 2004, Reinke and Barrett 2007). Therefore it is possible that these physiological alterations are also affecting the way in which female calling occurs or perhaps even changing the composition of the pheromone itself.

However, our studies found no difference between the treated and nontreated females as well as prior studies determining males to be much more sensitive to methoxyfenozide (Hoelscher and Barrett 2003). The disruption of normal sexual behavior and response could be due to an inability of the male moths to recognize and respond to pheromone plumes once exposed to methoxyfenozide. Further research is needed to determine the exact mechanisms behind the effects of methoxyfenozide on male response and sexual excitability to pheromone of the *C. pomonella*.

B. Laboratory Bioassay

It is well documented that neuroactive compounds have sublethal affects regarding moths mate-finding ability by reducing the males responsiveness or orientation to pheromone sources (Floyd and Crowder 1981, Haynes and Baker 1985, Linn and Roelofs 1985, Moore 1988, Evenden et al. 2005). Both tebufenozide and methoxyfenozide have also been reported to have deleterious affects on tortricid males sexual excitability as well as their ability to orient to pheromone (Hoelscher and Barrett 2003, Reinke and Barrett 2007, Barrett 2008). Additionally, exposure to these insect growth regulators has been shown to significantly reduce tortricid fertility and fecundity (Sun and Barrett 1999, Sun et al. 2000, Knight 2000).

The purpose behind our behavioral observations was to determine if methoxyfenozide also disrupts the close-range courtship behaviors of *C. pomonella*. In general, our results indicated that exposure to methoxyfenozide-treated surfaces did not stop the moths from initiating courtship behavior and was not disruptive to the mating process. However, when both the male and female were exposed to methoxyfenozide-treated surfaces (Treatment 4) there was a significant reduction in female behavior in regards to wing fanning and movement inside the mating arena.

Water-treated females paired with water-treated males moved towards and away from the male significantly more times than the methoxyfenozide-treated females paired with methoxyfenozide-treated males (see Table 24 and 28). Females movements way from the male should not be viewed strictly as rejection

of her partner as it was often observed during our behavioral trials that both the male and female would walk around each other in an excited state upon introduction to the mating arena. This walking in an excited state could play a part in sexual selection, as persistence in courtship displays the male's vigor, acting as an indicator of his gene quality (Rutowski 1981). Behavioral observations made on the obliquebanded leafroller, *Choristoneura rosaceana* (Harris), noted that one male attempted to mate with the female up to 27 times before being successful (Curkovic et al. 2006).

Studies have also shown that Lepidopteran males need a recovery period after mating wherein they will show less interest and persistence in mating with the female (Rutowski 1982, Perez and Wang 2004, Marcotte et al. 2007). Additionally, female fecundity in the black lyre moth, *Cnephasia jactatana* (Walker), was reduced by 40-50% if they had mated with a male that had previously mated (Perez and Wang 2004) Therefore, it is in the best interest of the female to mate with the most persistent and vigorous male to ensure her reproductive success. Our results show that treating females with methoxyfenozide and pairing her with a treated male made females less active in regards to walking around the mating arena. It is possible that methoxyfenozide is altering the females physiology in such a manner that she is less responsive to male stimulus and less willing to engage in sexual behavior that aid in mate choice.

This is further supported by our data showing that the methoxyfenozide-treated females paired with treated males also fanned their wings a significantly

less number of times than the females from the control group (see Table 33). Female moths utilize wing fanning to aid in the release of their pheromone, which also acts as a visual stimulus to male moths (Borden 1931, Baker and Carde 1979, Allan and Wang 2001). Studies have also found that methoxyfenozide negatively affects female calling by delaying the onset and frequency of pheromone release (Trimble et al. 2004, Dallaire et al. 2004). Suppression of both the walking and wing fanning of the female further support the possibility that methoxyfenozide is interacting with the female in such a way that her capability or willingness to display normal sexual behavior and pheromone release is diminished. More research is needed in the field, however, to determine if this same suppression of female mating behavior occurring in the lab would also take place in the wild. Apple volatiles stimulate female *C. pomonella* pheromone release (Yan et al. 2004) and it is possible that in the wild where the female has both stimulus from the male and kairomones from the host plant that methoxyfenozide may have little impact on the female.

It is interesting that there were no differences found in male wing fanning behavior amongst any of the different treatment types. Wing fanning in the presence of pheromone among males moth is considered a sign of sexual arousal, preparation for mating (Heath and Adams 1967, Judd et al. 2005, Curkovic et al. 2006) and correlated with the ability to hone in on pheromone sources (Baker and Carde 1979). However in such close proximity the male moths do not need to rely solely on 'smelling' the female (Traynier 1968, Doane 1968, Castrovillo and Carde 1980). Tactile sensations, such as seeing and touching the female, play a

larger role in inducing the short-range courtship behavior of the male. Castrovillo and Carde (1980) found that *C. pomonella* males will spend more time in close-range orientation when in proximity of dead female. Another study also found that blinding the moths, by covering their eyes with paint, affected the number of matings that occurred (Hutt and White 1977). Although our results indicate that methoxyfenozide does not suppress the male's sexual excitability when in close proximity to the female this may be in large part due to the visual and tactile stimulus the male is receiving.

Overall, we have concluded that methoxyfenozide will not prevent courtship behavior or mating in laboratory settings once the male and female are in close proximity. *C. pomonella* mating behavior is largely due to 'good' mating behaviors from the male and simple acceptance from the female. So although there was suppression of certain female sexual behaviors, these behaviors did not alter the female acceptance of the male *C. pomonella*. In regards to the male *C. pomonella* behavior, methoxyfenozide may hinder male reception of pheromone, however, when in close-range the female visual and tactile sensations can make up for inability to detect pheromone. More research into the physiological impacts of methoxyfenozide on both the male and female *C. pomonella* would be needed to determine the exact mode of actions.

LITERATURE CITED

Allan, Rachel A., and Q. Wang. 2001. Mating Behavior, and evidence for a female-released sex pheromone in *Wiseana copularis* (Meyrick) (Lepidoptera: Hepialidae). New Zealand Journal of Zoology Vol. 28: 257-262.

Ayoade, O., S. Morooka, and S. Tojo. 1996. Induction of macroptery, precocious metamorphosis, and retarded ovarian growth by topical application of precocene II, with evidence for its nonsystemic allaticidal effects in brown planthopper, *Nilaparvata lugens*. Journal of Insect Physiology Vol. 42: 529-540.

Babson, Al. 1963. Eradicating gypsy moth. Science Vol. 142: 447.

