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# MATING DISRUPTION OF THE LEAFROLLER COMPLEX (LEPIDOPTERA: TORTRICIDAE) IN MICHIGAN APPLE ORCHARDS AND IMPACTS ON NATURAL ENEMIES AND NON-TARGET PESTS

By

HAW LENG HO

# A THESIS

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Entomology

# ABSTRACT

# MATING DISRUPTION OF THE LEAFROLLER COMPLEX (LEPIDOPTERA: TORTRICIDAE) IN MICHIGAN APPLE ORCHARDS AND IMPACTS ON NATURAL ENEMIES AND NON-TARGET PESTS

By

HAW LENG HO

Disruption of obliquebanded leafroller behavior was most efficient with the Mec 240 pheromone treatment which also partially disrupted redbanded leafroller. Tufted apple bud moth was almost totally disrupted in the Generic II and Generic III pheromone blends. None of the pheromone or insecticide treatments provided commercially acceptable levels of fruit damage from leafrollers. The results indicated that no one pheromone provided effective disruption of multiple leafroller species.

Natural enemy and non-target pest populations were evaluated by suction sampling. All pheromone treated and untreated plots exhibited a significantly higher Shannon and Simpson's diversity indices and with a higher numbers of Hymenoptera, Araneae, and Diptera when compared to the insecticide treatment. Members of Tachinidae (Diptera), and Braconidae and Ichneumonidae (Hymenoptera) were parasites of the obliquebanded leafroller larvae. These studies indicate that mating disruption has potential as a pest management tool and is effective in conserving natural enemies. My special recognition to my wife and children for their love, understanding, patience and support none of it would have been possible.

To my parents who have always gives their love and support and believed in the importance of education.

•

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LITERATURE REVIEW

#### LITERATURE REVIEW

## Introduction

Throughout production regions in the US and Europe, leafroller species constitute a major pest problem in apple orchards. The situation is often rather complex because there are several leafroller species that cause damage to the fruit. In Michigan fruit damage at harvest can exceed 10 -15% of the harvested fruit (J. W. Johnson, unpublished), which causes a substantial reduction in returns to the grower. The leafroller complex consisting of the obliquebanded leafroller (OBLR), *Choristoneuera rosaceana* (Harris), redbanded leafroller (RBLR), *Argyrotaenia veluntinana* (Walker), tufted apple bud moth (TABM), *Platynota ideausalis* (Walker), and variegated leafroller (VLR), *P. flavedana* (Clemens), are the most significant pests of apple in Northeastern and Middle Atlantic parts of the US (Carde 1976). In Michigan, this complex in which OBLR predominates, is the major fruit pest after the codling moth, *Cydia pomonella* (L) has developed resistance to different organophosphate insecticides in many parts of the state making it difficult to be controlled (J. W. Johnson, unpublished).

Organophosphate insecticides have been used to control apple pests for about 35 years without evidence of organophosphate resistance in redbanded leafroller (Croft 1982). Most recently, a rise in the pest status of several other tortricids was attributed to increased tolerance or resistance to organophosphate insecticides (Croft and Hull 1991). Tufted apple bud moth and variegated leafroller are increasingly resistant to

organophosphate insecticides. Tufted apple bud moth has become a serious tortricid pest in apple because of its resistance to azinphosmethyl (Knight et al. 1990, Meagher and Hull 1986), a commonly used organophosphate insecticide for the control of certain insect pests in apple. Knight and Hull (1987) reported that organophosphate resistance is now widespread among the tufted apple bud moth populations within Adams County, Pennsylvania and North Carolina.

While redbanded leafroller is still controlled by the current conventional spray program, it has resulted in: costly application programs; disruption of natural enemies in the orchard ecosystem; pesticide residue on fruit; and finally contamination to the environment. Therefore, it has become necessary to develop a more effective and environmentally sound alternate pest control strategy such as mating disruption using pheromone. Mating disruption with pheromones prevent mating of a species by interrupting the normal process of mate location (Brunner 1991). Pheromone-based mating disruption appears to be a promising technique for controlling tortricids. Pheromones are currently used commercially to control several tortricid species in a number of countries (Audemard 1988; Campion et al. 1989; Ridgway et al. 1990). Mating disruption is one of the future scenarios in pest management in the fruit industry (Brunner 1990).

#### **Biology and life history of four leafroller species**

The obliquebanded leafroller is native to and widely distributed in temperate North America (Chapman et al. 1968), and feeds on foliage and fruit of many woody plants. Larvae are polyphagous but prefer hosts in the Rosaceae (Chapman and Lienk 1971).

Obliquebanded leafroller is bivoltine and overwinters on the host as second and third instars. These larvae emerge from hibernaculae in middle to late April when new plant growth appears. The first or summer generation begins in late June or early July, and the five instars develop during July. The second generation emerges in August and the larvae feed on fruit before overwintering. Larvae feed on both leaves and fruit, but fruit damage is of the greatest concern to apple.

The redbanded leafroller is widely distributed from the Atlantic provinces in Canada westward through Quebec, southern Ontario to western British Columbia. In US it occurs east of the 100th meridian (Weires and Riedl 1991). It is a polyphagous pest and overwinters in the pupal stage within fallen leaves under apple and other trees. Adults emerge in the spring at approximately the green tip stage of apple bud development and lay most of their eggs during the pink stage of development on the trunk or scaffold limbs (Weires and Riedl 1991). The eggs hatch during bloom and the larvae feed on leaves initially, but may switch to developing young fruitlets in the later instars. Pupation occurs in June and second generation moths are in flight by July. Larval damage occurs from the second generation and continues through August (Weires and Riedl 1991). In Michigan, there are usually two generations and there may be a partial third generation in an apple growing season (Howitt 1993). The first generation of redbanded leafrollers normally emerge in late April soon after the first green tissue shows in the buds. There are two generations in western New York, but farther south the number of generations may increase to as many as four. In North Carolina, redbanded leafroller is trivoltine (Rock & Yeargan 1974) and a pest of apple. Feeding by first generation larvae on the fruit can cause fruit drop or scarring; second generation larvae

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typically produce a scalloped shallow feeding pattern on apple.

Chapman and Lienk (1971) suggested that the primary hosts are among native Roseceae. Undoubtedly apple must now be considered a primary host. The redbanded leafroller does not seem to exhibit a preference for specific apple cultivars (Goonewardene et al. 1979).

Tufted apple bud moth overwinter as larvae in the ground cover under fruit trees. The larvae begin pupating from late April to early May, and the first-brood adult emergence peaks in early June. Subsequent mating and egg laying of this generation produce larvae that are active during July and early August. Second brood adult emergence peaks in late August, and larvae produced from these adults usually present the most serious problems as they feed on fruit just before and during harvest. Because tufted apple bud moth injury is characterized by fruit surface punctures and feeding marks, any tufted apple bud moth feeding can immediately result in decrease in grade and a financial loss to the grower for fresh market apples (Hull et al. 1988). Hull et al. (1988) also indicated that another economic impact of tufted apple bud moth feeding injury on processing apples may be its causing accelerated fruit maturity and decreased fruit quality.

The variegated leafroller was first reported by Riley in Missouri in 1869 (Howitt 1993). It is a common pest of strawberries in the Midwest and a major pest of apples in the Southeast. In orchards, the variegated leafroller overwinters as a dormant larvae in leaf litter on apple orchard floors. Pupation occurs in early May and adult moths begin emerging in early June and are present until late July. Second generation adults emerge in late August to early September (Howitt 1993).

## **Pest Status**

The obliquebanded leafroller was formerly not an important pest in commercial orchards in New York because numbers were low and larvae were considered to be foliage feeders since there were no records of them causing extensive injury to apple fruit (Powell 1964; Chapman et al. 1968; Chapman and Lienk 1971). Increasing numbers of OBLR larvae have been found in commercial orchards in Michigan and Western New York, and some significant fruit damage has occurred even though control sprays were regularly applied (Reissig 1978). It is one of a complex of sympatric tortricine moths that feed on apple in the Northern United States and southern Canada (Chapman and Lienk 1971) and has been of moderate economic importance. It has been increasingly difficult to control in Northeastern US orchards and has become the most important leafroller species in Western New York and Michigan apples.

The redbanded leafroller is a native pest and it rose to pest status in the 1950's, and occasional outbreaks continue to occur. It was first reported on grapes in 1870, and on apples in 1879 (Howitt 1993). It caused little or no damage to apples until about 1918.

In the mid-Atlantic states of the US, the most important leafrollers are tufted apple bud moth and variegated leafroller. Together they account for most of the injury to pples at harvest (Weires & Riedl 1991). The former species predominates in Pennsylvania and northern Virginia and the latter species in central Virginia.

Tufted apple bud moth was first reported as an apple pest in Pennsylvania by Forbes (1923). The first substantial economic damage from this insect in Pennsylvania occurred in 1969 (Bode et al. 1973), although it has been recorded as causing minor

damage to apple in Adams County since 1918 (Frost 1923). Economic injury was not observed again until 1969 when the insect appeared in a few scattered orchards in Adams and Franklin Counties. Since 1969 this pest has become a serious economic threat to apple throughout these two counties.

In recent surveys of 16 Pennsylvania orchards conducted in 1979, tufted apple bud moth injury to apple averaged 2.9 %, with a high of 12.4 % (Hull et al. 1983). Variegated leafroller was described by Clemens (1860), and has been reported as a general feeder in New Jersey (Smith 1910) and New York (Forbes 1923). Bottimer (1926) found it feeding on yellow ray flowers of *Helianthus* sp. and *Eupatorium compositfolium* in Texas, and Hamilton (1940) reported it as injurious to roses in a greenhouse in New Jersey. The species was recorded as injuring strawberries in Ohio by Neiswander (1944) and in New York by Wilde and Semel (1966 a, b). Summerland and Hamilton (1954) reported the species as injuring peaches in Indiana. However, this leafroller has not been recorded as seriously injuring tree fruits in the Northeast and Midwestern US.

Chapman et al., (1971) reported that the variegated leafroller is one of a sympatric complex of leafrollers occurring in the northeastern United States for which apple trees are a primary host. Bobb (1972) also reported that during 1970, this leafroller injured from 30 to 75 % of the apples in 3 orchards, totaling more than 250 acres, in the Piedmont of Virginia. The species had previously been noted as causing minor injury to apples in another orchard counties in 1961.

#### Pheromones as management tools

Pheromone are substances secreted by an organism that cause a specific reaction in a receiving organism of the same species (Karlson and Butenandt 1959; Karlson and Luscher 1959) or are substances produced by insects that have a specific effect on members of their own species (Bartell 1977). This type of chemical communication is common among insects. These chemical signals are secreted in minute quantities by the insects for communication with others of their kind. They can evoke many responses including mating, aggregation, alarm, trail-following, defense, feeding and reproduction (Kirsch 1988). The two classes of pheromones most exploited in pest control situations are the sex pheromones employed by insects during mating and the aggregation pheromones which bring both sexes together for feeding and reproduction (Campion 1984).

Pheromones have been employed and developed into tools for pest detection in pest management since the early 1970's (Wall 1984). Common applications include monitoring the spread of accidental species (Dickler 1982; Pasqualini et al. 1982; Bathon and Glas 1983), providing a means of estimating adult population density, the study of male movement (Sziraki 1984) and establishing a phenological model based on flight patterns (Rice et al. 1982; Baumgartner and Charmillot 1983). Monitoring of pests with pheromones has resulted in more efficient pesticide usage by timing pesticide applications correctly. Such systems have been used successfully to time sprays against tufted apple bud moth and variegated leafroller in Virginia (David 1985).

In addition to the use of insect pheromones in insect detection and monitoring, another important and recent use is mating disruption. The advantage of this technique,

when properly used, over the insecticide application is that the pheromone is non-toxic and only small amount per unit area are required (Kydonieus et al. 1982). In addition, it helps to eliminate the use of broadspectrum insecticides to control insects such as the leafroller species, allows survival of natural enemies to subsequently control secondary pests in the system (Rice and Kirsch 1990). Finally, this technique also reduces problems of pesticide residue in fruit, delays development of pesticide resistance and reduces exposure of pesticide operators to toxic chemicals.

Consistent success was observed in the using of mating disruption technique to control certain insect pests, such as pink bollworm (*Pectinophora gossypiella*). In California and Arizona yields were enhanced and only 5 % of the crop was damaged by pink ballworm compared with more than 30 % damage in conventionally treated fields (Doane et al. 1983). Vickers (1985) demonstrated that the oriental fruit moth (*Grapholita molesta*) was very susceptible to communication disruption in Australia. Finally, commercially acceptable control has been reported in Japan for tea tortrix and smaller tea tortrix (Kirsch 1988).

Other important uses of pheromone as a pest management tool includes monitoring of insecticide resistance using pheromones as attractants, luring insects to areas treated with insecticides and finally, luring insects to areas treated with pathogens, which are then spread by the infected individuals to the rest of the population (Kirsch 1988).

Finally, as with any management tool, the operational use of pheromones must be considered in the context of an integrated pest management system.

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#### Application of insect pheromone as pest management

The use of pheromone in traps for detection of pests is well established and pheromone traps are important tools in many pest management programs. The most obvious use of pheromones has been as a tool for detection and survey of insects in pest management since early 1970's (Wall 1984). Synthetic pheromone monitoring of the summer fruit moth *Adoxophyes orana*, a prominent pest of apples in the Netherlands has resulted in a marked decrease in the application of conventional insecticides, previously determined by calendar spray (Minks 1973).

Pheromones have been widely used in mass trapping for population suppression. Mass trapping relies upon the removal of sufficient males from the adult population to significantly limit the proportion of females that are able to mate. Many trials have been carried out with a number of tortrix species, with varying degrees of success (Charmillot and Vickers 1991). Excellent control of the redbanded leafroller was obtained through mass trapping in US by placing two traps per tree (180 traps per hectare) in apple orchards with low populations of the leafroller (Roelofs et al. 1970). The success of mass trapping is closely related with population density, and use of the technique might best be confined to situations where populations are very low. Mass trapping for redbanded leafroller in 2 vineyards in 1971 resulted in a substantial reduction in the percentage of damaged grapes compared to a check area (Roelofs 1974).

Insect sex pheromone as a baited trap can also be effectively used to monitor levels of insecticide resistance in the population (Riedl et al. 1985; Haynes et al. 1986, 1987). This method using various rates of insecticides into the trapping mechanism (sticky liner) requires little or no handling of insects and no need for topical dosage of insecticides. One of the most important and recent technique of using pheromones in pest management is mating disruption. The use of this technique has been proposed for well over 25 years (Bezora 1960; Wright 1964 a, b, 1965) following recognition that the olfactory guidance system of flying insects is a complex one and therefore potentially vulnerable to disruption. Control by mating disruption involves dispensing synthetic substance of the pest's pheromone, or a product having a similar effect, thus modifying behavior in such a way that males are unable to locate females for the purposes of mating (Charmillot et al. 1991). Disruption of long-distance communication by pheromones was viewed by Shorey (1973) as involving the following mechanism: peripheral sensory adaptation; diminished or lost behavioral response; and "confusion'. This pest control technique showed considerable promise in many cropping systems.

#### Types of sex pheromones in leafroller species

Most tortricid sex pheromones are chemicals produced by glands located at the female abdominal tip (Percy-Cunningham and MacDonald 1987) which excite the male and induce long range attraction and precopulatory behavior. Most of the tortricid compounds contain either 12 or 14 carbon atoms in the principal chain (Roelofs and Brown 1982) and at the end of the chain there are either Z (cis) or, E (trans) configuration with an alcohol (OH) or acetate (COOH) group attached. The presence of the double bond in the compounds can be anywhere between the number 2 carbon atom and at the end of chain. The (Z)-11-tetradecenyl acetate is the most widespread tortricid pheromone component, occurring in pheromones and attractants of about 90 species (Arn 1991). The known female sex pheromones of the tortricids are all composed of primary

aliphatic alcohols, their corresponding acetates and aldehydes. These components of a pheromone blend are often closely related to an alcohol and the corresponding acetate, a pair of homologues, of geometric or positional isomers.

