CHO

BIOLOGICAL CONTROL OF OBLIQUEBANDED LEAFROLLER, CHORISTONEURA ROSACEANA (HARRIS) (LEPIDOPTERA: TORTRICIDAE), IN MICHIGAN APPLE ORCHARDS

By

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ABSTRACT

BIOLOGICAL CONTROL OF OBLIQUEBANDED LEAFROLLER, CHORISTONEURA ROSACEANA (HARRIS) (LEPIDOPTERA: TORTRICIDAE), IN MICHIGAN APPLE ORCHARDS

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The obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris), is one of the major arthropod pests in Michigan apple production, due to OBLR's resistance to organophosphate insecticides. In 1999 and 2000 we conducted a survey of the parasitoid community of OBLR in Michigan apple orchards. A total of 9,044 OBLR larvae were collected of which 2,229 were parasitized. The most abundant parasitoids were *Bassus dimidiator* (Braconidae), *Macrocentrus linearis* (Braconidae), *Colpoclypeus florus* (Eulophidae), *Nilea erecta* (Tachinidae), and *Actia interrupta* (Tachinidae). Insecticide bioassays were conducted testing the direct effects of five insecticides currently used for control of OBLR on adult *B. dimidiator* and *M. linearis*. Ranking of the insecticides from least toxic to most toxic resulted in control (water) = Intrepid < Provado = Asana = SpinTor < Guthion. A final study was conducted to determine when adult *B. dimidiator* and *M. linearis* are present in the orchard. Parasitoid occurrence was compared to OBLR adult flight data. A total of 13 parasitoids were recovered from sentinel larvae 11 were an *Enytus sp*. and two were *M. linearis*. Only three *B. dimidiator* were captured in yellow bucket traps. *Macrocentrus linearis* appeared to have been present in the orchards shortly after peak OBLR adult flight that occurred in mid June. *Bassus dimidiator* appeared to be present in the orchards during peak OBLR flight.

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Chapter 1

Literature Review

Impact of Obliquebanded Leafroller on Michigan Apple Production

The biology of the obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris), coupled with its resistance to organophosphate insecticides has made this insect one of the most economically important pests in Michigan apple production. Michigan is the third leading apple producing state in North America, with 49,000 acres of working apple orchards (Klewno & Matthews 2001). Feeding damage from OBLR larvae causes a considerable amount of fruit loss. More than 15% of harvested fruit is unfit for retail distribution because of OBLR cosmetic damage (Ho 1996). Fruit that is damaged early in its development by OBLR feeding usually drops off from the tree before harvest. The most severe fruit damage occurs after petal fall as larvae feed on the developing fruit (Reissig 1978). As fruit increases in size it is more likely to stay on the tree until harvest because OBLR damage does not interfere with fruit development. Madsen et al. (1984) classifies this type of injury as either "early season," where fruit has deep "russetted" scars, or "summer" injury characterized by shallow feeding scars. A great amount of effort and resources are put into insect control with insecticides being the number one cost in fruit production (Brunner 1996).

OBLR Biology

The obliquebanded leafroller is a Tortricid moth native to North America, occurring throughout the United States and into southern Canada (Reissig 1978, Howitt 1993). The versatility of leafroller host plant utilization, their high fecundity, and their ability to disperse both as adults and larvae have contributed to their broad range pest st

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status in fruit production (Howitt, 1993, Suckling et al., 1998). OBLR larvae are phytophagous, commonly feeding on plants in the Rosaceae family, including apple, pear, cherry, peach, plum, rose, gooseberry, currant, strawberry, blueberry, and various weeds (Reissig 1978, Howitt 1993, Ohlendorf 1999).

A single egg mass contains an average of approximately 200 eggs, however a single adult female OBLR is capable of producing up to 900 eggs during her lifetime (Howitt 1993). To avoid competition after eclosion, larvae disperse from oviposition sites by ballooning away on silk strands (Howitt 1993). Therefore, OBLR larvae infesting orchards may have originated from a different host location, such as a bordering woodlot or an abandoned neighboring orchard (Mayer and Beirne 1974). Croft (1982) suggests that the more mobile an insect, the more it is to be exposed to insecticide levels that would provide sufficient selective pressure thereby increasing the probability of developing resistance.

Michigan OBLR are bivoltine (having two generations per year) while those at higher latitudes are univoltine and those in southern latitudes are multivoltine (Chapman et al. 1968). Differences in the number of generations per year at varying latitudes correspond to the differences in temperature and time available to complete a lifecycle. Insects like OBLR with multiple generations and high reproductive rates develop resistance to insecticides at a faster rate than those without these attributes (Croft 1982).