Baker, T.C., R.T. Carde, and W.L. Roelofs. 1976. Behavioral responses of male *Argyrotaenia velutinana* (Lepidoptera: Tortricidae) to components of its sex pheromone. Journal of Chemical Ecology Vol. 2: 333-352.

Baker, T.C., and R.T. Carde. 1979. Analysis of pheromone-mediated behavior in male *Grapholita molesta*, the oriental fruit moth (Lepidoptera: Tortricidae). Environmental Entomology Vol. 8: 956-968.

Barclay, Hugh J., and Gary J.R. Judd. 1995. Models for mating disruption by means of pheromone for insect pest control. Researches on Population Ecology Vol. 37: 239-247.

Barnes, Martin M., Jocelyn G. Millar, Philipp A. Kirsch, and David C. Hawks. 1992. *Cydia pomonella* (Lepidoptera: Tortricidae) control by dissemination of synthetic female sex pheromone. Journal of Economic Entomology Vol. 85: 1274-1277.

Barrett, B.A. 2008. Assessment of methoxyfenozide exposure on the sexual attractiveness and responsiveness of adult *Cydia pomonella* in small orchard blocks. Pest Management Science 64:916-922.

Bartell, R.J. 1982. Mechanisms of communication disruption by pheromone in the control of Lepidoptera: a review. Physiological entomology Vol. 7: 353-364.

Bau, J., D. Martinez, M. Renou, and A. Guerrero. 1999. Pheromone-triggered orientation flight of male moths can be disrupted by trifluoromethyl ketones. Chemical Senses Vol. 24: 473-480.

Bengtsson, Marie, Gerhard Karg, Philipp A. Kirsch, Jan Lofqvist, Arne Sauer, and Peter Witzgall. 1993. Mating disruption of pea moth *Cydia nigricana* F. (Lepidoptera: Tortricidae) by a repellent blend of sex pheromone and attraction inhibitors. Journal of Chemical Ecology Vol. 20: 871-887.

Beers, E.H., J.F. Brunner, M.J. Willet, and G.M. Warner. 1993 Orchard pest management: a resource book for the Pacific Northwest. Good Fruit Grower, Yakima, WA.

Beers, Elizabeth H., D. Max Suckling, Ronald J. Prokopy, and Jessus Avilla. 2003. Ecology and Management of Apple Arthropod Pests, pp.489-519. *In* D.C. Feree and I.J. Washington, eds. 2003. Apples: Botany, Production, and Uses. CABI publishing, Cambridge, MA.

Beroza, M. 1960. Insect attractants are taking hold. Journal of Agricultural Chemistry Vol. 15: 37-40.

Biddinger, D.J., and L.A. Hull. 1999. Sublethal effects of selected insecticides on growth and reproduction of a laboratory susceptible strain of tufted apple bud moth (Lepidoptera: Tortricidae). Journal of Economic Entomology Vol. 92: 314-324.

Biddinger, D.J., L.A. Jull, and B.A. McPheron. 1996. Cross-resistance and synergism in azinphosmethyl resistant and susceptible strains of tufted apple bud moth (Lepidoptera: Tortricidae) to various insect growth regulators and abamectin. Journal of Economic Entomology Vol. 89: 274-287.

Biddinger, D., L. Hull, H. Huang, B. McPheron, and M. Loyer. 2006. Sublethal effects of chronic exposure to tebufenozide on the development, survival, and reproduction of the tufted apple bud moth (Lepidoptera: Tortricidae). Journal of Economic Entomology Vol. 99: 834-842.

Borden, A.D. 1931. Some field observations on *Cydia pomonella* behavior. Journal of Economic Entomology Vol. 24: 1137-1145.

Borden, John H., and Catherine E. Slater. 2004. Induction of flight muscle degeneration by synthetic juvenile hormone in *Ips confuses* (Coleoptera: Scolytidae). Journal of Comparative Physiology Vol. 61: 366-368.

Bowers, W.S., Thompson, M.J., Uebel, E.C. 1965. Juvenile and gonadotropic activity of 10,11-epoxyfarnesenic acid methyl ester. Life Science Vol. 4: 2323-2331.

Bowers, W.S., Fales, H.M., Thompson, M.J., Uebel, E.C. 1966. Juvenile hormone: Identification of an active compound from balsam fir. Science Vol. 154: 1020-1021.

Bouvier, J.C., T. Boivin, D. Beslay, and B. Sauphanor. 2002. Age-dependent response to insecticides and enzymatic variation in susceptible and resistant *Cydia pomonella* larvae. Archives of Insect Biochemistry and Physiology Vol. 51: 55-56.

Brown, A.W.A. 1983. Nature and distribution of resistance, pp. 457-551. *In* G.P. Georghiou & T. Saito [eds.], Pesticide management and insecticide resistance. Academic, New York.

Brown, A.W.A. and R. Pal. 1971. Insecticide resistance in arthropods. Monograph Series no. 38. World Health Organization, Geneva, Switzerland.

Brown, T.M., and A.W.A. Brown. 1974. Experimental induction of resistance to a juvenile hormone mimic. Journal of Economic Entomology Vol. 67: 799-801.

Brunner, Jay, Steve Welter, Carrol Calkins, Rick Hilton, Elizabeth Beers, John Dunley, Tom Unruh, Alan Knight, Robert Van Steenwyk, and Phil Van Buskirk. 2002. Mating disruption of *Cydia pomonella*: a perspective from the Western United States. *In* Use of pheromones and other semiochemicals in integrated production. IOBC wprs Bulletin. Vol. 23.

Butt, B.A., and D.O. Hathaway. 1966. Female sex pheromone as attractant for male *Cydia pomonella*. Journal of Economic Entomology Vol. 24: 1137-1145.

Butt, B.A. 1975. Bibliography of the *Cydia pomonella*. United States Department of Agriculture, Agricultural Research Service, ARS W-31, 221pp.

Carde, Ring T. 1990. Principles of mating disruption, pp. 47-72. *In* Richard L. Ridgway, Robert M. Silverstein, May N. Inscoe [eds.], Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants. CRC Press, New York.

Carde, R.T., T.C. Baker and P.J. Castrovillo. 1977. Disruption of sexual communication in *Laspeyresia pomonella* (*Cydia pomonella*), *Grapholita molesta* (oriental fruit moth), and *G. prunivora* (lesser appleworm) with hollow fiber attractant sources. Entomologia Experimentalis Applicata. Vol. 22: 280-288.

Carde, R.T., T.C. Baker, and W.L. Roelofs. 2005. Ethological function of components of a sex attractant system for oriental fruit moth males, *Grapholita molesta* (Lepidoptera: Tortricidae). Journal of Chemical Ecology Vol. 1: 475-491.