Hill and Roelofs (1979) had identified the sex pheromone of the obliquebanded leafroller, *Choristoneura rosaceana* (Harris), from New York as a blend consisting of Z11-14:Ac and E11-14:Ac in a 95:5 ratio as the major attractant components + ca 5% Z11-14:OH, a 'secondary' compound synergizing the attractant action of the two former chemicals.

The sex pheromone of the redbanded leafroller, was characterized by Roelofs et al., (1975) as a mixture of two attractant components: Z11-14:Ac and E11-14:Ac in a ratio of 92:8, and 12:Ac as a secondary component, eliciting close range behavior (Baker et al. 1976). In RBLR, the attractiveness of a blend of (Z)- and (E)-11-tetradecenyl acetate was enhanced in the presence of dodecyl acetate (Roelofs et al. 1975).

Hill (1974) found extracted female abdominal tips of tufted apple bud moth containing trans11-tetradecen-1-ol and addition of trans-11 tetradecenyl acetate in a 2:1 ratio. However the VLR females were analyzed and found to contain a mixture of (E)-11-tetradecen-1-ol and (Z)-11-tetradecen-1-ol(9:1), as well as a mixture of (E)-11tetradecenyl acetate and (Z)-11-tetradecenyl acetate(2-3:1). A small amount of the tetradecyl acetate probably was also present (Hill et al. 1977).

The synthetic versions of compounds known to be components of the female sex pheromone to interfere with mate finding by moths and other pests had been used in most mating disruptions. Successful compounds have been termed parapheromones or antipheromones depending on whether they attract the insects or interfere with the way in which the insects perceive the natural pheromone (Gaston et al. 1972). In the first field trials over 25 years ago, Shorey and colleagues used simple steel planchettes mounted on stakes to evaporate what was then assumed to be the only constituent of pheromone of the cabbage looper, *Trichoplusia ni* (Gaston et al. 1967).

## **Mating disruption**

Disruption of pheromone-mediated mate finding in moths has been demonstrated in field trials for more that 20 years, beginning with the pioneering experiments of Shorey, Gaston and their colleagues (Gaston et al. 1967). It is assumed to be successful by permeating the area under treatment with a synthetic pheromone so as to reduce mate finding or aggregation, the end result being mating suppression. There are several possible ways that disruption could operate. An understanding of how mating disruption works is important because different mechanisms would suggest different approaches to the application of the pheromones in the field.

The main mechanism to explain the disruption or behavior by artificially released pheromone have been proposed (Bartell et al. 1982). First, it involves the direct neurophysiological effects involving sensory adaptation of pheromone receptors, and central nervous habituation brought about by relatively constant, high levels of pheromone experienced by the insects. The constant exposure of the insects to a relatively high level of pheromone leads to adaptation of the antennal receptors and habituation of the central nervous system. Under such circumstances, the responding insects would be unable to respond to any normal level of the stimulus.

The second mechanism was basically based on 'confusion' of responding insects

presented with a multiplicity of 'false' trails which divert them from calling females. A sufficiently high background level of the applied pheromone masks the natural pheromone plume and therefore trail following is impossible.

The third mechanism relates to the inability of the responding insects to distinguish between individual odor trail from calling females and a background of, in general, higher concentrations of the same odor. The synthetic pheromone is applied in a relatively large number of discrete sources so that insects flying within the treatment area can be diverted from the naturally occurring plumes (Campion 1984).

Most lepidoptera have a multi-component pheromone system. Mechanisms based on imbalance of the pattern sensory input when the ratios between chemical components of naturally occurring pheromone trails are distorted by the relatively massive 'control' liberation of a single component. Roelofs (1978) has observed that upwind flight behavior of the male redbanded leafroller, *Argyrotaenia veluntinana* (Walker), toward a source containing the normal blend of pheromone components in a wind tunnel is greatly altered when air containing only one component is circulated through the apparatus.

Finally, the mechanisms based on the modification of responses by chemical compounds other than those occurring naturally in the specific pheromone blend (antipheromones). These chemicals may structurally resemble the true pheromone or may have a completely different chemical structure. Some antipheromones may act by competing for the same receptor site on sensory organs as true pheromone. However, some antipheromones not structurally related to the true pheromone may block mate-finding in a complete manner (Brunner 1991).

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## Successes/achievements of mating disruption

Use of pheromones for mating disruption may have high potential in pest management (Reissig et al. 1978; Taschenberg and Roelofs 1978). Pheromones are currently used commercially to control several tortricid species in a number of countries (Audemard 1988; Campion et al. 1989; Ridgway et al. 1990) and research has provided encouraging results in preliminary trials for the control of several leafroller species (Deventer and Blommers 1992; Suckling and Shae 1992). Plant damage provides the most reliable information about the results of the mating disruption techniques (Audemard 1988). From the commercial point of view, the only criteria by which to judge success of disruption trials is whether or not the level of fruit damage at harvest is acceptable to the grower (Rothschild 1981). The economic threshold for fruit damage from leafrollers is 5 % for apples in Michigan (Johnson and Herr 1995).

Novak and Roelofs (1985) reported that in an experiment conducted in New York using laboratory-reared redbanded leafroller, males exhibited a significant reduction in orientation to sex pheromone-baited traps in small plots of 750 m<sup>2</sup>, at a release rate of 5 mg per hour Z11-14:Ac/ha from hollow fibers. However, this formulation was ineffective against obliquebanded leafroller. Pfieiffer et al. (1993), demonstrated that mating disruption for control of variegated leafroller, tufted apple bud moth, and redbanded leafroller were evaluated in Virginia using dispenser with 190 mg of a putative generic leafroller disruption blend consisting of 67.2 % E11-14:Ac, 28.8 % Z11-14:Ac, 2 % Z9-14:Ac, 1.4 % E11-14:OH, and 0.6 % Z11-14-OH (1000 dispenser/ha.) reduced trap captures by 97, 51, and 55 % for variegated leafroller, tufted apple bud moth, and redbanded leafroller respectively. Average leafroller injury (not attributable to individual species) in the pheromone blocks was acceptable; it ranged from 3.8 % in the interiors to 2.8 % at the edges, compared with 0.05 % for the conventional and 27.5 % for the abandoned control plots (Pfieiffer et al. 1993).

In the Netherlands, where the leafroller species *Adoxophyes orana* populations are usually rather modest, mating disruption with commercially available ampulle containing 200-300 g Z11-14:C/ha dispensers at a density of 550/ha provided acceptable control with results equal to fenoxycarb treated control plots in trials during 1989-1991 (Deventer et al. 1992). Tests in neighboring countries extended these positive results (Neumann et al. 1990).

Roelofs et al. (1975) reported that in redbanded leafroller moth, a microencapsulated spray of two pheromone components, cis-11-and trans-11tetradecenyl acetates (89:11), applied at 22 g pheromone/ha in an apple orchard effectively suppressed the ability of males to locate pheromone sources, but Carde et al. (1976) indicates that this same treatment was ineffective in eliminating ability of males to mate with females in very close proximity (confined to small cages).

Finally, success has been obtained in New Zealand against lightbrown apple moth, Epiphyas postvittana (Walker), where mating disruption enhanced resistance management (Suckling et al. 1990 a, b.).

# Benefits of mating disruption technique.

The economics of using pheromones for mating disruption as an alternative to conventional insecticides can only be determined if there are reliable criteria by which the effect of the target species on crop yields can be assessed. Although leafroller complex control by pheromones is slightly more expensive than conventional pesticides, Brooks et al., (1979) showed that pink bollworm *Pectinophora gossypiella* control by pheromone gave both improved yields and quality of crops and more than compensated the costs involved.

Reduced use of broadspectrum insecticides and reduced number of sprays would allow better survival of natural enemies. The potential use of pheromone mediated mating disruption (Hull and Felland 1993) together with the use of physiologically selective compounds such as insect growth regulators (Biddinger 1993) helps in the conservation of existing biological control agents, such as predators and parasitoids.

Using mating disruption may provide the added advantage of avoiding the problems of pest resistance to insecticides and destruction of beneficial insects that may lead to eruption of other pest species (Reynolds et al. 1982). Mating disruption could provide an alternative control tactic that would help to reduce selection pressure for resistance development to chemicals currently registered on tree fruit crops.

The use of mating disruption over several years could result in the suppression of a pest population to very low levels. This might allow the cessation of controls for a period of time until pest populations again increased to dangerous levels (Brunner 1991). Such an example has been reported in California where the Oriental fruit moth has been controlled by mating disruption for more than two years. The recovery of the pest population could be monitored with pheromone traps or fruit inspections at harvest.

Farm workers are an important part of the tree fruit production system and the exposure to insecticide residues during thinning and harvesting operations will be greatly reduced (Rice and Kirsch 1990). Pheromones are essentially non-toxic to mammals,

especially at the levels released in mating disruption programs. Since the chemicals are contained in the dispenser packet, direct exposure to workers will be minimized.

Pheromones are not applied directly to the fruit and as such, are not present as residues on the crop. The use of mating disruption to control key pests could help reduce already low levels of insecticide residue present on fruit.

Mating disruption promises to be an alternative pest control technique useful for some major agricultural pests. The other most important benefit of using pheromones is their low toxicity (Bezrozza et al. 1975; Knipling 1976; Kydonieus and Beroza 1982). In addition, due to the low rate and method of application, pheromones are not expected to have adverse effects on non-target species (Ghassemi et al. 1983).

Finally, how easily pheromones can be applied in the field is dependent on the type of formulation. Some formulations need to use expensive application equipment (Kydonieus et al. 1982) and this has limited their practicability in commercial use. Applicators do not need to have the special training and protective clothing that are required in the use of conventional pesticides.

#### Types of pheromone dispensers

There are several types of dispensers that have been used in mating disruption tests namely, microcapsules, capillaries, trilaminates, ropes, and liquid flowables. Microencapsulated pheromone used as spray formulation, which was developed by coacervation or interfacial polymerization techniques (Hall et al. 1982). The capsule shells have consisted of gelatin, polyurea, polyamide, or polyurea crosslinked with polyamide. Release rates from the microencapsules can be controlled by changing the

permeability of the polymer shell. The sizes of the microencapsules range from 2 to 400 um. This formulation offers the advantages that they can sprayed in large areas with conventional applicators, the pheromone was distributed in a relatively uniform manner and generally requires no special adhesive to ensure retention on the foliage (Roelofs 1977; Hall et al. 1982).

The microcapules formulation is applicable in situations to control insects by the disruption or bioirritant strategy (Weatherson 1991); it cannot be used in trap and kill situations because the formulation does not provide sufficiently strong point sources. Therefore, the modes of action imposed by microcapsules would be those of adaptation or habituation or camouflage (Weatherson, 1991).

Earlier studies with microencapsulated formulations were generally unsatisfactory (Campion et al. 1978). This was subsequently shown to be due, at least in some cases, to the degradation by sunlight of both the capsule wall and the contained pheromone. More recent stabilized microencapsulated formulations have been developed in the United Kingdom (Campion et al. 1981a, b; Hall et al. 1982) and when aerially applied have given satisfactory control of pink bollworm *Pectinophora gossypiella* in Egypt (Campion and Nesbitt 1982a; Critchley et al. 1983). Studies conducted in New York showed that redbanded leafroller of apple were disrupted between 75 to 99 % with microencapsulated cis and trans-11-tetradecenyl acetates (89 : 11) at 22g/ha (Carde et al. 1975).

The capillary type of formulation which consists of strong point source, can be used in either disruption or attracticide strategies, influencing the male moths by any or all the proposed modes of action (Weatherson 1991). This formulation was produced by

the Controlled Release Division of Albany Intentional (formerly Conrel) since 1974. The pheromone is held within the fiber by capillary action and is released by evaporation from the open end (Golub and Weatherston 1984). To make the fibers stick to the foliage they were mixed with special glue and sprayed using specially designed applicators attached to aircraft (Funkhouser 1979). When a formulation requires specialized application equipment it is a serious drawback. Evidence indicates that plastic capillary pheromone formulation significantly disrupted mating of the gypsy moth in 1-ha forest plots at the rate of 25 or 250 g (AI/ha) at low population density (Webb 1990).

The trilaminate formulation can be in the form of sheet, power wafer, tape, and confetti. As with capillary and rope formulations, the trilaminates can affect the male insects usually by the following mode of action:- adaptation/habituation, false trail following, and camouflage (Weatherson 1991). The advantage of this formulation is that it provides stability in field situations because it has the ability to protect labile pheromones from environmental conditions that can cause rapid degradation (Weatherson et al. 1985). The drawback of this formulation is that it requires specialized application equipment.

The rope formulations are mainly used for mating disruption techniques to control insects by all the three mechanisms mentioned above (Weatherson 1991). This type of formulation possesses the highest longevity of all the formulations because it contains relatively large amounts of active ingredients per rope. Manual application is required for the rope material and it may face difficulty in being accepted in countries where labor is expensive. The application rope formulation is suitable for orchards but not for certain forestry situations (Weatherson 1991). The rope seems to be the most expensive of all the

formulations discussed.

Finally, the liquid flowables formulation usually used as mating disruption technique, relies on either the adaptation/habituation or camouflage mode of action. Generally very little information was available regarding this formulation. However, this type of formulation can be applied using the simple conventional applicators.

Capillary fibers, laminated flakes and microencapsulated formulations as well as polyethylene ropes, have all been demonstrated as effective dispensing systems for the pink bollworm (Baker et al. in press; Critchley et al. 1985). Of the five formulation types mentioned above, the microcapsule formulation is the second largest amount of pheromone used (Weatherson 1991).

Today, the most common dispenser systems used in mating disruption incorporate pheromones in plastic tubes, ampules, or packets that are designed to provide a slow release of the product for several months (Brunner 1991). These dispenser systems are placed in the field by hand at a rate of 150 to 400 per acre.

## Factors that influence the mating disruption trials

There are several factors that can influence the mating disruption trials. One important factor that can influence the effects of mating disruption is the release rate of pheromone from the dispenser. In many disruption studies, release rates are cited in mg/ha/h or /day and are based on estimates obtained under laboratory conditions. It generally has been found that pheromone dispensers releasing the chemicals above a certain emission rate will catch fewer males. The optimum release rate or dispenser load

for trap catch varies greatly among species, ranging from those exhibiting attraction to lower emission rates, such as larch bud moth and grape berry moth, to species that are best lured to high release rates, such as the tufted apple bud moth (Roelofs et al. 1977). The chemical structure of the pheromone can also affect its release rate. Long chain compounds are released more slowly than shorter ones, and their functional groups also play an important role (Vickers and Charmillot 1991). Ioriatti et al., (1987) have shown that temperature influences the release rate of pheromones from plastic dispensers. Brown et al., (1992) showed that an increase in temperature produces n exponential increase in the release rate of each component of the pheromone.

The aerial distribution and concentration of behavior-modifying chemicals is another key factor in determining the outcome of mating disruption treatments. This will depend upon which mechanism of disruption is actually operating. If the males are disorientated at the time of calling by relatively larger quantities of pheromone present throughout the orchard that obscure the discrete female emissions, then the pheromone will need to be maintained at this high level at all times, unless each release station is operated on a time basis. If disruption works by habituating males to the odor of the pheromone during non-calling period (Bartell and Roelofs 1973), then small quantities in the air throughout the day should effect habituation and leave the males unstimulated at the normal mating time.