OBLR has a total of five instars and overwinter as second or third instar larvae between late August and late September (Howitt 1993). Overwintering OBLR can be found beneath the bark of the apple tree within a tightly spun silk shelter called a hibernaculum. From late April into early May overwintering larvae will emerge from the hibernaculum and begin feeding on developing fruit, leaves and flower parts (Reissig 1978, Howitt 1993, Ohlendorf 1999). Emergence of overwintering larvae is bimodal (AliNiazee 1986), which explains the occurrence of adults and late instars well into July. In Michigan, the first adult OBLR flight peaks from mid-June into July and the second flight occurs in the latter part of August into early September (Howitt 1993).

Larvae characteristically build leaf shelters for protection from parasitism, predation, and other environmental conditions. Leaf shelters are constructed by rolling the leaf over so that there is an opening at each end and binding it with silk. Larvae will also bind several leaves together or to nearby fruit to feed in the safety of the shelter (Howitt 1993, Ohlendorf 1999). When disturbed, OBLR larvae will drop down from the leaf surface on a strand of silk and later pull themselves back up onto the leaf they had previously abandoned

OBLR Insecticide Resistance

Organophosphate (OPs) and carbamate insecticides have been the leading method of insect control in Michigan orchards for 40 years (Gut et al. 1998). These insecticides have gradually become less effective throughout North America and in Canada, due to the development of insecticide resistance. Problems with increasing OBLR damage and declines in effectiveness of control started appearing in New York in the 1970's but were not documented until the 1980's, and only recently has resistance been reported in Canada (Lawson et al.1997, Smirle et al. 1998). Resistance by OBLR to OP insecticides was first detected in Michigan in the 1970's (Howitt 1993). In New York orchards, Reissig et al. (1986) found that OBLR resistant colonies were 115 times more resistant to

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OPs than were susceptible colonies. Michigan's OBLR have been reported to be 21 times more resistant to OPs than susceptible colonies (Gut et al. 1998).

Fitness costs associated with OBLR resistance include a decline in fecundity, decreased pupal and larval weight, and increased development time. The latter may increase mortality by providing more opportunities for parasitism and predation (Carriere et al. 1994), or decrease mortality by allowing OBLR to avoid exposure to insecticides (Carriere et al. 1995). New insecticides have been developed over the years for OBLR control but with continuous use these also are becoming ineffective. Waldstein and Reissig (2000) have detected resistance to the newest insect growth regulators (IGRs), tebufenozide, which interrupt the molting process by interfering with the insect's molting hormone, 20-hydroxy ecdysone.

Integrated Pest Management in Fruit Production

Traditionally, insecticides were applied regardless of insect abundance as a means of preventing damage (Prokopy et al. 1994). This practice was altered when resistance became evident, which led in part to the development of integrated pest management (IPM) in the 1960's (Croft 1982). At its beginning, IPM consisted of the application of insecticides only as needed, when population levels neared economic threshold, which is the point where insect damage causes unacceptable economic loss (Van Driesche et al.1998). Although this more prudent use of insecticides was a step in the right direction, the continued dependence on chemicals was far from sustainable and insufficient to solve the problem of resistance.

IPM in apple production has gradually evolved due to the inadequacies of relying on insecticides alone for pest control and observations of adverse effects on natural e

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enemy populations. In the last 20 years IPM has been approached from a more ecologically based method of insect control, reducing the use of broad-spectrum insecticides and using methods more favorable to biological control systems (Gruys 1982). Current alternatives to broad-spectrum materials for OBLR control include pheromone mating disruption (Gut et al. 1999), selective insecticides such as the IGRs (Sun et al. 2000), changes in cultural practices (Lawson et al. 1998), and biological control (Viggiani 2000). These methods must be properly integrated in order to be truly effective; no one method can stand alone. Insecticides, although decreasing in their effectiveness still remain the most immediately sought after method for quickly controlling increasing pest populations. Insecticide use is a short-term solution that can result in long-term reduction of natural enemies and ultimately a resurgence of the pest population. Growers should strive for long-term sustainable control practices where pests can be maintained below economic threshold by their natural enemies when low levels of insecticides are used along with pheromone mating disruption or other selective control tactics. Biological control of orchard pests has not been widely implemented in the past, but parasitoids naturally occurring in the orchard ecosystem can be a major mortality factor of OBLR with low insecticide use (Brunner 1998).

Biological Control and the Orchard Ecosystem

The frequent use of insecticides, fungicides and herbicides used to maintain tree health and increase fruit crop yields make the orchard ecosystem a highly disrupted and unfriendly place for natural enemies. Insecticides are probably the greatest limiting factor affecting the success of parasitoids and other natural enemies already present or entering the orchard in regulating pest populations. Landis and Menalled (1998) consider the "r