Carde, Ring T., and Albert K. Minks. 1995. Control of moth pests by mating disruption: successes and constraints. Annual Review of Entomology Vol. 40: 559-583.

Carlson, Glenn R., Tarlochan S. Dhadialla, Ricky Hunter, Richard K. Jansson, Christine S. Jany, Zev Lidert, Richard A. Slawecki. 2001. The chemical and biological properties of methoxyfenozide, a new insecticidal ecdysteroid agonist. Pest Management Science Vol. 57: 115-119.

Carpenter, J.E., and L.D. Chandler. 1994. Effects of sublethal doses of two insect growth regulators on *Helicoverpa zea* (Lepidoptera: Noctuidae) reproduction. Journal of Entomological Science Vol. 29: 428-435.

Castrovillo, P. J., and R.T. Carde. 1979. Environmental regulation of female calling and male pheromone response periodicities in the *Cydia pomonella*. Journal of Insect Physiology Vol. 25: 659-667.

Castrovillo, P.J., and R.T. Carde. 1980. Male *Cydia pomonella* orientation to visual cues in the presence of pheromone and sequences of courtship behaviors. Annals of the Entomological Society of America 73: 100-105.

Chapman, Kathleen L. and Bruce A. Barrett. 1997. Susceptibility of male *Cydia pomonella* (Lepidoptera: Tortricidae) to azinphosmethyl in Missouri. Journal of Agricultural Entomology Vol. 14: 441-447.

Chapman, P.J., and Lienk, S.E. 1971. Tortricid Fauna of Apple in New York; Including an Account of Apples' Occurrence in the Stat, Especially as a Naturalized Plant. New York State Agricultural Experiment Station Bulletin, 703:20-22.

Chang, F., C. L. Hsu, L. Jurd, and D.L. Williamson. 1984. Effect of precocene and benzyl-1, 3-benzodioxole derivatives on sex attractancy in the Mediterranean fruit fly (Diptera: Tephritidae). Annals of the Entomological Society of America Vol. 77: 147-151.

Chen, T.Y., and T.X. Liu. 2002. Susceptibility of immature stage of *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) to pyriproxyfen, a juvenile hormone analog. Journal of Applied Entomology Vol. 126: 125-129.

Cerf, D.C., and G.P. Georghiou. 1972. Evidence of cross-resistance to a juvenile hormone analogue in some insecticide-resistant houseflies. Nature Vol. 239: 401-402.

Cohen, Ephraim. 1987. Chitin biochemistry: synthesis and inhibition. Annual Review of Entomology Vol. 32: 71-93.

Cohen, Ephraim. 1992. Chitin synthesis and degradation as targets for pesticide action. Archives of Insect Biochemistry and Physiology Vol. 22: 245-261.

Cordero, Roberto J., Jeffrey R. Bloomquist, and Thomas P. Kuhar. 2007. Susceptibility of two diamondback moth parasitoids, *Diadegma insulare* (Cresson) (Hymenopter; Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera; Eulophidae), to selected commercial insecticides. Biological Control Vol. 42: 48-54.

Croft, B.A. and H.W. Riedl. 1992. Chemical control and resistance to pesticides in *Cydia pomonella*, pp. 371-87. *In* L.P.S. van der Geest and J.J. Evenbuss [eds.], Tortricid pests: their biology, natural enemies and control. Elsevier, Amsterdam.

Croft, B.A. and S. Hoyt. 1978. Considerations for the use of pyrethroid insecticides for deciduous fruit pest control in the U.S.A. Environmental Entomology Vol. 7: 627-630.

Curkovic, Tomislav, Jay F. Brunner, and Peter J. Landolt. 2006. Courtship Behavior in *Choristoneura rosaceana* and *Pandemis pyrusana* (Lepidoptera: Tortricidae). Annals of the Entomological Society of America Vol. 99: 617-624.

Cutler, Christopher G., Cynthia D. Scott-Dupree, Jeffrey H. Tolman, and C. Ronald Harris. 2005. Acute and sublethal toxicity of novaluron, a novel chitin synthesis inhibitor, to *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Pest Management Science Vol. 61: 1060-1068.

Dallaire, R., A. Labrecque, M. Marcotte, E. Bauce, and J. Delisle. 2004. The sublethal effects of tebufenozide on the precopulatory and copulatory activities of *Choristoneura fumiferana* and *C. rosaceana*. Entomologia Experimentalis et Applicata Vol. 112: 169-181.

Dhadialla, Tarlochan S., Glenn R. Carlson, and Dat P. Le. 1998. New insecticides with ecdysteroidal and juvenile hormone activity. Annual Review of Entomology Vol. 43: 545-569.

Dethier, V.G. 1957. Chemoreception and the behavior of insects. Survey of Biological Process Vol. 3: 149-183.

Dinan, Laurence, and Rene Lafont. 2003. Effects and applications of arthropod steroid hormones (ecdysteroids) in mammals. Journal of Endocrinology Vol. 191: 1-8.

Doane, C.C. 1968. Aspects of mating behavior of the gypsy moth. Annals of the Entomological Society of America. Vol. 61: 768-773.

Dong-Soon Kim, Deborah J. Brooks, and Helmut Riedl. 2006. Lethal and sublethal effects of abamectin, spinosad, methoxyfenozide, and acetamiprid on the predaceous plant but *Deraeocoris brevis* in the laboratory. Biocontrol Vol. 51: 465-484.

Duportets, L., M.C. Dufour, F. Couillaud, and C. Gadenne. 1998. Biosynthetic activity of corpora allata, growth of sex accessory glands and mating in the male moth *Agrotis ipsilon* (Hufnagel). Journal of Experimental Biology Vol. 201: 2425-2432.

Duthie, Bruce, Gerhard Gries, Regine Gries, Christian Krupke, and Shannon Derksen. 2003. Does pheromone-based aggregation of *Cydia pomonella* larvae help procure future mates? Journal of Chemical Ecology Vol. 29: 425-436.

Dyte, C.E. 1972. Resistance to synthetic juvenile hormone in a strain of the flour beetle, *Tribolium castaneum*. Nature Vol. 238: 48-49.

Evans, R. G., A. Sunley, C. Bradford, and R. I. Patmore. 1995. Effects of fenoxycarb on development and reproduction of the Oriental cockroach, *Blatta orientalis*. Medical and Veterinary Entomology Vol. 9: 235-240.

Evenden, M. L., J. R. McLaughlin, and D. Czokajlo. 2005. Effects of exposure to pheromone and insecticide constituents of an attracticide formulation on reproductive behavior of oriental fruit moth (Lepidoptera: Tortricidae). Journal of Economic Entomology 98: 334-341.