In Japan, studies on the distribution of pheromone concentration in small plot (less than 1 acre) field trials to control tortrix moths showed that it is uneven (Ogawa 1990). At the center of the field, the pheromone concentration is about 20 ng/m3, but at the border area, it is about 10 ng/m3 (Ogawa 1990). Therefore, to reduce boarder effects, a
larger area is very important. Ogawa (1990), had also shown that increased mating could have occurred near orchard edges because of low pheromone concentrations relative to the center of the orchard.

Besides the release rate and concentration of pheromone, other important factors such as pheromone components or ratios can affect the mating disruption trials. Increasing the pheromone concentration to unnaturally high levels may disrupt male orientation, but a change in component ratio can also be very disruptive. For example, the redbanded leafroller males respond to natural ratio of 92:8 (Z11/E11-14:Ac) in the laboratory at concentrations much lower than required with a ratio of 100:0 and 70:30 (Baker et al. 1976). Roelofs et al., (1976) in his studies on the male RBLR moth orientation disruption in vineyards with microencapsulated pheromone formulation, showed that the natural blend (ca. 92:8) of pheromone components, Z11-14:Ac/E11-14:Ac, was more effective in disrupting male RBLR orientation to pheromone traps than was a 50:50 mixture or the pure E11-14:Ac.

Pheromones related chemicals present in the compounds can affect the mating disruption treatment. Disruptants must be structurally very similar to the correct pheromone structure to provide effective disruption. It was first found with redbanded leafroller moths that some chemicals such as positional isomers or the alkyne analogue of a pheromone components, could drastically reduce trap catch when present in a 1:1 ratio to the pheromone (Roelofs et al. 1968).

The placement position of the disruptant on the crop will be another important factor influencing the result of the mating disruption trials. Shorey et al., (1995) in his studies indicated that most of the male *Platynota sultana* moths were active in the upper

third of the grapevine structure, and the disruptant placed at this height were most effective in preventing males from locating females.

Population density of pests present in the mating disruption system can affect the results of the trials. High adults densities leading to increased opportunities for sexual encounters, particularly in zones where the aerial concentration of behavior-modifying compounds is low and not only through male activity but as a result of female-initiated sexual encounters (Barrer and Hill 1980) or females aggregating near sources of synthetic pheromone (Birch 1977).

Pheromones are very specific, and it is difficult to apply them in fields when many types of insects exist. Insecticides can be applied at the same dose in different countries, but dosage levels of pheromone are determined by different environmental conditions. For example, in China, a dosage less than half of that used in the Imperial Valley, United States is effective and has a longer life to control the pink bollworm of cotton (Ogawa 1990).

The other crucial factor that affects the mating disruption trial is the prevalence of immigration by mated females from adjacent field or areas (Rothschild 1981). This technique provides no safeguards against the immigration of mated females from outside the area treated with disruptants. In many moth pests, the intensity of migration can vary seasonally or with population density (Carde et al. 1995).

The environmental conditions and the structure of the crop can also affect the results of the mating disruption trials. The nature of the canopy and the shifts in wind direction determine the plume's turbulent structure. For example, cotton has little foliage to generate turbulence early in the season, and wind speed within the canopy is typically

higher in the summer (Carde et al. 1995). The distance over which males attract females may therefore vary with seasons.

Finally, the behavior of the insects can affect the results of mating disruptions. Some species of insect are active during either the day or night. However some insect species are attracted to pheromone during both daylight hours and at night (Carde et al. 1975). At night, wind speeds are on the average lower, and obviously there is less light, which reduces the moths ability to utilize the optomotor reaction, the only mechanism by which the flying moth can judge wind directions (Baker 1989). Both of these factors suggest that at night a male's ability to follow a plume to its source is diminished over daytime conditions.

### Summary

The reason why I have chose mating disruption research is because this technology of pest management is fairly new to me and my country. My objective is to seek more effective and better alternatives to pesticides in pest management systems that can help to conserve natural enemies, minimizing pesticide hazard to pesticide operators and finally minimize pesticide contamination of our food, fiber, wildlife, and the environment.

The leafroller complex is of interest to me because lately it has become a more important pest of apple in most parts of orchards in the US. Significant fruit damage has occurred even though controls were regularly applied. The conventional spray program to control the leafroller complex has resulted in the disruption of natural enemies and has caused a surge of secondary pests such as mites in the system. In addition, because of the

development of resistance to organophosphate insecticides of the obliquebanded leafroller, it has become necessary to develop an effective and environmentally sound alternative control pest strategy such as mating disruption.

After reviewing the literature, I realized that only a handful of research studies have been carried out regarding mating disruption on leafroller complex of apple. This finding has led me to choose and do this research project. Any information regarding the use of mating disruption on leafroller complex of apple may become of paramount importance in future pest management programs and to the apple industry in Michigan.

### The objectives of my study were to :

- 1. a. Compare the efficacy of pheromone disruption blends (Generic II, Generic III and OBLR Spiral) and untreated plot for the control of leafroller complex in apple.
  - b. Compare the efficacy of pheromone disruption blends (Generic II, OBLR Spiral and a microencapsulated pheromone formulation [Mec]), to commercial insecticides and the untreated control for the control of leafroller complex in apple.
- Evaluate the efficacy of three rates of a microencapsulated pheromone formulation with OBLR Spiral pheromone disruption blend for control of obliquebanded leafroller and redbanded leafroller in apple.
- 3. Compare the impact of mating disruption on the populations of natural enemies and non-target insects in the apple orchard, with commercial insecticides.
- 4. Identify the parasitoids parasitizing the egg mass and larval stages of obliquebanded leafroller in the mating disruption, insecticide and untreated plots in apple orchard.
- 5. Compare the impact of mating disruption on parasitism of egg mass and larval stages of obliquebanded leafroller in apple orchard, with commercial insecticides.

**CHAPTER 1** 

### **CHAPTER 1**

### Field evaluation of mating disruption with pheromones to control leafroller complex (Lepidoptera: Tortricidae) of apple

### **INTRODUCTION**

Tortricid leafrollers are major fruit pests in Michigan, second only to codling moth. Four leafroller species make up the pest complex: obliquebanded leafroller (OBLR), *Choristoneura roseaceana* (Harris); redbanded leafroller (RBLR), *Argyrotaenia velutinana* (Walker); tufted apple bud moth (TABM), *Platynota idaeusalis* (Walker) and variegated leafroller (VLR), *Platynota flavedana*. The larvae of leafroller species do the bulk of their damage to foliage, and later feed on apple fruit and other tree fruit. Variegated leafroller caused injury to 30-70 % of fruits in several orchards of the Piedmont region in Virginia (Bobb 1972). Fruit damage by leafroller complex at harvest can exceed 10-15 % of harvested fruit in Michigan, which causes an economic loss of US \$400 - US \$1200 per acre to the grower annually (J. W. Johnson, unpublished data).

The present control programs for the leafroller complex, relying on broad spectrum pesticides including organophospate and carbamate mixtures (Biddinger et al. 1994), has resulted in the development of resistance to these chemicals in obliquedbanded leafroller in Michigan (Howitt 1979). Organophosphate resistance in tufted apple bud moth was reported in Pennsylvania (Biddinger 1993) and North Carolina (Knight et al. 1989). In addition, organophosphate spray programs lead to significant increases in non-target secondary pests through the reduction of natural enemy populations in the system, consequently increasing the cost of mite control programs because additional applications of pesticides must be applied.

Clearly, more environmentally sound and effective alternative control strategies are needed to control the leafroller complex. One alternative method is mating disruption with pheromones (Hull et al. 1993). Mating disruption can selectively affect a narrow range of arthropods and cause less disturbance in the orchard system (Croft 1990). This alternative control strategy does not disturb existing biological control agents, such as predators and parasitoids of various species of leafrollers, including tufted apple bud moth (Biddinger et al. 1994). The use of pheromones for mating disruption and their incorporation with other methods of control in integrated pest management have clear control implications for a variety of major pests (Rothschild 1981).

Most of the studies on mating disruption of leafrollers use a single blend of pheromone for disrupting a single species of leafroller. The development of multiple species pheromone blends for disruption could make this promising technology more economically viable (Pfeiffer et al. 1993).

The objectives of my study were to:

- 1. a. Compare the efficacy of pheromone disruption blends (Generic II, Generic III and OBLR Spiral) and untreated plot for the control of leafroller complex in apple.
  - b. Compare the efficacy of pheromone disruption blends (Generic II, OBLR Spiral and a microencapsulated pheromone formulation [Mec]), to commercial

insecticides and an untreated control for the control of the leafroller complex in apple.

2. Evaluate the efficacy of three rates of a microencapsulated pheromone formulation with OBLR Spiral pheromone disruption blend for control of obliquebanded leafroller and redbanded leafroller in apple.

#### **MATERIALS AND METHODS**

# A. I. Determine the efficacy of three pheromone disruption blends for control of leafroller complex of apple -1994

This study was conducted at the Trevor Nichols Research Complex of Michigan State University, near Douglas, MI in 1994. Three pheromone blends, Generic blends II & III and a standard OBLR spiral pheromone (Ecogen Inc. Litchfield, Arizona) were tested for the control of mating and damage to fruit and foliage by four leafroller species (OBLR, RBLR, TABM, and VLR) in apples (Table 1). The three treatment pheromones were compared to an untreated check without a mating disruption treatment. The treatments were replicated four times in a randomized complete block design. Each replicated plot size was ranged from 0.25 to 0.38 ha per plot depending on tree spacing in each apple block (Figure 1), and plots were separated by at least a 20 m between each block. Each block was at least 1.35 ha in size and was separated from the other blocks by at least a 20 meter buffer zone. All blocks were composed of mixed varieties of mature semi-dwarf apples. No insecticides were applied to any of the blocks, but all received a standard fungicide maintenance program, including Dithane M-45 and Bayleton 50 DF throughout the season.

	Species				1994 Disruption Blends			1995 Disruption Blends		
Chemical structure of pheromone <sup>a</sup>	VLR	TABM	RBLR	OBLR	OBLR	Gen II	Gen III	OBLR	Gen II	Mec
	% <sup>*</sup>				% <sup>b</sup>			•⁄• <sup>b</sup>		
E11 - 14:Ac	-	50	8	2	2	30	40	2	30	4
Z11 - 14:Ac	-	-	92	96	95	40	20	95	40	96
E11 - 14: OH	84	50	-	-	-	30	40	-	30	-
Z11 - 14: OH	16	-	-	1.5	3	-	-	3	-	-
Z11 14: AL	-	-	-	1	-	-	-	-	-	-
12.Ac	-	-	0.15	-	-	-	-	-	-	-

**Table 1.** Pheromone disruption blends used in the 1994 and 1995 mating disruption trials with composition of sex pheromones of the four leafrollers in Michigan

Abbrevation: For species - VLR = Variegated leafroller, TABM = Tufted apple bud moth, RBLR = Rebanded leafroller, OBLR = Obliquebanded leafroller

> For disruption blends - OBLR = Obliquebanded leafroller pheromone, - Gen II = Generic II pheromone,

- Gen III = Generic III pheromone,

- Mec = Microencapsulated pheromone formulation

For chemical structure of pheromone :-

E11 - 14:Ac = (E)-11-tetradecenyl acetate Z11 - 14:Ac = (Z)-11-tetradecenyl acetate E11 - 14:OH = (E)-11-tetradodecadien-1-ol Z11 - 14:OH = (Z)-11-tetradodecadien-1-ol Z11 - 14:Al = (Z)-11- tetradecenal 12.AC = dodecyl acetate

<sup>a</sup> As reported in literature.

<sup>b</sup>As given by commercial source.



Block C

Pheromone disruption dispensers were placed in treatment plots on May 9, 1994, at a rate of 400 ties per acre, placed approximately 2 m high around the perimeter of each tree. The number of ties ranged from 3 to 4 per tree depending on the spacing of trees in each plot. Treatment plots were monitored for OBLR, RBLR, TABM and VLR adults on a weekly basis from May 9 to October 24, 1994 placing standard Trece pheromone lures of each species in wing traps (Pherocon 1), 2 m high in four separate trees in the center of each plot. Lures and trap bottoms were replaced each month throughout the season.

### Assessment of mating disruption

The efficacy of treatments was assessed by comparing catches of OBLR, RBLR, TABM, and VLR adult males with pheromone-baited traps (Pherocon I). One trap per species was placed at random in the center four trees of each plot on May 9, 1994. The traps were baited with standard red rubber septa obtained from Trece. All rubber septa were replaced after 4 weeks. The traps were hung on the apple trees at approximately 1.5 m above ground. Traps were examined once a week and the number of males for each of the four leafroller species caught in each trap was recorded and then removed. Trap bottoms were changed when the sticky surface became dirty.

Mean numbers of moths per trap per season caught during the 10 weeks experiment were compared for each leafroller species between the Generic II, Generic III, OBLR spiral pheromones, and untreated check treatments. Analysis of variance (ANOVA) was performed on the mean catches and where treatment differences were significant, means were compared using LSD test (alpha = 0.05) using SAS statistical software (SAS Institute 1989).

### Assessment of terminal infestation

Evaluation of larval terminal infestations of the leafroller complex were assessed on July 29, 1994 by randomly selecting 100 growing shoot terminals from four selected tree per replicate in the field. For each tree, 5 terminals were sampled from each of the four cardinal directions and at the center portion of the tree (25 terminals per tree). Each shoot was visually inspected and any leafroller infestation recorded. The rate of infestation, expressed as percentage was subjected to an arcsin transformation and to an analysis of variance (SAS Institute 1989). When the treatment differences were significant, means were compared using LSD test (alpha = 0.05) (SAS Institute 1989).

### Assessment of fruit damage

Larval fruit damage was assessed on 4 randomly selected trees per replicate. For each tree, 5 fruits were randomly collected from each of the four cardinal direction and at the center of each tree (25 fruits per tree) and assessed for visible external fruit damage by the leafroller complex. Larval fruit damage was evaluated for second generation leafrollers at harvest on September 20 in all the plots. For statistical analysis SAS software (SAS Institute 1989) was used, the data were subjected to arcsin (x) transformation, where x is the percentage of fruit damaged. Analysis of variance (ANOVA) was performed on the mean percentage of damaged fruit and where treatment differences were significant, means were compared using LSD test (alpha = 0.05) (SAS Institute 1989).

### A. II. Evaluation of three different blends of pheromones for disruption versus insecticide sprays and untreated check plot for control of leafroller complex -1995

The mating disruption study was conducted at the Trevor Nichols Research Complex of Michigan State University, near Douglas, MI in 1995. Two pheromone dispenser blends (Ecogen Generic II and OBLR Spiral) and a microencapsulated pheromone formulation (Mec) obtained from Ecogen Inc. Litchfield, Arizona (Table 1) were tested in apples for the control of mating and damage to foliage and fruit by the leafroller complex (OBLR, RBLR, TABM, and VLR).

In this experiment, treatments were arranged in a randomized complete block design with 4 blocks and 4 replicates. Treatment plot sizes ranged from 0.25 to 0.38 ha depending on tree spacing in each apple block. Each block was at least 1.35 ha in size, composed of mixed varieties of mature semi-dwarf apples 3 to 7 m in height. Blocks were separated from each other by at least a 20 meter buffer zone, and replicate plots were at least 20 m apart (Figure 2).

The two pheromone dispenser blends (Ecogen Generic II and OBLR) were placed on April 27 at a rate of 400 dispensers per 0.45 ha, before the first emergence of RBLR adults. The dispensers were placed approximately 2 m above the ground around the perimeter of each tree and the number of ties per tree ranged from 3-4 depending on the spacing of trees in each block. The microencapsulated pheromone formulation (Mec) was sprayed on June 15 at a rate of 240 ml formulation per 0.45 ha. The insecticide plot was treated with Lannate 90 SP at 0.45 kg formulation per 0.45 ha on June 7 (petal fall); with Lannate 90 SP and Lorsban 50 W at 0.23 kg and 0.68 kg formulation per 0.45 ha on



June 29 (2nd cover), and with Lannate LV 2.4 EC and Lorsban 50W at 1.4 l and 0.68 kg formulation per 0.45 ha on August 21 (6th cover). The microencapsulated pheromone spray and insecticide treatments were applied with an FMC 1029 air blast sprayer at 680 liters per 0.45 ha for insecticides, 136 liters per 0.45 ha for the Mec. Traps were removed temporarily during spraying of the microencapsulated pheromone formulation and insecticide treatments. The untreated check plots did not receive any insecticide or pheromone treatments during the trial period. All blocks received a standard fungicide maintenance program, including Dithane M-45 and Bayleton 50 DF throughout the season. The insecticide imidacloprid (Provado 1.6) at a rate of 177 ml formulation per 0.45 ha was sprayed on July 12 on all plots to control potato leafhopper, *Empoasca fabae* (Harris) damage to terminal foliage.

### Assessment of mating disruption

Evaluations of disruption of mating were made by monitoring the ability of OBLR, RBLR, TABM and VLR adult males ability to orientate to point sources of pheromone with pheromone traps (Pherocon I). One trap per species was placed at random in the center four trees of each plot on April 27, 1995. The traps were baited with standard red rubber septa lure from Treces Inc. Salinas, CA. All rubber septa were replaced after 4 weeks. The traps were placed on the apple trees at approximately 1.5 m above ground level. Trap bottoms were changed every month or when the sticky surface became dirty. Synthetic pheromone-baited traps were examined once a week and the number of males of each of the four leafrollers species caught in each trap was recorded.

All leafrollers species were removed from the traps after each recording. Mean numbers of moths per trap per season caught during the 10 weeks experiment were compared for each leafroller species between disruption, insecticide and control treatments. Analysis of variance (ANOVA) was performed on the mean catches and the means were compared using LSD test (alpha = 0.05) using SAS statistical software (SAS Institute 1989).

### Assessment of terminal infestations

Larval terminal infestations of leafroller complex were assessed on May 31, July 14 and July 31 by observing 100 growing shoot terminals per replicate per treatment in the field, and recording the number of terminals infested with leafroller larvae. Terminals were sampled from 4 selected trees per replicate and 5 terminals (100 terminals per replicate) were randomly sampled from each of the four cardinal directions and at the center portion of each tree. Each shoot was visually inspected for damage, all larvae found were collected and reared to adults for identification. The rate of infestation, expressed as percentage were subjected to an arcsin transformation and to an analysis of variance (SAS Institute 1989).

### Assessment of fruit damage

Larval fruit damage was assessed on four randomly selected trees per replicate . For each tree, five fruits were randomly collected from each of the four cardinal direction and at the center of each tree (25 fruits per tree) and assessed for visible external fruit damage by the leafroller complex. Larval fruit damage was evaluated for first generation leafrollers on June 21 and for second generation leafrollers at harvest on September 9 in all the plots. For statistical analysis SAS software (SAS Institute 1989) was used, the data were subjected to arcsin (x) transformation, where x is the % of fruit damaged.

### B. Evaluating efficacy on three rates of microencapsulated pheromone formulation with a spiral OBLR blend to control obliquebanded and redbanded leafrollers in 1995.

This field study was conducted at the Trevor Nichols Research Complex, near Fennville, MI. Each test plot was approximately 0.24 ha in size and was composed primarily of 'Delicious'. The apple trees were 8 years old and planted on a 6.4 m by 3.0 m spacing. The experiment was arranged in a complete randomized design with 3 replicates (Figure 3). Replicate plots were at least 10 m apart. Treatments consisted of a single application of microencapsulated pheromone formulation (Mec) at three different rates (240 ml, 60ml, and 15 ml formulation per 0.45 ha) for control of OBLR and RBLR, applied prior to initiation of the adult flight period on June 14; A treatment of the OBLR pheromone disruption blend dispenser placed on June 14 and an untreated check.

All field applications with the microencapsulated pheromone formulation were made by using FMC 1029 air blast sprayer at 136 liters per 0.45 ha. A pheromone trap (Pherocon I) with monitoring lures for OBLR and RBLR was placed at random in each plot. An additional pheromone trap for codling moth was also placed at random in the 240 ml microencapsulated pheromone formulation and untreated check plot for evaluation of codling moth flight in a pheromone environment. Male moth catches in the trap were monitored and recorded weekly.



Fig. 3 Location of treatments, replications and traps of the experiment near Fennville 1995

References for pheromone trap: o = obliquebanded leafroller, r = redbanded learoller, and c = codling mothTreatments : Control = untreated plot, Mec 15 = 15 ml formulation (form.) of microencapsulated pheromone formulation(Mec)/0.45 ha, Mec 60 = 60 ml form. Mec/0.45 ha, Mec 240 ml form. Mec/0.45 ha, and OBLR = obliquebanded leafroller pheromone. x = missing trees

### Assessment of mating disruption

Evaluation of mating disruption were carried out by monitoring weekly the number of OBLR, RBLR and CM in the pheromone-baited traps. The number of males of each of the leafrollers species caught in each trap was recorded. The OBLR and RBLR pheromone-baited traps were placed in all treatment plots. The CM pheromone traps was placed in the OBLR spiral disruption blends, 240 ml Mec and the untreated plots. Each trap was placed at random on the apple trees at 1.5 m above ground level in the center of each plot on June 14. All pheromone lures and trap bottoms were replaced after 4 weeks.

Mean numbers of moths per trap per season caught during the 10 weeks experiment were compared for each species between different rates of microencapsulated pheromone formulation, the OBLR spiral disruption blend with the untreated plots. Analysis of variance (ANOVA) was performed on the mean catches; and where treatment differences were significant, means were compared using LSD test (alpha = 0.05) using SAS statistical software (SAS Institute 1989).

#### RESULTS

## A. I. Efficacy of three pheromone disruption blends for control of the leafroller complex of apple -1994

### **Assessment of mating disruption**

The relative disruption of the male moth's ability to orient to point sources of pheromone was measure by captures in standard monitoring traps. The three pheromone blends, OBLR Spiral, Gen II and Gen III had significantly lower (df = 3, F = 14.28, P <

0.05) mean numbers of OBLR caught in traps for the season compared to the untreated check plot but they were not significantly different from each other (Figure 4). The three pheromone blends did not provide effective trap shut down of OBLR for the first generation and the OBLR populations in the second generation were too low to provide meaningful results (Figure 5).

Similarly, mean number of RBLR males caught in traps in the OBLR, Gen II and Gen III pheromone blend plots were significantly different (df = 3, F = 42.45, P < 0.05) than the untreated check plot (Figure 4) but they were not significantly different from each other. The untreated check plot had the highest number in mean number of RBLR trap catch of 148 RBLR per season as compared to the Gen II, Gen III and OBLR treatments (Figure 4). The Gen II and Gen III provided effective trap shut down of RBLR for the first generation of RBLR (Figure 6).

Both Gen II and Gen III treatments provided significantly lower (df = 3, F = 4.33, P < 0.05) mean number of tufted apple bud moth males in traps for the season compared to the OBLR pheromone blend and the untreated check plot (Figure 4) but the OBLR and untreated check were not significantly different from each other. The Gen II and Gen III treatments provided an effective control of tufted apple bud moth throughout the season (Figure 7). Variegated leafroller populations were too low for evaluation.

### **Assessment of terminal infestations**

There were no significant differences (df = 3, F = 0.65, P < 0.05) in mean percent terminal infestations between all the three pheromone blends treatment and the untreated plot. In addition, all the treatments were below the economically of 5 percent infested



Figure 4. Mean (± SE) number of four leafroller species caught per pheromone traps per season in mating disruption treatments OBLR = Obliquebanded leafroller pheromone. For leafroller species, OBLR = Obliquebanded leafroller, RBLR = Redbanded Abbrevations : For treatments, Control = Untreated plot, Gen II = Generic II pheromone, Gen III = Generic III pheromone, in apple orchards, Douglas (1994). Means followed by the same letter are not significantly different (LSD test, P<0.05) leafroller, TABM = Tufted apple bud moth, VLR = Variegated leafroller.





Abbrevations: For treatments, Control = Untreated plot, Gen II = Generic II pheromone, Gen III = Generic III pheromone, OBLR = Obliquebanded leafroller pheromone.





Figure 6. Mean number of adult male redbanded leafrollers (RBLR) caught in pheromone traps each Abbrevations: For treatments, Control = Untreated plot, Gen II = Generic II pheromone, Gen III = Generic III pheromone, OBLR = Obliquebanded leafroller pheromone. week in the mating disruption treatments in Douglas (1994).





terminals (Johnson and Herr 1995). The low percentage of terminal infestation in all the treatments does not indicate good control and is likely due to low leafroller populations in the plots.

### Assessment of fruit damage

The Gen II and Gen III treatments showed significantly lower (df = 15, F = 3.52, P < 0.05) mean percentage fruit damaged by the leafroller complex at harvest compared to OBLR and the untreated plots (Figure 8). The OBLR and untreated treatments were not significantly different from each other. All the pheromone treatments and the untreated plots provided fruit damage above the 5 percent economic threshold (Johnson and Herr 1995).

## A II. Efficacy of three different blends of pheromones for disruption versus insecticide and untreated plots for control of leafroller complex of apple-1995

### Assessment of mating disruption

During the growing seasons, there were no significant differences (P < 0.05) in mean number of OBLR trap catches per season between the Gen II, insecticide treatment and untreated check plot (Figure 9). In addition, OBLR and Gen II treatments were not significantly different (P < 0.05) from untreated plot. The microencapsulated pheromone formulation (Mec 240) treatment was significantly lower (df = 4, F = 3.53, P < 0.05) in mean number of OBLR trap catches compared to the insecticide treated and untreated check plot. However the OBLR and Gen II and Mec 240 pheromone treatments were not







Mean moths caught/trap/season



Figure 9. Mean (± SE) number of each of four leafroller species caught pe pheromone traps per season in mating disruption and insecticide treatments in apple orchards, Douglas (1995). Means followed by the same letter are not significantly different (LSD test, P<0.05).

pheromone formulation, OBLR = Obliquebanded leafroller pheromone. For leafroller species, OBLR = Obliquebanded leafroller RBLR = Redbanded leafroller, TABM = Tufted apple bud moth, VLR = Variegated leafroller Abbrevations: For treatments, Control = Untreated plot, Gen II = Generic II pheromone, Mec 240 = 240 ml Microencapsulated

significantly different (P < 0.05) in the mean number of OBLR trap catch per season. OBLR captures were lowest in plots treated with Mec 240 pheromone, however captures were also low in plots treated with OBLR pheromone, although to a lesser degree (Figure 9).

In the 1995 season, Mec 240 pheromone treatment provided effective trap shut down for OBLR for the whole season (Figure 10). However, OBLR pheromone treatment provided control of only second generations OBLR. The Gen II pheromone, insecticide and untreated check plots showed the highest mean number of OBLR males per week.

In this study, all pheromone and insecticide treatments were significantly different (df = 4, F = 10.19, P < 0.05) in the mean number of RBLR trap catches per season compared to untreated plots (Figure 9). The OBLR pheromone treatments had significantly lower catch of RBLR males when compared to insecticide and untreated check plots, and the Gen II and Mec 240 were intermediate between the OBLR and insecticide treatments. The results in Figure 11, shows that the OBLR pheromone blend provided the most effective shut down of RBLR throughout the season as compared to other treatments. While Mec 240 pheromone also showed good trap shut down for RBLR for 5-6 weeks, it did not disrupt flight of the second generation RBLR.

The Gen II pheromone treatments showed significant differences (df = 4, F = 2.26, P < 0.05) in mean number of trap catches of TABM per season compared to the Mec 240 pheromone and OBLR treatments. There were no significant differences (P < 0.05) in mean trap catches of TABM between the Gen II, insecticide and untreated check plots (Figure 9). The Gen II pheromone blend gave excellent trap shut-down of TABM





Microencapsulated pheromone formulation, OBLR = Obliquebanded leafroller pheromone, and Insecticide = Insecticide treatments.





Abbrevations: For treatments, Control = Untreated plot, Gen II = Generic II pheromone, Mec 240 = Mec 240 ml Microencapsulated pheromone formulation, OBLR = Obliquebanded leafroller pheromone, and Insecticide = Insecticide treatments.



Mean number TABM/trap/week

Abbrevations: For treatments, Control = Untreated plot, Gen II = Generic II pheromone, Mec 240 = Mec 240 ml Figure 12. Mean number of adult male tufted apple bud moth (TABM) caught in pheromone traps each week Microencapsulated pheromone formulation, OBLR = Obliquebanded leafroller pheromone, and in mating disruption and insecticide treatments in Douglas (1995). Insecticide = Insecticide treatments. through the season as compared to all the treatments (Figure 12). Variegated leafroller trap catch were monitored, but the populations was too low for evaluation (Figure 9).

### Assessment of terminal infestations

The Gen II pheromone blend was significantly lower (df = 4, F = 6.04, P < 0.05) mean percentage infested terminals when compared to the untreated check, insecticide and Mec 240 pheromone treatments on May 31 (Figure 13) but there were no significant differences (P < 0.05) between the Gen II and OBLR treatments. The Gen II treated plot provided the lowest mean percent larval infestation on terminals on the May 31 sample.

Mean percent infested terminals of OBLR, Gen II, Mec 240 pheromone and insecticide treatments were significantly different (df = 4, F = 10.86, P < 0.05) from the untreated check plot on July 14 (Figure 13).

Gen II treatments were not significantly different (P < 0.05) from the untreated check in mean percent infested terminals on July 31, but both had significantly higher (df = 4, F = 27.42, P < 0.05) infestations than the other treatments (Figure 13). The OBLR pheromone treatments provided terminal infestation below the five percent economic threshold (Johnson and Herr 1995) but was higher than insecticide treated plots.

### Assessment of fruit damage

The untreated, Gen II and OBLR treatments showed no significant differences (P < 0.05) in mean percent fruit damage at harvest (September 9) but these had significantly (df = 4, F = 3.91, P < 0.05) higher damage than the Mec 240, OBLR and insecticide treatments (Figure 14). None of the pheromones or insecticide treatments provide commercially acceptable control fruit damage.





Figure 13. Mean ( $\pm$  SE) percentage infested terminals of apple by leafroller complex for the three dates Mean followed by the same letter are not significantly different (LSD test, P<0.05). in mating disruption and insecticide treatments in Douglas (1995).

Abbrevations: For treatments, Control = Untreated plot, Gen II = Generic II pheromone, Mec 240 = Mec 240 ml

Microencapsulated pheromone formulation, OBLR = Obliquebanded leafroller pheromone, and Insecticide = Insecticide treatments.

Leafroller complex includes obliquebanded, redbanded, variegated leafrollers and tufted apple bud moth.





Insecticide = Insecticide treatments.

Leafroller complex includes obliquebanded, redbanded, variegated leafrollers and tufted apple bud moth.

provide commercially acceptable control fruit damage.

All treatments, had significantly lower (df = 4, F = 9.03, P < 0.05) fruit damage than the untreated check plot on June 21, and there were no differences (P < 0.05) among the pheromone blends. Damage in the untreated check was above the economic threshold.

# B. Efficacy of three rates of microencapsulated pheromone formulation (Mec) and OBLR dispenser to control OBLR and RBLR in 1995.