Ferree, D.C., and I.J. Warrington. 2003. Apples: botany, production and uses. CAB International. Cambridge, Massachusetts.

Friedrich, Louis and Karl-Josef Schirra. 2001. Mating disruption of *Lobesia botrana* (Lepidoptera: Tortricidae) in vineyards with very high populations densities. *In* Pheromones for Insect Control in Orchards and Vineyards. IOBC wprs Bulletin. Vol. 24: 75-79.

Floyd, J.P., and L.A. Crowder. 1981. Sublethal effects of permethrin on pheromone response and mating of male pink bollworm moths [*Pectinophora gossypiella*]. Journal of Economic Entomology Vol. 74: 634-638.

Fluri, P.E., E. Mani, T. Wildbolz, and H. Arn. 1974. Untersuchungen uber dad paarungsverhalten des apfelwicklers (*Laspeyresia pomonella* L.) und uber den einfluss von kunstlichen sexuallockstoff auf die kopulationshaufig keit. Mitt Schweiz EntoMolecular Ges Vol. 51: 315-326.

Foster, S.P, and M.O. Harris. 1997. Behavioral Manipulation Methods for Insect Pest-Management. Annual Review of Entomology Vol. 42: 123-146.

Gaston, L.K., H.H. Shorey, and C.A. Saario. 1967. Insect population control by the use of sex pheromones to inhibit orientation between the sexes. Nature Vol. 213: 1155.

Geest, L.P.S., and H.H. Evenhuis, eds. 1991. Tortricid pests: their biology, natural enemies, and control. Amsterdam: New York: Elsevier.

Glancey, B.M., and W.A. Banks. 1988. Effect of the insect growth regulator fenoxycarb on the ovaries of queens of the red imported fire ant (Hymenoptera: Formicidae). Annals of the Entomological Society of America Vol. 81: 642-648.

Glare, Travis R. and Maureen O'Callaghan. 1999. Environmental and health impacts of the insect juvenile hormone analogue, S-methoprene. Report for the Ministry of Health. Grasslands Division, AgResearch, Lincoln, Nebraska.

Gotz, B. 1951. The sexual odoriferous substance of Lepidoptera. Experientia Vol. 7: 406-18.

Gore, J., and J.J. Adamczyk, Jr. 2004. Laboratory selection for beet armyworm (Lepidoptera: Noctuidae) resistance to methoxyfenozide. The Florida Entomologist Vol. 87: 450-453.

Grant, C.C., and V.E. Brady. 1975. Courtship behavior of phycitid moths. I. Comparison of *Plodia interpunctella* and *Cadra cautella* and role of male scent glands. Canadian Journal of Zoology Vol. 53: 813-826.

Gut, L. J. and J. F. Brunner. 1998. Pheromone-based management of *Cydia pomonella* (Lepidoptera: Tortricidae) in Washington apple orchards. Journal of Agricultural Entomology 15: 387-406.

Harmon, Margaret A., Marcus F. Boehm, Richard A. Heyman, and David J. Mangelsdorf. 1995. Activation of mammalian retinoid X receptors by the insect growth regulator methoprene. Proceedings of the National Academy of Sciences 92: 6157-6160.

Haseeb, M., H. Amano, and H. Nemoto. 2000. Pesticidal effects on mortality and parasitism rates of *Diadegma semiclausum*, a parasitoid of the diamondback moth. BioControl Vol. 45: 165-178.

Haseeb, M. and H. Amano. 2002. Effects of contact, oral and persistent toxicity of selected pesticides on *Cotesis plutella* (Hym., Braconidae), a potential parasitoid of *Plutella xylostella* (Lep., Plutellidae). Journal of Applied Entomology Vol. 126: 8-13.

Haynes, K.F. and T.C. Baker. 1985. Sublethal effect of permethrin on the chemical communication system of the pink bollworm moth, *Pectinophora gossypiella*. Archives of Insect Biochemistry and Physiology Vol. 2: 283-293.

Haynes, J.W., and J.W. Smith. 1994. Diflubenzuron plus cottonseed oil: effects on boll weevil (Coleoptera: Curculionidae) cuticle hardness, mating, and flight. Journal of Economic Entomology Vol. 87: 339-344.

Heath, E.H., and P.A. Adams. 1967. Regulation of heat production by large moths. Journal of Experimental Biology Vol. 47: 21-23.

Heath, R.R., P.J. Landolt, B.D. Dueben, R.E. Murphy, and R.E. Schneider. 1992. Identification of male cabbage looper sex pheromone attractive to females. Journal of Chemical Ecology Vol. 18: 441-453.

Hoelscher, Jennifer A., and Bruce A. Barrett. 2003. Effect of methoxyfenozide-treated surfaces on the attractiveness and responsiveness of adult codling. Journal of Economic Entomology Vol. 96: 623-628.

Horowitz, A. Rami, and Isaac Ishaaya. 1994. Managing resistance to insect growth regulators in the sweetpotato whitefly (Homoptera: Aleyrodidae). Journal of Economic Entomology Vol. 87: 866-871.

Horowitz, A.R., Z. Mendelson, M. Cahill, I. Denholm, and I. Ishaaya. 1999. Managing resistance to the insect growth regulator, pyriproxyfen, in *Bemisisa tabaci*. Pesticide Science Vol. 55: 272-276.

Howell J.F., A.L. Knight, T.R. Unrug, D.F. Brown, J.L. Krysan, C.R. Sell, P.A. Kirsch. 1992. Control of *Cydia pomonella* in apple and pear with sex pheromone-mediated mating disruption. Journal of Economic Entomology Vol. 85: 918-925.

Howell, J.F., and K.D. Thorp. 1972. Influence of mating on attractiveness of female *Cydia pomonella*. Environmental Entomology Vol. 1: 125-126.

Howell, J.F., R.B. Hutt, and W.B. Hill. 1978. *Cydia pomonella*: mating behaviour in the laboratory. Annals of the Entomological Society of America Vol. 3: 522-524.

Hutt, R.B., and L.D. White. 1977. Mating response to visual stimulus in the male *Cydia pomonella*. Environmental Entomology Vol. 1: 125-126.

Irigaray, Francisco-Javier Saenz-de-Cabezon, Vicente Marco, Frank G. Zalom, and Ignacio Perez-Moreno. 2005. Effects of methoxyfenozide on *Lobesia botrana* Den & Schiff (Lepidoptera: Tortricidae) egg, larval and adult stages. Pesticide Science Vol. 61: 1133-1137.