All rates of the microencapsulated pheromone formulation (Mec) were not significantly different (P < 0.05) from the untreated check in the total number of RBLR males caught for the entire season (Figure 15). However, the OBLR, Mec 240, and Mec 60 treatments were not significantly different from each other in the capture of RBLR.

The OBLR pheromone blend provided excellent trap shut down for RBLR throughout the season, after application of the OBLR dispenser (Figure 16). The Mec 240 also provided good trap shut down for RBLR, but only for a period of about 5 to 6 weeks. The Mec 60 and Mec 15 treatments were similar to the control, providing no effective trap shut down.

OBLR populations were too low to provide meaningful results. No significant differences in mean number of codling moth trap catches were observed throughout the season in the Mec 240, OBLR treatments and the untreated plot.

### DISCUSSION

General trend was observed indicating that the application of the pheromones using Gen II, Gen III and OBLR do not provide effective trap shut down of








Mean number RBLR/trap/week

obliquebanded leafroller in the first generation in the 1994 and 1995 trials. Similarly, no significant different in the mean number of obliquebanded leafroller per trap per season were observed between Gen II, Gen III and OBLR pheromone blends in 1994 and between Gen II and OBLR pheromone blends 1995 trials. However, application of the microencapsulated pheromone formulation (Mec 240 ml) greatly disrupted leafroller behavior as evidenced by the dramatically lower catch of males obliquebanded leafroller in pheromone-baited traps within treated plots in 1995 trial (Figure 9). The pheromone provided effective trap shut down for obliquebanded leafroller for the whole season. The OBLR pheromone blend seem to give control only for second generations obliquebanded leafroller. The untreated control plot indicated that the highest mean number of obliquebanded leafroller males catch per season of 72 in 1994 trial. In 1995 trials the untreated control and insecticide plots had 48 and 56 males caught per season respectively (Figure 9).

The microencapsulated pheromone formulation (Mec 240 ml) and OBLR may be more effective against obliquebanded leafroller is that they containing only the E 11-14: Ac and Z 11-14:AC may enhance the mating disruption compared to the Gen II and Gen III. Taschenberg et al., (1974) reported successful disruption of orientation to traps and reduction in damage by leafrollers when the proper pheromone blend was used. In the Gen II and Gen III in addition to the above two compounds, the present of other components ratio, Z11-14:OH for OBLR pheromone and E11-14:OH for Gen II and Gen III pheromone may reduced the degree of mating disruption. The isomeric ratio is critical in eliciting male response in redbanded leafroller (Baker et al. 1976).

In both the trials in 1994 and 1995, including the trial at Fennville using different

rates of microencapsulated pheromone, the same pattern in mating disruption treatment was observed for effective trap shut down of redbanded leafroller males for the first generation of redbanded leafroller by the OBLR pheromone blend (Figure 6 and 11). Although the Mec 240 ml provided effective disruption of redbanded leafroller for 5-6 weeks but did not provide effective control for the second generation redbanded leafroller (Figure 11). This trend was also been observed in the mating disruption trial carried out at Fennville in 1995. From these results, it is evidence that the efficacy of Mec 240 ml treatment could maintain their efficacious for five to six weeks from the initiation of the application. In the 1994 and 1995 trial indicated that there were significant difference in the mean number of redbanded leafroller male caught in traps per season in OBLR, Gen II or Gen III compared to the untreated plot (Figure 4 and 9). However OBLR pheromone blends showed significant difference in mean number of redbanded leafroller trap catch compared to insecticide and untreated plots in both trials.

The Gen III pheromone blend used in 1994 trial and Gen II pheromone blend used in both 1994 and 1995 trials showed excellent disruption of tufted apple bud moth from the beginning of the application of the pheromone (Figure 7 and 12). However, there was also a pattern indicating that OBLR treatment in both trials did not provide significant control of tufted apple bud moth. Both OBLR and Mec 240 treatments showed no significant difference in trap catch of tufted apple bud moth compared to the untreated and insecticide plots.

The Gen II and Gen III pheromone blends showed effective disruption of tufted apple bud moth may be attribute to the present of the E11-14:Ac and E11-14:OH components which are similar to the sex pheromone produced by the female tufted apple

bud moth. Mating disruption can be successful when proper pheromone blend was used (Tashenberg et al. 1974).

The insecticide treatment did not provide effective control of the moth population for the obliquebanded leafroller, redbanded leafroller and tufted apple bud moth in the 1995 trial.

Three generations of redbanded leafroller were observed in the two years trial, one small first generation appeared on the first week of May, the second generation begins on the first week of June and finally in the middle of August is the third generation (Figure 6 and 11). However, the obliquebanded leafroller had two generations, a large generation began on the second week of June and a small second generation emerged on the second week of August in the 1994 trial. A small bimodal generation of obliquebanded leafroller emerged between the month of August to September in the 1995 trial.

Although all the treatments, Gen II, Gen III and OBLR pheromones showed a low percentage of terminal infestations in 1994 trial, it does not seem to provide good control and is likely due to low leafroller populations in the plots. Another possible explanation of low infestation may be the trees were not pruned in the recent years. Lack of regeneration of new terminals in the plots could reduce populations of leafroller larvae.

In the 1995 trial, terminal injury by the leafroller complex was light during the early part and increased in the later part of the first generation. Similar results were observed in the injury of terminals caused by *Platynota* spp. where infestation was low during the first generation, increasing in the fall as described by Chapman and Link (1971).

The Gen II and OBLR pheromone treatments showed low level of mean percent infested terminals on May 31 1995 because both treatments provided good trap shut down for RBLR at the early apple growing season (Figure 11) and the other three leafroller species were not present at that particular time. The other three treatments, Mec 240, insecticide and untreated plots had terminal infestations exceed the five percent economic threshold due to the present of adult populations of redbanded leafroller in the plots beginning of May 2 (Figure 11). The presence of the adult moth population activity could have resulted in mating, egg laying and early larval feeding on the young terminals. The insecticide plot had terminal infestations because no insecticide was applied to the plot at that particular time.

On July 14 evaluations of the terminal infestations, the insecticide treatment showed the lowest level of terminal infestations among the treatments (Figure 13). The untreated plot had the highest mean percent infested terminals at 12 percent. This high injury is due to the presence of high population of the three leafroller species (OBLR, RBLR and TABM) in the plots (Figure 10, 11 and 12). The Gen II, OBLR, and Mec 240 treatments were not significantly different in mean percent terminal infestations and provided terminal infestations below the five percent economic threshold (Figure 13) (Johnson and Herr 1995). The Mec 240 and OBLR treatments had lower level terminal infestation because the treatment provided effective trap shut down of obliquebanded and redbanded leafroller, while the Gen II treatments provided effective trap shut down of only tufted apple bud moth.

Only OBLR and insecticide treatments showed terminal infestations below five percent economic threshold on the July 31 assessment of terminal infestation (Figure 13).

Although there was moth activity throughout the season in the insecticide treatment there were no terminal infestations by leafroller species (Figure 13). The possible reason could be persistent insecticides Lannate and Lorsban which were applied on August 21, which may have killed the larvae present in the plot. The possible explanation of the Gen II, Mec 240 and the untreated plots having terminal infestations above five percent economic threshold may be due to immigration of mated females into the relatively small trial plot (Ogawa 1990, Trimble 1991).

In 1994 trials although Gen II and Gen III treatments were significantly different in mean percent damaged fruit compared to OBLR and untreated plots at harvest, however none of the treatments provided commercially acceptable control of fruit damage.

Similarly, none of the treatments showed any commercially acceptable control of fruit damage in the 1995 trial. It appears that damage may have resulted from immigration of mated females into this relatively small plot, a common problem in mating disruption efforts (Ogawa 1990, Trimble 1991). The probable source of pests was from plots of abandoned apple tree adjacent to the trial plots. Alternatively, increased mating could have occurred near orchard edges because of lower pheromone concentrations relative to the center of the orchard (Ogawa 1990, Trimble 1991).

In summary, mating disruption show some efficacy on certain species of leafroller and for part of the season. No one pheromone blend provided disruption of multiple species of leafroller. None of the pheromones or the insecticide treatment provided commercially acceptable control of fruit damaged by leafroller.

OBLR pheromone blend disrupted flight of redbanded leafroller more effectively

than all treatments and provided some disruption of obliquebanded leafroller. The Gen II and Gen III provided inadequate disruption of obliquebanded and redbanded leafrollers, but excellent disruption of tufted apple bud moth in both 1994 and 1995 trials. The OBLR pheromone blend provided terminal infestation below economic thresholds but higher than insecticide treatment in 1995 trial. Obliquebanded leafroller disruption was most effective with the Mec 240 treatment in 1995 trial. This treatment provided effective disruption of redbanded leafroller for five to six weeks but did not the disrupt flight of second generation redbanded leafroller. Further studies should evaluate reducing the application rates of Mec and making an additional application five to six weeks after the first. This may provide effective control of the second generation leafrollers, especially the obliquebanded and redbanded leafrollers.

Finally, future studies on the release rate, amount of concentration of pheromone present in the plot, larger plot size or other factors should be carried out to determine the effectiveness of mating disruption of leafroller complex in apple orchard.

**CHAPTER 2** 

#### **CHAPTER 2**

# Impact of mating disruption for control of the leafroller complex on natural enemies and non-target pests

### **INTRODUCTION**

Conventional control programs for primary pests of apple such as codling moth, plum curculio and oriental fruit moth have resulted in a significant disruption of the nontarget and natural enemy populations. This subsequently caused a substantial rise of secondary insect pest complex such as obliquebanded leafroller (OBLR), *Choristoneura roseaceana* (Harris); redbanded leafroller (RBLR), *Argyrotaenia velutinana* (Walker); tufted apple bud moth (TABM), *Platynota idaeusalis* (Walker); and variegated leafroller (VLR), *Platynota flavedana* (Clemens) in the orchard.

A good example of this is the rise of RBLR to pest status after use of various organic insecticides against codling moth and other pests had reduced the impact of its natural enemies (Paradis 1956). The frequent use of broad spectrum insecticides including organophosphates, pyrethroids, carbamates and mixtures of these compound to control the pests in the apple orchards has resulted in the development of resistance to these chemicals. The development of resistance to organophosphate insecticides by OBLR in Michigan apple orchards was reported by Howitt (1979). Knight et al. (1989) and Biddinger (1993), reported that organophosphate resistance is now wide spread

among the TABM populations in North Carolina and Pennyslvania.

The results of broad spectrum insecticide usage, such as organophosphate resistance in the leafroller complex, along with public health concerned led to a search for safer and effective alternative methods of control. Two very promising alternatives are the potential use of pheromone-mediated mating disruption (Hull et al. 1993) and the use of physiologically selective compounds such as insect growth regulators (Biddinger 1993). These alternatives assist in the conservation of existing biological control agents, such as predators and parasitoids of various species of leafrollers, including TABM (Biddinger et al. 1994). Insect growth regulators and mating disruption can selectively affect a narrow range of arthropods and cause less disturbance in the orchard system (Croft 1990).

A fundamental principle of integrated pest management programs is to curtail the pesticide usage and hence reduce the adverse effect on the natural enemies. The use of pheromone for mating disruption and the incorporation with other methods of control in integrated pest management have clear potential for control of a variety of major pests (Rothschild 1981). Using mating disruption for control of a leafroller complex might allow natural control agents to supplement control of the pest and assist in reducing non-target pests below damaging levels.

Allen (1962), in a survey of the parasitoids of oriental fruit moth, *Grapholita molesta* (Busck), indicated that before biological control agents can be integrated into workable integrated pest management programs, identification of key parasitoids and the interaction with the hosts on associated crops need to be determined throughout the season. Doganlar et al. (1978) examined parasitoids of OBLR in Vancouver District,

British Columbia. Pogue (1985) studied parasitoids of OBLR and other leafrollers in Wyoming. Finally, Maltais et al. (1989) studied the seasonal biology of *Meteorus trachynotus* (Viereck) on overwintering OBLR in Quebec.

There have been no studies of natural enemies, parasitoids and parasitism rate of OBLR in Michigan apple orchards. I have chose OBLR on my study because it has become the most important leafroller species in Michigan and Western New York during the last several years. Any information regarding natural enemies, parasitoids and parasitism rate of OBLR may become of paramount importance in future pest management programs in Michigan.

The objectives of my study were to :-

- Compare the impact of mating disruption on the populations of natural enemies and non-target insects in the apple orchard, with commercial insecticides.
- 2) Identify the parasitoids parasitizing the egg mass and larval stages of obliquebanded leafroller in the mating disruption, insecticide and untreated plots in apple orchards.
- 3) Compare the impact of mating disruption on parasitism of egg mass and larval stages of obliquebanded leafroller in an apple orchard with commercial insecticide.

#### **MATERIALS AND METHODS**

The three studies reported here were conducted in the same mating disruption efficacy test plots described in Chapter 1 shown in Figure 2.

### Assessment of natural enemies and non-target pests.

Beneficial and other arthropods were sampled every two weeks with a modified

leaf blower/vacuum as a suction sampling device, from May 19 to September 30, 1995. Four apple trees from each replicate (Figure 2) were selected and sampled repeatedly for the duration of this study. Each tree was sampled for 30 seconds(2 minutes for 4 trees) from trunk to tip along tree branches located approximately 1.5 m above the ground. Samples were collected in nylon stockings in the suction device and immediately frozen to kill arthropods and prevent sample deterioration. Samples were sorted within 24 hours to assure positive identification of fresh specimens and preserved in 70 % ethyl alcohol solution for identification. The arthropod samples were identified to the family level. To measure the diversity in the system, the Shannon diversity index (H') as described by Magurran 1988 and Simpson's index (Simpson 1949) were calculated using the following equation:

Shannon diversity index:

$$\mathbf{H'} = -\mathbf{p'} \ln \mathbf{p'}$$

where quantity p' represents the proportion of individuals found in the `th species and H' depends on the number of species and their abundance (Magurran 1988). Simpson's index :

$$D = \sum [n_i(n_i - 1)/N(N - 1)]$$

where  $n_i$  = the number of individuals in the *i*th species and

N = the total number of individuals

For statistical analysis, the number of individuals per order was transformed by log (X + 1) to test for normality and homogeneity of variance. The data were analyzed using analysis of variance and where the mean differences were significant, means were

compared using a LSD test (alpha = 0.05) (SAS Institute 1989).

# Assessment of parasitism of the obliquebanded leafroller larvae in mating disruptions, insecticide and the untreated plots in Michigan apple orchard.

Obliquebanded leafroller larvae were collected on August 1, 1995, by timed searches (6 min./tree) around the perimeter of two apple trees per replicate (Biddinger et al. 1994). The leaves on the trees were visually searched for obliquebanded leafroller larvae. When located, larvae together with the infested leaves from the tree were transferred into a plastic ziplock bag. The larvae collected from four replicates of each treatment were pooled because of the low number of larvae obtained per replicate. Larvae that were collected from the bags were reared on codling moth diet (Bio Serv Inc.) in an environmental chamber at 21 or 27 °C and 17 hr light and 7 hr dark period cycles (Glass et al. 1962). Samples were observed twice weekly for adult eclosion, death and parasitoid emergence.

All parasitoids were pin mounted and identified to the family level. Data was not statistically analyzed because of low number of obliquebanded leafroller larvae and all larvae in each replicate had been pooled.

Assessment of parasitism of the obliquebanded leafroller eggs in mating disruptions, insecticide and the untreated plot in Michigan apple orchard.

## Collection of Sentinel Egg Masses

A total of 30 male and 21 female obliquebanded Douglas, MI on June 20 and 21, 1995. Adult moths were immediately placed in a rearing cage (Karpel et al. 1968).