Ishaaya, I., and A.R. Horowitz. 1992. Novel phenoxy juvenile hormone analog (pyriproxyfen) suppresses embryogenesis and adult emergence of sweetpotato whitefly (Homoptera: Aleyrodidae). Journal of Economic Entomology Vol. 85: 2113-2117.

Johansen, Carl A. 1957. History of Biological Control of Insects in Washington Northwest Science Vol. 31: 57-79.

Judd, G.J.R., M.G.T. Gardiner, and D.R. Thomson. 2004. Control of *Cydia pomonella* in organically-managed apple orchards by combining pheromone-mediated mating disruption, post-harvest fruit removal and tree banding. Entomologia Experimentalis et applicata Vol. 83: 137-146.

Judd, Gary J.R., Mark G.T. Gardiner, Naomi C. Delury, and Gerhard Karg. 2005. Reduced antennal sensitivity, behavioral response, and attraction of male *Cydia pomonella* to their pheromone (E,E)-8, 10-dodecadien-1-ol following various pre-exposure regimes. Entomologia Experimentalis et applicata Vol. 114: 65-78.

Jumean, Z., R. Gries, T. Unruh, E. Rowland, and G. Gries. 2005. Identification of the larval aggregation pheromone of *Cydia pomonella*. Journal of Chemical Ecology 31: 911-924.

Kettlewell, H.B.D. 1960. Further observations on *Panaxia dominula*. The Florida Entomologist Vol. 79: 31-32.

King, Edward J. 2005. Ovicidal activity of Noviflumuron when fed to adult german cockroaches (Dictyoptera: Blattellidae). Journal of Economic Entomology Vol. 98: 930-932.

Knight, A. L., M. Weiss, and T. Weissling. 1994a. Diurnal patterns of adult activity of four orchard pests (Lepidoptera: Tortricidae) measured by timing trap and actograph. Journal of Agricultural Entomology 11: 125-136.

Knight, A. L., J.F. Brunner, and D. Alston. 1994b. Survey of azinphosmethyl resistance in *Cydia pomonella* (Lepidoptera: Tortricidae) in Washington and Utah. Journal of Economic Entomology 87: 285-292.

Knight, A. 1996. The impact of *Cydia pomonella* (Lepidoptera: Tortricidae) mating disruption on apple pest management in Yakima Valley, Washington. Journal of the Entomological Society of British Columbia Vol. 92: 29-38.

Knight, A.L. 2000. Tebufenozide targeted against *Cydia pomonella* (Lepidoptera: Tortricidae) adults, eggs, and larvae. Journal of Economic Entomology Vol. 93: 1760-1767.

Kobayashi, S., Aida, S., Kobayashi, M., Nonoshita, K. 1992. Studies on resistance of diamondback moth to insect growth regulators. In Talekar, N.T. (ed). 1992. Management of Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop. Shanhua, Taiwan: Asian Vegetable Research and Development Center, pp 383-390.

Landolt, Peter J. 1995. Attraction of female cabbage looper moths (Lepidoptera: Noctuidae) to males in the field. The Florida Entomologist Vol. 78: 96-100.

Landolt, P.J., and Robert R. Heath. 1990. Sexual role reversal in mate-finding strategies of the cabbage looper moth. Science Vol. 249: 1026-1028.

Landolt, P.J., R.W. Hofstetter, and P.S. Chapman. 1998. Neonate *Cydia pomonella* larvae (Lepidoptera: Tortricidae) orient anemotactically to odor of immature apple fruit. The Pan-Pacific Entomologist 74: 140-149.

Lawson, D.S., W.H. Reissig, A.M. Agnello, J.P. Nyrop, and W.L. Roelofs. 1996. Interference with the mate-finding communication system of the obliquebanded leafroller (Lepidoptera: Tortricidae) using synthetic sex pheromones. Environmental Entomology Vol. 25: 895-905.

Leonhardt, B.A., V.C. Mastro, D.S. Leonard, W. McLane, R.C. Reardon, and K.W. Thorpe. 1996. Control of low-density gypsy moth (Lepidoptera: Lymantriidae) populations by mating disruption with pheromone. Journal of Chemical Ecology Vol. 22: 1255-1272.

Linn, C. E., Jr., and W. L. Roelofs. 1984. Sublethal effects of neuroactive compounds on pheromone response thresholds in male Oriental fruit moths. Archives of Insect Biochemistry and Physiology Vol. 1: 331-344.

Liu, T.X., and P.A. Stansly. 1997. Effects of pyriproxyfen on three species of *Encarsia* (Hymenoptera: Aphelinidae), endoparasitoids of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Journal of Economic Entomology Vol. 90: 404-411.

Liu, T.X. and P.A. Stansly. 2004. Lethal and sublethal effects of two insect growth regulators on adult *Delphastus catalinae* (Coleoptera: Coccinellidae), a predator of whiteflies (Homoptera: Aleyrodidae). Biological Control Vol. 30: 298-305.

Liu, Tong-Xian, and Tian-Ye Chen. 2000. Effects of the chitin synthesis inhibitor buprofezin on survival and development of immatures of *Chrysoperla ruvilabris* (Neuroptera: Chrysopidae). Journal of Economic Entomology Vol. 92:234-239.

Liu, Tong-Xian, and Tian-Ye Chen. 2001. Effects of a juvenile hormone analog, pyriproxyfen, on the apterous form of *Lipaphis erysimi*. Entomologia Experimentalis et Applicata Vol. 98: 295-301.

Machado, J., J. Coimbra, F. Castilho and C. Sa. 2005. Effects of diflubenzuron on shell formation of the freshwater clam, *Anodonta cygnea*. Archives of Environmental Contamination and Toxicology Vol. 19: 35-39.

Marcotte, M., J. Delisle, and J. N. McNeil. 2006. Effects of different male remating intervals on reproductive success of *Choristoneura rosaceana* males and females. Journal of Insect Physiology Vol. 53: 139-145.

Masner, P., M. Angst, and S. Dorn. 1987. Fenoxycarb, an insect growth regulator with juvenile hormone activity: a candidate for *Heliothis virescens* (F.) control on cotton. Pesticide Science Vol. 18: 89-94.

McBrien, H.L., G.J.R. Judd, and J.H. Borden. 1998. Development of pheromone-based mating disruption for control of the eye-spotted bud moth, *Spilonota ocellana*. Entomologia Experimentalis et Applicata Vol. 88: 101-107.

Medina, Pilar, Guy Smagghe, Flor Budia, Pedro del Estal, Luc Tirry, and Elisa Vinuela. 2002. Significance of penetration, excretion, and transovarial uptake to toxicity of three insect growth regulators in predatory lacewing adults. Archives of Insect Biochemistry and Physiology Vol. 51: 91-101.

Metcalf, Robert L., and William H. Luckmann, ed. 1975. Introduction to Insect Pest Management. A Wiley-Interscience Publication.

Minks, A.K., and R.T. Carde. 1988. Disruption of pheromone communication in moths: is the natural blend really most efficacious? Entomologica Experimentalis Applicata Vol. 49: 25-36.

Miyamoto, J., M. Hirano, Y. Takimoto and M. Hatakoshi. 1993. Insect growth regulators for pest control, with emphasis on juvenile hormone analogs: Present status and future prospects. ACS Symposium Series, ACS, Washington, DC. 524: 144-168.

Moffitt, H. R. & P. H. Westigard. 1984. Suppression of the *Cydia pomonella* (Lepidoptera: Tortricidae) population on pears in southern Oregon through mating disruption with sex pheromone. Journal of Economic Entomology Vol. 77: 1513-1519.

Moffitt, H. R., P.H. Westigard, K.D. Mantey, and H.E. Van de Baan. 1988. Resistance to diflubenzuron in the codling moth (Lepidoptera: Tortricidae). Journal of Economic Entomology Vol. 81: 1511-1515.

Mondal, K.A.M.S.H and Selina Parween. 2000. Insect growth regulators and their potential in the management of stored-product insect pests. Integrated Pest Management Reviews Vol. 5: 255-295.

Mommaerts, Veerle, Guido Sterk, and Guy Smagghe. 2006. Hazards and uptake of chitin synthesis inhibitors in bumblebees *Bombus terrestris*. Pest Management Science Vol. 62: 752-758.

Moore, R. F. 1988. Inhibition of chemical communication between male and female bollworms (Lepidoptera: Noctuidae) by sublethal amounts of permethrin. Journal of Economic Entomology Vol. 81: 78-82.

Moulton, John K., David A. Pepper, Richard K. Jansson, and Timothy J. Dennehy. 2002. Pro-active management of beet armyworm (Lepidoptera: Noctuidae) resistance to tebufenozide and methoxyfenozide: baseline monitoring, risk assessment, and isolation of resistance. Journal of Economic Entomology Vol. 95: 414-424.

Mulder, R. and M.T. Gijswijt. 1973. The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. Pesticide Science Vol. 4: 737-745.

Nates, S.F., C.L. McKenney. 2000. Growth, lipid class and fatty acid composition in juvenile mud crabs (*Phtihropanopeus harrisii*) following larval exposure to fenoxycarb, insect juvenile hormone analog. Comparative Biochemistry and Physiology Vol. 127: 317-325.

Perng, F.S., M.C. Yao, C.F. Hung, and C.N. Sun. 1988. Teflubenzuron resistance in diamondback moth (Lepidoptera:Plutellidae). Journal of Economic Entomology Vol. 81:1277-1282.

Perveen, F. 2000. Sublethal effects of chlorfluazuron on reproductivity and viability of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Journal of Applied Entomology Vol. 124: 223-231.

Pfeiffer, D.G., W. Kaakeh, J.C. Killian, M.W. Lachance, P. Kirsch. 1993. Mating disruption for control of damage by *Cydia pomonella* in Virginia apple orchards. Entomologica Experimentalis Applicata Vol. 67: 57-64.

Pedigo, L.P, and M. E. Rice. 2006. Integrated Pest Management, 5th edition. Hamiliton Printing, Columbus, OH.

Perez, A.J., and Q. Wang. 2004. Male remating behavior and its effect on female reproductive fitness in *Cnephasia jactatana* Walker (Lepidoptera: Tortricidae). Journal of Insect Behavior Vol. 17: 685-694.

Pidea, Samuel, Marcela-Ines Schneider, Guy Smagghe, Ana-Mabel Martinez, Pedro Del Estal, Elisa Vinuela, Javier Valle, and Flor Budia. 2007. Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae). Journal of Economic Entomology Vol. 100: 773-780.

Pree, D.J., R.M. Trimble, K.J. Whitty, P.M. Vickers. 1994. Control of Oriental fruit moth by mating disruption using sex pheromone in the Niagara Peninsula, Ontario. The Canadian Entomologist Vol. 126: 1287-1299.

Proverbs, M.D. 1965. The sterile male technique for *Cydia pomonella* control: 10 years of progress. Fruit Grower Vol. 19: 10-20.

Reede, R. H. de, R. F. Groendijk, and A.K.H. Wit. 1984. Field tests with the insect growth regulators, epofenonane and fenoxycarb, in apple orchards against leafrollers and side-effects on some leafroller parasites. Entomologia Experimentalis et Applicata Vol. 35: 275-281.

Reed, H. C., and P. J. Landolt. 2002. Attraction of mated female *Cydia pomonella* (Lepidoptera: Tortricidae) to apples and apple odor in a flight tunnel. The Florida Entomologist 85: 324-329.

Reinke, Michael D., and Bruce A. Barrett. 2007. Sublethal exposure to methoxyfenozide-treated surfaces reduces the attractiveness and responsiveness in adult oriental fruit moth (Lepidoptera: Tortricdae). Journal of Economic Entomology Vol. 100: 72-78.

Reissig, W.H., M. Novak, and W.L. Roelofs. 1978. Orientation disruption of *Argyrotaenia velutinana* and *Choristoneura rosaceana* male moths. Environmental Entomology Vol. 7: 631-632.

Retnakaran, A., J. Granett, and T. Ennis. 1985. Insect growth regulators, pp. 529-601. *In* G.A. Kerkut and L.I. Gilbert [eds.], Comprehensive insect physiology, biochemistry, and pharmacology. Pergamon Press, Oxford; New York.

Retnakaran, Arthur, Peter Krell, Qili Feng, and Basil Aril. 2003. Ecdysone agonists: mechanism and importance in controlling insect pests of agriculture and forestry. Archives of Insect Biochemistry and Physiology Vol. 54: 187-199.

Retnakaran, Arthur, Ivan Gelbic, Meenakshi Sundaram, William Tomkins, Tim Ladd, Mark Primavera, Qili Feng, Basil Arif, Reddy Palli, Peter Krell. 2000. Mode of action of the ecdysone agonist tebufenozide (RH-5992), and an exclusion mechanism to explain resistance to it. Pest Management Science Vol. 57: 951-957.

Rodriguez, L.M., J.A. Ottea, and T.E. Reagan. 2001. Selection, egg viability, and fecundity of the sugarcane borer (Lepidoptera: Crambidae) with tebufenozide. Journal of Economic Entomology Vol. 94: 1553-1557.