The rearing cage consisted of a coarse polystyrene foam frame (12 x 8 in. OD and 10  $1/2 \ge 6 1/2$  in. ID) (Figure 17). A trough cut in the bottom served as a wick or cotton holder by which honey solution as a diet medium was supplied for the adults. A slit in the top allowed placement of waxed paper for oviposition, the addition of honey solution to the wick, or adding more insects to the cage. A length of cheesecloth was placed around the cage and held in place with 4 rubber bands (Figure 18). As an added precaution the cage was placed in a plastic box with perforated top to prevent contamination of the cage and adults. The plastic box and polystyrene foam were sterilized by soaking in 50 % bleach for 24 hours and dried before use. Adult male and female obliquebanded leafrollers in the rearing cage were placed in an environmental chamber at 25 °C and 19 °C with a 18 hr light and 6 hr dark period on June 22, and were monitored three times weekly for egg mass deposition. A total of 40 OBLR egg masses were collected from the rearing cage on June 25 and 26. Each egg mass was cut out from the wax paper, washed with distilled water, and placed into separate vials and capped. The vials were placed in a container with water to prevent the egg masses from desiccating and held in an environmental chamber at 25 °C (Glass et al. 1962) for an interim period until the egg masses were placed in the field.

Two egg masses on waxed paper (one egg mass per tree) per replicate for each treatment were stapled on perimeter leaves of an apple tree approximately 1.5 m above the ground on June 27. All the egg masses were collected on June 29 after being in the field for 48 hours and were placed back into labeled petri dishes. The petri dishes were sealed with cellophane tape to prevent smaller parasitoids from escaping if they emerged from the egg masses. The petri dishes were placed in an environmental chamber at 25 °C



Figure 17. Adult cage frame (Karpel and Hagmann 1968)



Figure 18. Adult cage in use (Karpel and Hagmann 1968)

and were monitored daily for egg parasitism. The egg masses were assessed and the number of eggs parasitized was counted under a dissecting microscope. The data were subjected to an arcsin (x) transformation, where x was the % of parasitism. Percent parasitism was analyzed using ANOVA (SAS Institute 1989).

#### RESULTS

#### Assessment of natural enemies and non-target insects.

In general, the higher the diversity index, the more equal in abundance the arthropod community (Krebs 1985). In the early growing season, general trends were observed in all treatments that the Shannon diversity index values increase from May 19 to June 4 1995 (Figure 19a), with the highest index value of 3.05 in the OBLR treatment and the lowest of 2.60 in the insecticide treatment. However the index value decreased at the end of the growing season on September 30 with the highest index value of 1.59 in the Gen II treatment and the lowest of 0.58 in the insecticide treatment.

After June 4 1995, the analysis of the Shannon diversity index values in the mating disruption treatments and untreated plot showed little variation throughout the season (Figure 19a). The diversity index value of all mating disruption treatments, Gen II, Mec 240 and OBLR varied throughout the season from 2.92, 2.71 and 3.04 to 1.08, 1.24 and 1.16 respectively. In general, the diversity index value of all the mating disruption treatments for all dates tended to fall within the values of the untreated and the insecticide treatment with the exception of July 18 and August 30. The Gen II and Mec 240 treatments on July 18 and all the mating disruption treatments on August 30 exceeded the index value of untreated and insecticide treated plot. The untreated plot had





Abbrevations : For treatment, Control = Untreated plot, Gen II = Generic II pheromone, Mec 240 = 240 ml Microencapsulated pheromone formulation per 0.45 ha, OBLR = Obliquebanded leafroller pheromone and Insecticide = Insecticide treatments. Figure 19a. Shannon diversity index of apple arthropods in mating disruption, insecticide and untreated plots. index values within dates followed by the same letter are not significantly different (LSD test, P<0.05). Note: Lannate and Lorsban was applied to insecticide plots and Provado 1.6 Flowable to all plots.





Simpson's Diversity Index

a diversity index value varying from 2.96 to 1.30.

The insecticide treatment showed a significantly lower Shannon diversity index throughout the season with values varying from 2.60 to 0.50 (Figure 19a). A clear decrease in its value was observed immediately after the first insecticide treatment on June 7, 1995 (at the petal fall of apple). Further decrease in the value followed the second application on June 29, the lowest value of 0.50 was observed on August 2, 1995. The diversity index values of all the treatments decreased to a low level on August 2, 1995. The insecticide treatment was at the lowest index value of 0.50, followed by OBLR, Mec 240, Gen II and untreated of 1.19, 1.24, 1.27 and 1.47 respectively (Figure 19a). All the mating disruption treatments including the untreated plot showed higher diversity index value compared to the insecticide treatment on same date.

The Shannon index value of insecticide treatment was low compared to all other treatments after the third insecticide treatments on August 21 1995. However the insecticide treatment showed no differences in index value compared to untreated, Gen II and OBLR treatments on August 30 1995, while differences (df = 4, F = 2.17, P < 0.05) were observed in Mec 240 treatments. Subsequently, the following 14 days of insecticide treatment did not show significant differences in index value compared to all the mating disruption treatments. They were different compared to the untreated plot.

However, the Simpson's index value of all the treatments showed no different from May 19 to June 16 1995 and from July 18 to August 30 1995 (Figure 19b). All the mating disruption and insecticide treatments showed a significantly (df = 4, F = 1.3, P < 0.05) lower index value compared to untreated plot on June 29. The OBLR pheromone treatments was at the lowest index value of 0.64, followed by insecticide, Mec 240, Gen

II and untreated of 0.67, 0.83, 0.84 and 0.91 respectively (Figure 19b).

The Simpson's index value of insecticide treatment was significantly (df = 4, F = 6.06, P < 0.05) lower compared to all other treatments on September 16 and 30, 1995 after the third insecticide treatments on August 21, 1995 (Figure 19b). However, the insecticide treatment had more dominant family species compared to all the other treatments.

The responses of different insect orders varied by treatment (Table 2).

Hymenoptera: Hymenoptera were consistently lower in number in the insecticide treatment compared to all the other treatments from the beginning to the end of growing season (Figure 20). No differences (df = 4, F = 1.48, P < 0.05) in numbers of Hymenoptera were observed between any of the treatments on May 19 and June 4 evaluations. On June 16, 1995 Gen II treatment showed the highest number of Hymenoptera compared to all other treatments. Gen II treatment had a two and half fold higher in number compared with untreated, Mec 240 and insecticide treatments. Finally, Gen II was three fold higher in numbers of Hymenoptera compared to the OBLR treatment. No difference (df = 4, F = 1.26, P < 0.05) was noted in the Gen II, Mec 240, untreated and insecticide treatment on June 16, 1995 nine days after the insecticide treatment was applied to the insecticide plot. Hymenoptera in the insecticide treatment were significantly (df = 4, F = 9.79, P < 0.05) lower than all the other treatments on August 2, 1995 after two applications of insecticides were applied to the insecticide plots. The first and second insecticide applications were two months and one and half months before the evaluation date. Subsequently, on August 30 and September 16 1995 the insecticide treatments showed a significantly (df = 4, F = 7.55, P < 0.05) lower mean

1995
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Mean
Table 2.

		5/19	6/4	6/16	6/29	<u>7/18</u>	8/2	8/16	8/30	9/16	9/30
ORDERS HYMENOPTER/	Treatment \										
	Control	2.00 a	10.50 a	8.25 ab	5.50 a	7.25 a	10.75 a	7.75 a	3.75 a	7.00 a	0.50 b
	Gen II	1.25 a	6.00 a	19.75 a	7.25 a	7.50 a	9.75 a	4.00 bc	3.00 a	3.25 ab	1.50 ab
	<b>Mec 240</b>	2.00 a	7.75 a	8.00 ab	7.75 a	6.75 a	<b>4</b> .50 a	7.75 a	5.25 a	2.50 ab	2.00 a
	OBLR	0.25 a	6.00 a	6.25 b	8.75 a	4.00 a	6.00 a	6.75 ab	2.75 a	2.25 b	1.50 ab
	Insecticide	0.50 a	4.50 a	7.50 ab	2.50 a	3.50 a	0.50 b	1.75 c	0.25 b	0.00 c	0.50 b
ARANEAE											
	Control	1.25 a	0.50 a	0.75 a	0.75 a	0.75 bc	3.25 a	6.75 a	2.00 ab	2.75 ab	5.25 a
	Gen II	0.75 a	1.50 a	0.25 a	1.00 a	<b>4.00 a</b>	6.00 a	6.00 ab	3.50 ab	2.75 ab	5.50 a
	<b>Mec 240</b>	2.25 a	0.75 a	0.75 a	0.75 a	1.75 b	<b>4</b> .50 a	5.00 ab	5.00 a	<b>4.</b> 25 a	<b>3.25 ab</b>
	OBLR	0.75 a	0.75 a	0.50 a	0.25 a	0.75 bc	3.00 a	3.00 bc	4.50 a	<b>4.</b> 50 a	2.75 ab
	Insecticide	1.25 a	1.00 a	0.00 a	0.00 a	0.00 c	0.00 b	1.25 bc	1.25 b	1.00 b	0.50 b
DIPTERA											
	Control	<b>4.</b> 50 a	23.75 a	22.00 a	15.00 a	25.00 ab	20.50 a	6.50 a	<b>1.50 a</b>	1.25 ab	2.50 a
	Gen II	3.00 a	27.50 a	18.00 ab	20.00 a	39.25 a	9.75 ab	5.50 a	1.25 a	1.50 ab	1.75 a
	Mec 240	<b>4</b> .00 a	23.75 a	13.75 ab	14.50 a	29.75 ab	8.50 ab	5.25 a	<b>2.</b> 50 a	2.75 a	1.50 a
	OBLR	7.25 a	33.00 a	11.75 ab	20.50 a	19.25 ab	6.25 ab	<b>4</b> .75 a	1.75 a	1.75 ab	1.00 a
	Insecticide	3.25 a	24.25 a	9.75 b	6.75 a	16.25 b	2.25 b	5.00 a	1.25 a	0.25 b	2.00 a
HOMOPTERA											
	Control	0.85 a	<b>4</b> .75 a	5.75 a	7.00 b	3.75 b	1.75 b	6.50 a	0.75 a	4.75 ab	2.50 a
	Gen II	0.70 a	7.00 a	9.00 a	21.75 a	<b>4.50 ab</b>	2.50 b	5.50 a	1.50 a	2.75 ab	2.00 a
	Mec 240	1.50 a	1.75 a	3.75 a	6.75 b	12.25 a	9.75 a	<b>4</b> .00 a	2.25 a	1.50 b	2.00 a
	OBLR	<b>1.50 a</b>	1.75 a	<b>4.</b> 25 a	24.25 a	8.25 ab	<b>4.</b> 50 ab	3.75 a	0.75 a	3.50 ab	2.75 a
	Insecticide	2.00 a	3.00 a	7.25 a	4.00 b	7.75 ab	8.50 a	5.75 a	0.25 a	6.50 a	3.75 a

Mean within dates followed by the same letter are not significantly different at P<0.05 (LSD test)





number of Hymenoptera compared to all the other treatments (Figure 20). Dutcher (1975) and Hagley et al. (1981) reported that many apple orchards insecticides, including phosmet and diazinon, are toxic to hymenopterous parasitoid adults of spotted tentiform leafminer, *Phyllonorycter blancardella* (Fabr.).

Hymenoptera populations were largely dominated by the superfamily of Chalcidoidea, followed by the families Braconidae and Ichneumonidae. Specimens of Chalcidoidea were predominately from Pteromilidae, Perilampidae, Eulophidae, Eupelmididae, Encyrtidae, Eurytomidae, Cynipidae, Mymaridae and Trichogrammatidae. The Chalcidoidea gradually increased in all treatments in the early growing season from May 19 to June 4. Although no significant differences in mean number was observed in all treatments on June 16, the Gen II treatment had the highest number compared to all other treatments (Figure 21). Chalcidoidea were significantly (df = 4, F = 4.62, P < 0.05) lower in the insecticide treatment compared to all other treatments on August 2, 1995 after two application of insecticides to the insecticide plots. The insecticide treatment had low numbers of Chalcidoidea compared to all the treatments throughout the season.

The populations of Braconidae and Icheneumonidae were too low to provide meaningful results (Table 3). Similarly, other families in Hymenoptera, including Ceraphronidae, Scelionidae, Formicidae and Apidae had populations too low for meaningful evaluations.

<u>Araneae:</u> The mean number of Araneae was low and no difference was observed in all treatments from May 19 to June 29, 1995. However no Araneae were found from June 16 to August 2, 1995 in the insecticide treatment after three applications of insecticide on June 4, June 29 and July 12 1995 (Figure 22). From June 29 to August



Figure 21. Mean numbers of Chalcidoidae per sample in mating disruption, insecticide and untreated plots. Abbrevations: For treatments, Control = Untreated plot, Gen II = Generic II pheromone, Mec 240 = 240 ml Note: Lannate and Lorsban was applied to insecticide plots and Provado 1.6 Flowable to all plots. Microencapsulated pheromone per 0.45 ha, OBLR = Obliquebanded leafroller pheromone and Mean within dates followed by the same letter are not significantly different (LSD test, P<0.05). Insecticide = Insecticide plot.

Mean numbers of Hymenoptera per sample in leafroller mating disruption treatments 1995 Table 3.