Rojas, M.G., and J.A. Morales Ramos. 2004. Effects of 3 chitin synthesis inhibitors on egg viability and survival of *Coptotermes formosanus*, (Isoptera: Rhinotermitidae) incipient reproductive adults. Annals of the Entomological Society of America. Publication Request.

Ross, M.H. and D.G. Cochran. 1991. Effects on German cockroach nymphs of contact exposure to IGRS, singly and in combination. Entomologia Experimentalis et Applicata Vol. 61: 117-122.

Rothschild, G.H.L. 1982. Suppression of mating in *Cydia pomonella* with synthetic sex pheromone and other compounds. In: A.F. Kydonieus and M.B. Beroza, [eds.]. Insect Suppression with controlled release pheromone systems. CRC Press, Boca Raton, FL.

Rumpf, S., C. Franipton, and D.R. Dietrich. 1998. Effects of conventional insecticides and insect growth regulators on fecundity and other life-table parameters of *Micromus tasmaniae* (Neuroptera: Hemerobiidae). Journal of Economic Entomology Vol. 91: 34-40.

Rupes, V., J. Zdarek, E. Svandova, and J. Pinterova. 1976. Cross-resistance to a juvenile hormone analogue in wild strains of the housefly. Entomologia Experimentalis et Applicata Vol. 19: 57-64.

Rutowski, R. L. 1981. Mate choice and Lepidopteran mating behavior. Florida Entomologist Vol. 65: 72-79.

SAS Institute. 2004. Version 9.1. SAS Institute Inc., Cary, North Carolina.

Satoh, G.T., F.W. Plapp, J.E. Slosser. 1995. Potential of juvenoid growth regulators for managing cotton aphids (Homoptera: Aphididae). Journal of Economic Entomology Vol. 88: 254-258.

Sauphanor, Benoit and Jean C. Bouvier. 1995. Cross-resistance between benzoylureas and benzoylhrdrazines in the *Cydia pomonella*. 1995. Pesticide Management Science Vol. 45: 369-375.

Sauphanor, B, V. Brosse, C. Monier, and J.C. Bouvier. 1998. Differential ovicidal and larvicidal resistance to benzoylureas in the *Cydia pomonella*. Entomomlogia Experimentalis et Applicata Vol. 88: 247-253.

Schneider, Marcela; Guy Smagghe, Antonio Gobbi, and Elisa Vinuela. 2003. Toxicity and pharmacokinietics of insect growth regulators and other novel insecticides on pupae of *Hyposoter didymator* (Hymenoptera: Ichneumonidae), a parasitoid of early larval instars of lepidopteran pests. Journal of Economic Entomology Vol. 96: 1054-1065.

Schmialek, P. 1961. Die Identifizierung zweier in Tenebriokot und in Hefe vorkommender Substanzen mit juvenilhormonwirkung. Zeitschrift fur Naturforschung Vol. 16: 461-464.

Schwink, I. 1958. A study of olfactory stimuli in the orientation of. moths. 10th Proceedings of the International Conference of Entomology 2: 577-582.

Seth, R.K., J.J. Kaur, D.K. Rao, and S.E. Reynolds. 2004. Effects of larval exposure to sublethal concentrations of the ecdysteroid agonist RH-5849 and tebufenozide (RH-5992) on male reproductive physiology in *Spodoptera litura*. Journal of Insect Physiology Vol. 50,

Shalom, U., and M.P. Pener. 1986. Endocrine manipulations and ontogenesis of male sexual behaviour in *Locusta*: studies on precocene-induced early adult forms. Physiological Entomology Vol. 11: 441-452.

Shorey, H.H. 1973. Behavioral responses to insect pheromones. Annual Review of Entomology Vol. 18: 348-380.

Shorey, H.H., L.K. Gaston, and R.N. Jefferson. 1968. In "Advances in Pest Control Research." (Ed., R.L. Metcalf). Interscience Publishers, New York.

Smagghe, Guy and Danny Degheele. 1994. Effects of the ecdysteroid agonists RH-5849 and RH-5992, alone and in combination with a juvenile hormone analogue, pyriproxyfen, on larvae of *Spodoptera exigua*. Entomologia Experimentalis et Applicata Vol. 72: 115-123.

Smagghe, G., D. Bylemans, P. Medina, F. Budia, J. Avilla, and E. Vinuela. 2004. Tebufenozide distorted *Cydia pomonella* larval growth and reproduction, and controlled field populations. Annals of Applied Biology 145: 291-298.

Smirle, M.J., D.T. Lowery, and C.L. Zurowski. 2002. Resistance and cross-resistance to four insecticides in populations of obliquebanded leafroller (Lepidoptera: Tortricidae). Journal of Economic Entomology Vol. 92: 820-825.

Soltani, N., and N. Soltani-Mazouni. 1992. Diflubenzuron and oogenesis in the *Cydia pomonella* (L.). Pesticide Science 34: 257-261.

Staal, G.B. 1975. Insect growth regulators with juvenile hormone activity. Annual Review of Entomology Vol. 20:417-460.

Sun, X., and B. A. Barrett. 1999. Fecundity and fertility changes in adult *Cydia pomonella* (Lepidoptera: Tortricidae) exposed to surfaces treated with the ecdysteroid agonists tebufenozide and methoxyfenozide. Entomologia Experimentalis et Applicata Vol. 94: 75-83.

Sun, X., B.A. Barrett, and D.J. Biddinger. 2000. Fecundity and fertility reduction in adult leafrollers exposed to surfaces treated with the ecdysteroid agonists tebufenozide and methoxyfenozide. Entomologia Experimentalis et Applicata Vol. 94: 75-83.

Tadic, M. 1963. The biology of the *Carpocapsa pomonella* (L.) as the basis for its control. OTS 60-21681 NOLIT Publ., Belgrade. 96p.

Teal, P.E.A., Y. Gomez-Sumuta, and A.T. Proveaux. 2000. Mating experience and juvenile hormone enhance sexual signaling and mating in male Caribbean fruit flies. Proceeding of the National Academy of Sciences Vol. 97: 3708-3712.

Thompson, Don, Jay Brunner, Larry Gut, Gary Judd, and Alan Knight. 2001. Ten years implementing *Cydia pomonella* mating disruption in the orchards of Washington and British Columbia: starting right and managing for success! Pheromones for Insect Control in Orchards and Vineyards. IOBC wprs Bulletin, Vol. 24: 23-30.

Traynier, R.M.M. 1968. Sex attraction in the Mediterranean flour moth, *Anagastas kuhniella*: location of the female by the male. The Canadian Entomologist Vol. 100: 50-10.