Grantly         5/19         6/4         6/16         6/29         7/18         8/2         8/1           Family         Treatment         5/19         6/16         6/29         7/18         8/2         8/1           Family         Control         0.25         a         0.56         a         0.25         c         0.25         c         0.25         c         0.25         c         0.25         c         0.25         c         0.25         a         0.25         a         0.25         c         0.25 <td< th=""><th>lchnaumonoidaa</th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th>2442</th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th></td<>	lchnaumonoidaa								2442									
Chneumonoidea       Treatment         Family       Control       0.25       a       0.50       a       0.25       a       2.56       a       0.50         Braconidae       Control       0.25       a       0.25       a       0.50       a       0.51         Braconidae       Control       0.25       a       0.55       a       0.56       a       0.25         Rec 240       0.00       a       0.00       a       0.00       a       0.75       a       0.75       a       0.75       a       0.75       a       0.25       c       1.00         Mec 240       0.00       a       1.00       a       0.00       a       0.25       a       0.75       a       0.25       c       0.25 </th <th>Ichneimonoidea</th> <th></th> <th>5/19</th> <th>6/4</th> <th></th> <th>6/16</th> <th></th> <th>6/29</th> <th>7/18</th> <th>8/2</th> <th></th> <th>8/16</th> <th></th> <th>8/30</th> <th></th> <th>9/16</th> <th></th> <th>9/30</th>	Ichneimonoidea		5/19	6/4		6/16		6/29	7/18	8/2		8/16		8/30		9/16		9/30
Braconidae         Control         0.25         a         0.25         a         0.25         a         2.25         a         0.50           Gen II         0.00         a         0.00         b         0.00         a         0.75         a         2.26         a         0.25           Mec 240         0.00         a         0.25         ab         0.05         a         1.50         a         0.75         a         2.50         a         0.25         c         0.75         bc         0.76         bc         0.25         c         0.25         c         0.26 <td>Family</td> <td>Treatment</td> <td></td>	Family	Treatment																
Gen II       0.00 a       0.00 b       0.00 a       0.75 a       2.50 a       0.25 bc       0.75 bc       0.74 bc       0.76 bc       <	Braconidae	Control	0.25 a	0.50	ab	0.25	_	0.25 a	2.25 a	2.2	s ab	0.50	æ	0.00		8.0	-	0.0
Mec 240         0.00         a         0.25         a         1.50         a         0.75         bc         0.75           OBLR         0.000         a         1.50         a         0.000         a         0.255         a         0.75         a         0.25         c         1.00           OBLR         0.000         a         1.50         a         0.000         a         0.25         a         0.25         c         1.00           Family         0.000         a         1.00         ab         0.000         a         0.25         a         0.25         c         0.25         d         0.00         c		Gen II	0.00 a	0.0	٩	0.00	-	0.75 a	2.00 a	2.5(	a	0.25	68	0.00		0.50	0	0.0
OBLR       0.00 a       1.50 a       0.00 a       0.25 a       0.75 a       0.25 c       1.00         Family       1.00 a       1.00 a       0.00 a       0.25 a       0.75 a       0.25 c       0.25         Family       0.75 a       0.75 a       0.75 a       0.25 c       0.25 c       0.25         Family       0.75 a       0.50 b       0.25 a       0.00 a       0.00 a       0.25 a       0.00 a         Gen II       0.50 a       0.00 a       0.25 a       0.00 a       0.25 a       0.00 a       0.00 a         Mec 240       0.25 a       0.00 a       0.25 a       0.00 a       0.00 a       0.00 a       0.00 a         Mec 240       0.25 a       0.00 a       0.25 a       0.00 a       0.00 a       0.00 a       0.00 a       0.00 a         Mec 240       0.25 a       0.00 a       0.25 a       0.25 a       0.25 a       0.00 a		<b>Mec 240</b>	0.00 a	0.25	ap	0.00	_	0.25 a	1.50 a	0.7	<u>ک</u>	0.75	6	0.25		8.0		0.25
Family         Insecticide         0.00         a         1.00         ab         0.00         a         0.25         a         0.25         c         0.25           Family         Eamily         0.75         a         0.50         b         0.25         a         0.00         a		OBLR	0.00 a	1.50	đ	0.00	_	0.25 a	0.75 a	0.2	U IC	1.00	æ	1.50	ں م	8.0	-	0.00
Family       Control       0.75 a       0.50 b       0.25 a       0.00 a       0.25 a       0.00 a		Insecticide	0.00 a	1.00	ab	0.00	-	0.25 a	0.75 a	0.2	U IC	0.25	<b>a</b>	0.00		0.00	-	0.00
Icheneumonidae       Control       0.75 a       0.50 b       0.25 a       0.00 a       0.00 a       0.25 a       0.00         Gen II       0.50 a       0.00 a       0.25 a       0.00 a       0.75 b       0.00 a       0.00         Mec 240       0.25 a       0.00 a       0.25 a       0.00 a       0.00 a       0.00 a       0.00         Mec 240       0.25 a       0.00 a       0.25 a       0.00 a       0.00 a       0.00         Nec 240       0.25 a       0.00 a       0.25 a       0.00 a       0.00 a       0.00         Nec 240       0.00 a       0.00 a       0.25 a       0.25 a       0.00 a       0.00         Nec 240       0.00 a       0.00 a       0.00 a       0.00 a       0.00       0.00         Nec 240       0.00 a       0.00 a       0.00 a       0.00 a       0.00 a       0.00         Insecticide       0.00 a       0.00 a       0.00 a       0.00 a       0.00 a       0.00         Chalcidoidea*       Control       1.00 a       8.50 a       5.75 a       5.25 a       5.57         Gen II       0.75 a       6.00 a       13.00 a       5.77       5.77	Family																	
Gen II       0.50 a       0.00 a       0.25 a       0.00 a	<b>Icheneumonidae</b>	Control	0.75 a	0.50	٩	0.25	-	0.00 a	0.00 a	0.2	8	0.0	æ	0.00		0.50		0.00
Mec 240         0.25 a         0.00 a         0.20 a         0.00 a		Gen II	0.50 a	0.00	65	0.25	_	0.00 a	0.75 b	0.0	a	0.00	6	0.00		8.0	-	8.0
OBLR       0.00 a       0.00 a       0.25 a       0.25 a       0.25 a       0.00 a		<b>Mec 240</b>	0.25 a	0.0	<b>(5</b>	0.20	_	0.00 a	0.00 a	0.0	a	0.0	6	0.00		0.25 4		0.00
Insecticide 0.00 a 0.00 a 0.00 a 0.25 a 0.00 a 0.00 a 0.00 Chalcidoidea* Control 1.00 a 8.50 a 5.75 a 4.75 a 3.25 a 5.25 a 5.56 Gen II 0.75 a 6.00 a 13.00 a 5.75 a 4.25 a 5.25 a 2.77		OBLR	0.00 a	0.0	8	0.25	-	0.25 a	0.25 al	b 0.2	8	0.0	æ	0.00	-	8.0	-	0.0
Chalcidoidea* Control 1.00 a 8.50 a 5.75 a 4.75 a 3.25 a 5.25 a 5.50 Gen II 0.75 a 6.00 a 13.00 a 5.75 a 4.25 a 5.25 a 2.7		Insecticide	0.00 a	0.0	6	0.00	-	0.25 a	0.00 a	0.0	a	0.0		0.00	_	8.0	-	0.00
Gen II 0.75 a 6.00 a 13.00 a 5.75 a 4.25 a 5.25 a 2.7?	Chalcidoidea*	Control	1.00 a	8.50		5.75	a	4.75 a	3.25 a	5.2	8	5.50	~	1.75 \$	ģ	5.25	-	0.00
		Gen II	0.75 a	6.00	æ	13.00		5.75 a	<b>4.25 a</b>	5.2	8	2.75	٤	2.00 å	ą	2.50	ą	2.00
Mec 240 0.25 a 6.00 a 7.25 a 6.25 a 4.75 a 4.00 a 5.00		<b>Mec 240</b>	0.25 a	6.00	65	7.25	4	6.25 a	<b>4.75 a</b>	4.0	a	5.00	ab	3.00 4		2.00	ğ	2.00
OBLR 0.25 a 5.50 a 5.75 a 7.00 a 1.75 a 4.00 a 6.00		OBLR	0.25 a	5.50	đ	5.75	a	7.00 a	1.75 a	4.0	a	6.00	a	2.50 å	٩	1.75	X	1.25 8
Insecticide 0.25 a 3.50 a 7.25 a 2.00 a 2.50 a 0.00 b 1.50		Insecticide	0.25 a	3.50	9	7.25	a	2.00 a	2.50 a	0.0	q (	1.50	υ	0.25 1	0	8.0		0.50

Note: \* includes families of Pteromalidae, Perilampidae, Eulophidae, Eupelmidae, Encyrtidae, Eurytomidae, Cynipidae, Mymaridae and Trichogrammatidae.

Mean within dates followed by the same letter are not significantly different at P<0.05 (LSD test)

16, 1995 the Araneae consistently increased in number in all mating disruption treatments and untreated plots compared to the insecticide treatment. The mean number of Araneae in untreated and all mating disruption treatment were significantly higher (df = 4, F = 14.88, P < 0.05) compared to the insecticide treatment on August 2, 1995. On that date, the Gen II treatment had the highest number of Araneae (6 specimens) followed by Mec 240, untreated, OBLR and insecticide treatments with 4.5, 3.25, 3.00 and 0 specimens respectively (Figure 22). The number of Araneae was low in the insecticide plot compared to all other treatments at the end of the season, after the insecticide was applied on August 21 1995. At the end of the growing season on September 30, significant differences (df = 4, F = 3.62, P < 0.05) were observed in Gen II and untreated plot compared to insecticide treatment (Fig. 22). However no differences were noted between Mec 240, OBLR and insecticide treatments.

The predominant families of Araneae; namely, Amaurobiidae, Lycosidae, Salticidae, Dysderidae, Pholcidae, Oxyopidae and Oonopidae could not provide meaningful differences between families due to low populations.

<u>Diptera:</u> Although all treatments showed no differences in the number of Diptera from May 19 to June 14, 1995, the population in all treatments gradually increased (Figure 23). The Diptera were consistently low in the insecticide treatments compared to all other treatments as noted after the four insecticide applications to the insecticide plot on June 4, June 29, July 12 and August 21, 1995. On July 18, the Gen II pheromone treatment was significantly different (df = 4, F = 1.54, P < 0.05) from insecticide treatment but not different from the treatments (Figure 23). Differences (df = 4, F = 2.54, P < 0.050) in the number of Diptera were observed in the untreated compared to the





Insecticide = Insecticide plot.

ean # Araneae per sample





Mean numbers of Diptera per sample in leafroller mating disruption treatments 1995

Table:

Suborder Nemtorer	TREATMENT	5/19	6/4	6/16	6/29	<u>Date</u> 7/18	8/2	8/16	8/30	9/16	9/30
	Control Gen II Mec 240 OBLR Insecticide	0.75 a 0.50 a 2.55 b 2.50 b 0.25 a	6.25 b 8.75 ab 13.75 a 10.75 ab 4.75 b	5.25 a 4.75 a 2.50 a 4.25 a 2.00 a	6.25 a 5.75 a 8.75 a 5.25 a 1.75 a	20.75 a 27.00 a 14.75 a 17.50 a 15.50 a	7.70 a 65.0 a 5.50 a 2.25 a	4.25 a 3.50 a 3.25 a 3.00 a	1.50 a 0.75 a 2.00 a 1.25 a 0.75 a	1.25 ab 1.25 ab 2.75 a 1.75 ab 0.25 b	2.25 a 1.25 a 1.25 a 2.00 a
Suborder Cyclorrhapha	Control Gen II Mec 240 OBLR Insecticide	2.25 a 2.00 a 4.75 a 2.25 a	17.00 ab 16.50 ab 8.50 b 19.75 a 18.00 a	11.50 a 9.75 ab 6.00 ab 4.25 b	9.00 a 15.25 a 11.00 a 16.50 a 6.75 a	4.00 b 10.50 a 11.25 a 0.00 b	7.75 a 2.75 ab 2.75 a 1.00 b 0.50 b	2.25 a 2.00 a 3.00 a 1.00 a	0.00 a 0.50 a 0.50 a 0.55 a 0.25 a	0.00 a 0.25 a 0.00 a 0.00 a 0.00 a	0.25 a 0.50 a 0.55 a 0.75 a
Suborder Brachycera	Control Gen II Mec 240 OBLR Insecticide	1.50 a 0.50 a 0.25 a 0.25 a 0.75 a	1.50 a 0.50 ab 0.25 ab 0.00 b 0.75 ab	5:25 a 3.50 a 3.55 a 3.55 a 3.50 a	0.00 a 0.25 a 0.50 a 0.00 a	0.25 c 1.75 b 3.75 a 0.75 bc 0.75 bc	5.25 a 0.50 b 0.02 b 0.00 b 0.00 b	a 00.0 a 00.0 a a 00.0 a a 00.0	a 0000 a 0000 a a 0000 a a 0000	a 00.0 a 00.0 a a 00.0 a a 00.0 a a	e e e e

Mean within dates followed by the same letter are not significantly different at P<0.05 (LSD test)

insecticide plot on August 2 but no difference was observed compared to all the disruption treatments. Numbers of Diptera were low in all treatments after August 16 until September 30, 1995.

In general, Diptera were low in numbers in the insecticide treatments compared to all the other treatments after the four insecticide treatments (Table 4 and Figure 23). Numbers of Nematocera and Cyclorrhapha increased gradually in all the treatments during the early growing season from May 19 to June 4 and decreased at the end of the growing season from August 16 to September 30, 1995.

Numbers of Nematocera were not different in number between the untreated and the insecticide treatment but those under Mec 240 treatment were higher compared to the untreated and insecticide treatment (Table 4). For Cyclorrhapha, the mating disruption treatment had a higher number compared to the untreated and insecticide treatment from the third week of June to the third week of July 1995. In Brachycera, all the treatments had too low populations to give meaningful results.

<u>Homoptera</u>: No significant difference in numbers of Homoptera was observed in all treatments including the insecticide plot from May 19 to June 16, 1995 even though insecticide was applied to the insecticide plots on June 7 (Figure 24). However on June 29 1995, the OBLR and Gen II treatments were significantly higher (df = 4, F = 4.78, P < 0.05) compared to untreated, Mec 240 and insecticide treatments, with the highest number of 24.25 in OBLR treatment, followed by Gen II, untreated, Mec 240 and insecticide treatments of 21.75, 7.00, 6.75 and 4.00 respectively.

A large reduction in the number of Homoptera was noted in the OBLR and Gen II treatments after June 29 to August 2, 1995. However a small increase in number was





Mean # Homoptera per sample

observed in the Mec 240 and insecticide treatments on the same date. The insecticide treatment was higher than all other treatments towards the end of the season from August 30 to September 30 1995 (Figure 24).

Populations of Coleoptera, Hemiptera, Neuroptera, Dermaptera, Acari, Thysanoptera and Lepidoptera were too low to provide meaningful results.

# Assessment of parasitism of the obliquebanded leafroller eggs mass in mating disruptions, insecticide and untreated plots, in Michigan apple orchard.

Only one parasitoid (Hymenoptera: Trichogrammatidae) was noted to parasite the egg mass of the obliquebanded leafroller in all the treatments in the 1995 study; however, the results showed no significant difference in parasitism rates in all the treatments.

# Assessment of parasitism of the obliquebanded leafroller larvae in mating disruptions, insecticide and untreated plot in Michigan apple orchard.

The data was pooled for each treatment to provide a better representation of the results obtained and was not statistically analyzed due to the low number of larvae per replicate.

### 2nd - 3rd Instar Parasite of Obliquebanded Leafroller Larvae

A total of two larval parasitoid species were reared from the 2nd -3rd instar of obliquebanded leafroller in all the treatments except the insecticide treatment on August 1, 1995 study (Table 5). No obliquebanded leafroller larvae were found in the insecticide treatment. The parasitoid species obtained were Braconidae (Hymenoptera)

Table 5.	Number and obliqueband	l percentage p ed leafroller in	barasitized on 2 n mating disrup∖	nd - 3rd instar of tion treatments (8/	1/95)	
Treatment		Total		Family of	<sup>,</sup> parasite	
	No. larvae	No. larvae parasitized	Percent parasitized	Braconidae	Tachinidae	
Control	25	10	40	7	œ	
Gen II	21	12	57.1	4	œ	
<b>Mec 240</b>	17	12	70.6	S	7	
OBLR	26	17	65.4	S	12	
Insecticide	0	0	0	0	0	

Number and percentage parasitized on 4th - 5th instar of	obliquebanded leafroller in mating disruption treatments (8/1/95)
rable 6.	

	Tachinidae	9	4	m	S	0
mily of parasite	Ichneumonidae	2	2	7	-	0
Ę	Braconidae	4	m	7	0	0
	Percent parasitized	60	100	86	80	0
Total	No. larvae parasitized	12	თ	7	ø	0
	No. larvae	20	თ	œ	10	0
Treatment		Control	Gen II	<b>Mec 240</b>	OBLR	Insecticide

and Tachinidae (Diptera). Tachinidae were the major parasites of 2nd-3rd instar obliquebanded leafroller. Braconidae obtained was a multi-endoparasitoid of the obliquebanded leafroller larvae while the Tachinidae was a solitary endoparasitoid.

It was observed that 65.4 percent (n=26) of the larvae were parasitized in the OBLR treatments (Table 6). Out of the 17 parasitized larvae Tachinidae parasitized 12 larvae and five parasites were Braconidae.

In the Mec 240 treatment there were a parasitism level of 70.6 percent (n=17). Of the parasitized larvae, seven of the same parasitoids were from the Tachinidae, and five from the Braconidae.