Trimble, R.M., D.J. Pree, and N.J. Carter. 2001. Integrated control of oriental fruit moth (Lepidoptera: Tortricidae) in peach orchards using insecticide and mating disruption. Journal of Economic Entomology Vol. 94: 476-485.

Trimble, R. M., A. M. El-Sayed, and D. J. Pree. 2004. Impact of sub-lethal residues of azinphos-methyl on the pheromone-communication systems of insecticide-susceptible and insecticide-resistant obliquebanded leafrollers *Choristoneura rosaceana* (Lepidoptera: Tortricidae). Pest Management Science Vol. 60: 660-668.

Trisyono, A. and G.M. Chippendale. 1998. Effect of the ecdysone agonists, RH-2485 and tebufenozide, on the southwestern corn borer, *Diatraea grandiosella*. Pesticide Science Vol. 53: 177-185.

Trumble, John T., and Benito Alvarado-Rodriguez. 1993. Development and economic evaluation of an IPM program for fresh market tomato production in Mexico. Agriculture, Ecosystems & Environment Vol. 43: 267-284.

Tunaz, Hasan and Nedim Uygun. 2004. Insect growth regulators for insect pest control. Turkish Journal of Agriculture and Forestry Vol: 28: 377-387.

Verloop, A., and C.D. Ferrell. 1977. Benzoylphenyl ureas—a new group of larvicides interfering with chitin deposition. In: Pesticide Chemistry in the 20th Century. Plimmer J.R., ed. ACS Symposium, Series 37. American Chemical Society, Washington, D.C. 237-270.

Vinson, Bradleigh S., and Frederick W. Plapp. 1974. Third generation pesticides: the potential for the development of resistance by insects. Journal of Agriculture and Food Chemistry Vol 22: 356-359.

Villavaso, E.J., J.W. Haynes, W.L. McGovern, R.G. Jones, and J.W. Smith. 1995. Difulbenzuron effects on boll weevils (Coleoptera: Curculionidae) in small field cages. Journal of Economic Entomology Vol. 88: 1631-1633.

Wakgari, W. 1997. A comparison of efficacies of bran-based baits containing diflubenzuron, teflubenzuron, and fenitrothion against desert locust, *Schistocerca gregaria* (Forsk.) (Orthoptera: Acridida). International Journal of Pest Management Vol. 43: 163-167.

Waldstein, D.E., W.H. Reissig, J.G. Scott, and R.W. Straub. 1999. Susceptibility of obliquebanded leafroller (Lepidoptera: Tortricidae) populations from commercial apple orchards and an unsprayed habitat in New York to tebufenozide. Journal of Economic Entomology Vol. 92: 1251-1255.

Wearing, C. Howard. 1999. Cross-resistance between azinphos-methyl and tebufenozide in the greenheaded leafroller, *Planotortix octo*. Pesticide Science Vol. 54: 203-211.

Webb, R.E., B.A. Leonhardt, J.R. Plimmer, K.M. Tatman, V.K. Boyd, D.L. Cohen, C.P. Schwalve, L.W. Douglass. 1990. Effect of racemic disparlure released from grids of plastic ropes on mating success of gypsy moth (Lepidoptera: Lymantriidae) as influenced by dose and by population density. Journal of Economic Entomology Vol. 83: 910-916.

Webb, T.J., S. Shu, S.B. Ramaswamy, and S. Dorn. 1999. Influence of juvenile hormone and mating on oogenesis and oviposition in the *Cydia pomonella*. Archives of Insect Biochemistry and Physiology Vol. 41: 186-200.

Weiland, R. Tim, F. David Judge, Teun Pels, and Arnold C. Grosscurt. 2002. A literature review and new observations on the use of diflubenzuron for control of locusts and grasshoppers throughout the world. Journal of Orthoptera Research Vol. 11: 43-54.

Wigglesworth, V.B. 1936. The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (Hemiptera). Quarterly Journal of Microscopic Science Vol. 77: 91-121.

Williams, C.M. 1956. The juvenile hormone of insects. Nature Vol. 178: 212-213.

Williams, C.M. 1967a. The juvenile hormone II. Its role in the endocrine control of molting, pupation, and adult development in the cecropia silkworm. Biological Bulletin Vol. 121: 572-585.

Williams, C.M. 1967b. Third-generation pesticides. Scientific American Vol. 217: 13-17.

Willis, Mark A., and Martin C. Birch. 1982. Male lek formation and female calling in a population of the arctiid moth *Estigmene acrea*. Science Vol. 218: 168-170.

Willis, M.A., and T.C. Baker. 1994. Behavior of flying oriental fruit moth males during approach to sex pheromone sources. Physiological Entomology Vol. 19: 61-69.

Wilson, T.G., M.H. Landers, and G.M. Happ. 1983. Precocene I and II inhibition of vitellogenic oocyte development in *Drosphila melanogaster*. Journal of Insect Physiology Vol. 29: 249-254.

Wilson, Thomas G. and Jason R. Cryan. 1998. Lufenuron, a chitin-synthesis inhibitor, interrupts development of *Drosophila melanogaster*. The Journal of Experimental Zoology Vol. 278: 37-44.

Wilson, Thomas G. 2004. The molecular site of action of juvenile hormone and juvenile hormone insecticides during metamorphosis: how these compounds kill insects. Journal of Insect Physiology Vol. 50: 111-112.

Wright, R.M. 1958. The olfactory guidance of flying insects. The Canadian Entomologist Vol. 90: 81-89.

Wright, RH. 1964. Marchon-new term for class of non-toxic pest control agents. Nature Vol. 204: 603.

Yan, Fengming, Marie Bengtsson, and Peter Witzgall. 1999. Behavioral Response of Female *Cydia pomonella*, to Apple Volatiles. Journal of Chemical Ecology Vol. 25: 1343-1351.

Yang, Zhihua, Marie Bengtsson, and Peter Witzgall. 2004. Host Plant Volatiles Synergize Response to Sex Pheromone in *Cydia pomonella*. Journal of Chemical Ecology Vol. 30: 619-629.

Yokoyama, V.Y., and G.T. Miller. 1991. Potential of pyriproxyfen as a quarantine treatment for *Cydia pomonella* and oriental fruit moth (Lepidoptera: Tortricidae). Journal of Economic Entomology Vol. 8: 942-947.

Zungoli, P.A., A.L. Steinhauer, and J.J. Linduska. 1983. Evaluation of diflubenzuron for Mexican bean beetle (Coleoptera: Coccinellidae) control and impact on *Pediobius foveolatus* (Hymenoptera: Eulophidae). Journal of Economic Entomology Vol. 76: 188-191.