The Gen II treatment demonstrated 57.1 percent (n=21) parasitism of obliquebanded leafroller. Of these eight were from Tachinidae, and four from Braconidae. Finally, the untreated plot had 40 percent (n=25) parasitism on the 2nd-3rd instar obliquebanded leafroller, eight from Tachinidae and two from Braconidae (Table 5).

### 4th-5th Instar Parasite of Obliquebanded Leafroller Larvae

A study of the parasitism rate of 4th-5th instar obliquebanded leafroller on August 1, 1995 showed that a total of three species of parasitoids from Braconidae, Ichneumonidae and Tachinidae were observed in all treatments except the insecticide treatment (Table 6). It was observed that Gen II treatment had a 100 percent parasitism(Table 6). Of the nine larvae parasitized, three were parasitized by Braconidae, two by Ichneumonidae, and six larvae were parasitized by Tachinidae.

The Mec 240 treatment had 86 percent (n=8) of larvae parasitism. Of all the parasitized larvae, three were Tachinidae, and two each were Braconidae and Tachinidae.

Although OBLR treatment had 80 percent (n=10) parasitism, only one larva was parasitized by the Ichneumonidae, two by Braconidae and a high number of five larvae was parasitized by Tachinidae. The untreated plot had 60 percent (n=20) parasitism. From the 12 parasitized larvae, two were parasitized by Ichneumonidae, four were parasitized by Braconidae and six were parasitized by Tachinidae. Finally, no larvae were collected from the insecticide treatment (Table 6).

#### DISCUSSION

Based on the extensive data presented here, a general pattern was observed in all mating disruption treatments that the Shannon diversity index value fell within the values of the untreated and the insecticide treatment with the exception of July 18 and August 30 (Figure 19a). The value was higher in the Gen II and Mec 240 treatments on July 18, probably due to the increase in number and family in the Order of Hymenoptera, Diptera, Araneae and Homoptera. Similarly, the Mec 240 treatment on August 30 was higher than all other treatments. This was most likely due to the increase in numbers and families in the Order of Hymenoptera, Araneae, Diptera and Homoptera.

The greater diversity indicates a more equal distribution among the insect populations in the orchard. Families of Hymenoptera, Araneae, Diptera and Homoptera were nearly equally abundant in the mating disruption treatments and the untreated plot (Table 2). The Shannon diversity index value was generally higher in the mating disruption and the untreated treatments compared to the insecticide treatment. This situation may be due to the effects of the insecticide application in the plots with detrimental effects on the insect community. These results are supported by the Kozar

and Szentkiralyi (1992) study in Hungary indicating that species richness was reduced almost by half in experimentally treated and commercial apple orchards compared to the abandoned and experimental non-treated orchards. Maye and Beirne (1974) in their study on the ecology of apple leafroller in the Okanagan Valley, British Columbia indicated that the leafroller population increases caused by parasite decreases are ultimate consequences of pesticide treatments. Croft and Hull (1983) reported that the use of insecticides may potentially reduce the insect species population occupying the orchard ecosystem and provide advantages to organisms with high dispersal capabilities and pesticide resistant forms. Hull and Straner (1983) noted that the predators in the apple orchard were 20 to 40 times more susceptible to insecticide spray than were their prey. Croft and Hull (1983) reported that only 5 to 15 pest species were present at high densities, and most natural enemies were absent in a commercial orchards in Wisconsin. On the other hand Oatman et al. (1964) indicated that there were approximately 763 arthropod species in unsprayed apple orchards in the same region.

Generally, the Shannon index value in all treatments increased gradually in the early growing season from May 19 to June 4 (Figure 19) primarily due to the abundance of Hymenoptera and Diptera (Table 2). The other possible reason was most likely due to the onset of the apple flowering season which acted as a food source for the Hymenoptera and Diptera. The index value increased from May 19 to June 4 in the insecticide treatment because no insecticide was applied to the insecticide plots for that duration. However the index value decreased at the end of the growing season in September 30. This could be due to the emigration of some species out of the orchards, or the onset of overwintering. Tolstova and Atanov (1982) noted that the reduction in
species diversity and change in arthropod numbers in an intensive orchard cultivation was brought about by varying sensitivity to pesticides and the degree of migratory activity and the breadth of trophic specialization.

The Shannon index value in Gen II and Mec 240 treatment was low in June 29, possibly due to the reduced number of Hymenoptera (Table 2 and Figure 20) and Diptera (Table 2 and Figure 23). On August 2, Gen II and Mec 240 were low probably due to the decrease in the number of Diptera (Table 2 and Figure 23) and Homoptera (Table 2 and Figure 24). Other possible explanations for low numbers on both events may be due to the high mobility of the insects or they were at the egg or immature stage of their life cycle.

In general all the mating disruption and untreated plot had higher Simpson's diversity index compared to the insecticide treatment. However, the insecticide treatment was more dominant in the family species on September 16 and 30, 1995 probably due to the present of dominant family species of Diptera and Homoptera.

Basically, a general pattern of a higher populations throughout the growing season was observed in the Hymenoptera, Araneae and Diptera in all the mating disruption treatments and the untreated plot compared to the insecticide treatment (Figure 20, 22 and 23). Decreasing populations in the insecticide plot were probably due to the effects of insecticide applications which were toxic to the insects. Madsen and Madsen (1982), in their study on apple orchard in British Columbia, indicated that there were far more beneficial species in the pesticide free orchard both on the trees and in the cover crop compared to the sprayed orchard. Oatman et al., (1964) found that in unsprayed apple orchard in Wisconsin, that Hymenoptera was the largest order, represented by 28

families.

The Gen II treatment showed a higher populations of Hymenoptera compared to all other treatments, probably due to the presence of high population of parasitoids of Pteromalidae (Chalcidoidae) (Figure 21). The population of Hymenoptera in all the treatments decreased at the end of the season in September 30, 1995 due to the lack of hosts, the onset of overwintering and emigration to other orchard area.

Spider populations seems to peak between end of July and in the middle of August in all the mating disruption treatments and the untreated plot. The spider populations in the insecticide treatment were very low. There were no Araneae found between June 16 to June 2, due to spider susceptibility to insecticide applications. Madsen and Madsen (1982) reported that spiders increased dramatically in the organic (pesticide free) orchard and relatively few spiders were found in the sprayed apple orchard in British Columbia. Dondale et al. (1979) in their 6-year study of spiders in a Quebec apple orchard found that the spiders population declined by two-thirds owing probably to several factors including the use of broad spectrum insecticides for the control of particular orchard pests.

Spiders have been often considered less than ideal biological control agents (Riechert and Lockley 1984) partly because they have long generation times relative to species, and, therefore, individual species have not been observed to exhibit a timely numerical response to changes in pest densities. However, in Australia, the small theridiid *Achaearaneae veruculata* (Urquhart), under certain conditions becomes abundant and effects economic control of a tortricid moth (MacLellan 1973).

A similar pattern of increased Diptera populations was observed in all mating

disruption treatments and the untreated plot. In contrast, the insecticide treatment had a low population throughout the growing season. The possible explanation could be due to their susceptibility to pesticides. The Gen II treatments had the highest populations of Diptera on July 18 due to the presence of high numbers Nematocera, Cyclcorrhapha and Brachycera. In Nematocera, the Culicidae, Mycetophilidae, Anisopodidae were the only families present. In the Cyclorrhapha, families collected were Chloropidae, Drosophilidae, Calliphoridae, and Tachinidae. Families of Brachycera collected included Stratiomyidae and Dolichopodae. Populations of Diptera gradually decreased from middle August to the end of the growing season, possible due to the onset of overwintering or some other environmental factors.

In this study, only one Trichogrammatidae was observed to parasitize the egg mass of obliquebanded leafroller. Trichogrammatidae has a potential for effective pest control either alone or integrated with other control measures. Trichogramma was reported as an egg parasite of tufted apple bud moth (*Platynata idaeusalis*) in Pennsylvania apple orchards (Biddinger et al. 1994). No difference of parasitism rate of the Trichogrammatidae on obliquebanded leafroller egg masses occurred in any of the treatments. The low percentage of egg parasitism in any of the treatments may be due to the timing of laying out the egg masses into the field where low populations of Trichogrammatidae were present or the material (wax paper) where egg masses were attached may not be a suitable substrate to attract the parasitoid to the egg masses. Another possible reason could be the location and height periphery of the canopy of placing the egg masses on the apple tree. Ground releases of 12 million *T. minutum* (Family Trichogrammatidae) per hectare in 12 to 20 year old white spruce, *Picea alba*, in

Canada resulted in 87 % parasitism of spruce budworm, *Choristoneura funiferana*, egg masses, while the natural parasitism level was less than 4 % (Smith et al. 1987; Olkowski and Zhang 1990).

In general, only two parasitoids from Braconidae and Tachinidae were found to parasitize the 2nd-3rd instar of obliquebanded leafroller in all the treatments with the exception of the insecticide treatment. Pogue (1985), found that larvae of obliquebanded leafroller were most commonly parasitized by *Microcentris cerasivoranae* Viereck (Hymenoptera: Braconidae). Maltis et al. (1989) reported that the braconid parasitoid *Meteorus trachynotus* Vier. was found in overwintered larvae of obliquebanded leafroller on foliage of a variety of deciduous species. Doganlar (1978) reported that a braconid parasitoid *Apanteles longicauda* (Wesm.) was reared from larvae of obliquebanded leafroller on apple. The Tachinidae showed large number and high percent parasitism on 2nd-3rd instar of obliquebanded leafroller in all the mating disruption treatments and the untreated plot. The insecticide treatment showed no larvae collected therefore no parasitism value was reported.

In all the treatments except the insecticide treatment, Ichneumonidae also parasitize the 4th-5th instar larvae of obliquebanded leafroller. In this study, Tachinidae showed higher percent of parasitism on 4th-5th instar larvae of obliquebanded leafroller.

In summary, higher diversity index values were observed in all of the mating disruptions and the untreated plot throughout the growing season, indicating a more balanced insect community in the system. However more dominant family species was observed in the insecticide treatment compared to all the mating disruption and untreated plots. The most significant groups of parasitoids found in the Hymenoptera included

members of the Chalcidoidea (several families), Braconidae and Ichneumonidae. In Diptera, the Tachinidae stand out as the most significant parasitoids. No difference in egg parasitism on obliquebanded leafroller was observed in all treatments;

Trichogrammatidae were the only egg parasitoid found.

Braconids and Tachinids were found to parasitize both 2nd-3rd and 4th-5th instar of obliquebanded leafroller. On the other hand, Ichneumonidae were the only parasitoids that parasitized only the 4th-5th instar of obliquebanded leafroller. In this study, the Tachinidae seemed to have higher numbers and higher in percentage of parasitism on both early and late instar of obliquebanded leafroller. Parasitoids are one of the most important biological control agents for many pests. Overall, this study indicates that the mating disruption technique may be able to help preserving natural enemies, thus they may assist in regulating the pest populations in the apple orchard. SUMMARY AND CONCLUSIONS

# SUMMARY AND CONCLUSIONS

The overall objectives of my study were to evaluate whether any of the pheromone blends show the disruption of multiple species of leafroller, to compare the feasibility of mating disruption technique to the conventional insecticide spray program used commercially on apple and finally to determine the impact of mating disruption on the arthropod populations.

From these studies based on the seasonal trap catch, terminal infestations, fruit damage, egg and larval parasitism and suction sample of arthropod populations, I come to the following conclusions:

The results clearly indicated that the Mec 240 (240 ml microencapsulated pheromone formulation) treatment provided effective reduction in trap captures of the obliquebanded leafroller for the whole season. It also showed good trap shut down for the redbanded leafroller for five to six weeks but did not disrupt the second generation.

I feel that the Mec 240 treatments has the potential to provide the disruption of two species of leafrollers (i.e. obliquebanded and redbanded leafrollers) provided that a second application is timed for redbanded leafroller second generation. Further study is needed to evaluate the reduced rates and extended intervals of application of the Mec pheromone to provide effective disruption of the second generation redbanded leafroller. If the research results can show that Mec treatment with the reduced rates helps to disrupt redbanded leafroller in both the generations then it will not only disrupt two species of

leafrollers, but at a reduced cost.

To promote the application and use of pheromone the cost and registration is important. At present it seems that cost of using pheromone for mating disruption is higher than the cost of conventional application programs. The registration requirements of pheromone is quite stringent at present. Until the cost of pheromone is more affordable to growers and the registration process is relaxed by the regulatory agency concerned, there is not an adequate incentive for growers to increase the efforts in using pheromone for mating disruption in a larger scale.

An added advantage of mating disruption using Mec pheromone is particularly promising for orchard pest management because its compatible with the conventional spray equipment.

Other results showed that the OBLR pheromone disrupted the flight of redbanded leafroller throughout the apple growing season but did not adequately disrupt the obliquebanded leafroller. The Gen II and III treatments showed effective control of tufted apple bud moth.

The overall results indicate that no one pheromone blend provided disruption of multiple species of leafrollers. None of the treatments provided commercially acceptable control fruit damaged by leafrollers at harvest. The OBLR treatment provided terminal infestation below economic threshold but higher than insecticide treatment.

The diversity index was generally higher in the mating disruption and the untreated plot compared with the insecticide treatment. This agrees with a study done by Kozar and Szentkiralyi (1992) which indicated that the species richness was reduced almost by half in the experimentally treated commercial apple orchards compared to the

abandoned and experimentally untreated orchards. Similarly, throughout the growing season higher populations in the Order of Hymenoptera, Araneae and Diptera was observed in all mating disruption treatments and the untreated plot compared to the insecticide treatment. These studies indicate that mating disruption is effective in conserving natural enemies.

Several specimens of Trichogrammatidae (Hymenoptera) parasitized the egg masses of the obliquebanded leafroller. In this study, members of Tachinidae (Diptera) and Braconidae and Ichneumonidae (Hymenoptera) parasitized the larvae of obliquebanded leafroller. The parasitoids of egg mass and larval of obliquebanded leafroller can be a potential biological control agents of obliquebanded leafroller.

From the human and the agricultural stand point, the orders of Hymenoptera, Araneae and Diptera (Tachinidae) are probably the most beneficial in the insect class (Debach and Rosen 1991). They contains a great many species that are valued as parasites and predators of insect pests. These natural enemies in agriculture may have provided a largely unrecognized service to agricultural through the years. It has been recognized that unmanaged natural enemies have provided the service of controlling minor and potential pests (Debach and Rosen 1991). As more toxic chemical were added to the orchard system, those benefits were lost. The beneficials insect will be disrupted when chemicals were used against leafroller species, so secondary pests had to be controlled, too.

As the options for insect management and control become limited due to increased resistance and decreased numbers of chemicals products, better alternatives of pest control strategy will become more important to successful growers.

Our concept of orchard farming system should be expanded to include the used of more environmentally sound and effective alternative pest management strategies such as mating disruption using pheromone. This control technique can be incorporated in the integrated pest management system by the conservation of natural enemies in the orchard ecological system.

Finally, in future research efforts, I would recommend that a much larger mating disruption trial area be utilized to deal with immigration and emigration of adult moths to and from the treated and untreated areas. Other factors that need to be determined or evaluated include the placement of pheromone traps, pheromone release rate, the amount of concentration of pheromone required in the air to be effective for mating disruption.

APPENDIX

### APPENDIX 1

#### Record of Deposition of Voucher Specimens\*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1996 - 5/10

Title of thesis or dissertation (or other research projects):

Mating Disruption of the Leafroller Complex (Lepidoptera:Tortricidae) in Michigan Apple Orchards and Impacts on Natural Enemies and Non-Target Pests.

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed) Ho Haw Leng

Date May 15, 1996

\*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation. Museum(s) files. Research project files.

# 102 APPENDIX 1.1 Voucher Specimen Data Page <u>1</u> of <u>1</u> Pages

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