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"management of insecticide impacts as the most important conservation measure to preserve viable and effective parasitoid communities." Diversity of parasitoids is also dependent on the diversity of the vegetation in and around the orchard. According to the Natural Enemies Hypothesis, an increase in plant diversity may increase the number of natural enemies and reduce herbivore density (Root 1973). Orchards with a greater degree of vegetational diversity (even with a high level of disturbance) have a larger number of natural enemies than those with low vegetational diversity (Szentkiralyi and Kozar 1991). This is most likely due to the differences in the available refuge and food resources. The parasitoid communities present in these refuges are an important source for recolonization and replacement of parasitoids lost to insecticide treatments within the orchard (Landis and Menalled 1998). Van Driesche et al. (1998) reported that the greatest concentration of parasitoids is at the perimeter of an orchard, due either to movement within the orchard between blocks or from an influx of parasitoids from outside the orchard. Some OBLR parasitoids may be entering the orchard in search of hosts to overwinter in. Maltais et al. (1989) reported that the parasitoid, *Meteorus trachynotus* Veireck, which attacks the spruce bud worm, *Choristoneura fumiferana* (Clemens), uses OBLR as its host for overwintering. The OBRL parasitoid, *Colpoclypeus florus* (Walker), on the other hand, must leave the orchard in order to locate suitable overwintering hosts (Gruys and Vaal 1984, Dijkstra 1986). Pfannenstiel et al. (2000) have found that *C. florus* is able to use the strawberry leafroller, *Ancylis comptana* Froelich, as an overwintering host. Collectively, these are examples of how the types of vegetation surrounding the orchard ecosystem can affect parasitoid numbers and diversity. Vegetational diversity in co

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commercial orchards is kept to a minimum by mowing and herbicide applications in order to decrease competition between trees and weed species, and avoid yield reduction.

Biological control usually focuses on establishing stable populations of already existing parasitoids (Landis and Menalled 1998). If stable populations of parasitoids are to be maintained in an orchard ecosystem, more attention needs to be paid to the types of insecticides used and the effect they have on parasitoids as well as the provision of appropriate vegetational diversity.

OBLR Control and Potential Impacts on Parasitoids

The International Organization of Biological Control, West Palaearctic Region Section, created the working group, "Pesticides and Beneficial Organisms" to develop standard methods for testing the effects of insecticides on parasitoids and to determine if any are suitable for use in IPM (Hassan 1998). Testing the effects of agricultural chemicals on natural enemies is mandatory in several countries before the chemicals can be registered for use (Hassan 1998). Such testing of insecticides on parasitoids prior to use in the orchard should be an integral part of all IPM programs. Growers could then avoid using insecticides harmful to natural enemies and thereby achieve high levels of pest suppression by parasitoids already present in the orchard. Houk (1954) reported parasitoid releases in Michigan orchards from the 1930's until 1943 when DDT became a dominant form of insecticide control.

Alternative methods for OBLR control are actively being developed as OP's are being slowly phased out as a result of the Food Quality Protection Act (1996). These methods include pheromone mating disruption (Gut et al. 1999), microbial insecticides (*Bacillus thuringiensis* or Bt) (Li et al. 1995), and insect growth regulators (Sun et al.

2000). In addition summer pruning and thinning of apples (Lawson et al. 1998) and biological control (Viggiani 2000) have been explored. The level of safety for natural enemies exposed to the new selective insecticides is questionable, while there is little doubt that pheromone mating disruption and the cultural practices of summer pruning and thinning for OBLR control have no impact on natural enemy health. Summer pruning and thinning reduces OBLR damage by removing its favored food source, succulent leaves; in addition the removal of fruit clusters forces OBLR to search more actively for other fruit to damage (Lawson et al. 1998). Lawson et al. (1998) also demonstrated that these practices improve fruit quality, giving fruit greater access to the sun and more room to grow. Gut et al. (1999) have found that orchards where pheromone mating disruption was used incurred less OBLR feeding damage than those that used insecticides as their only means of control.

OBLR Parasitoids

Parasitoids require an insect host in order to complete their lifecycle. Competition between parasitoids is reduced by differential resource utilization, by attacking different life stages and by attacking different species. The Trichogrammatidae, for instance, parasitize the host's eggs, preventing larval emergence and thus parasitism by a larval parasitoid. More importantly parasitoids have diverged into two distinct evolutionary pathways: endoparasitism and ectoparasitism (Mills 1992). Ectoparasitoids develop outside of the host's body and endoparasitoids develop within the host's body. These two groups can be categorized further into idiobionts, which terminate the host's development upon ovipostion and koinobionts, which allow the host to continue development after oviposition (Mills 1992). Ectoparasitoids are primarily idiobionts, and most end

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endoparasitoids are koinobionts (Mills 1992). Both lifestyles have their advantages and disadvantages. Koinobionts have a longer development time because the host is allowed to grow as the parasitoid develops; however, extended development time increases the chances of predation. Idiobionts often have a more rapid development time because they lack the protection that koinobionts have within the host's body. Ectoparasitoids have an advantage in that they do not have to contend with its host's immune responses and therefore they can have a broader host range than endoparasitoids (Mills 1992). The widest host ranges occur in egg and pupal parasitoids (Mills 1992), because hosts at these two stages in their development are undergoing rapid morphological change, which impairs immune responses.

Parasitoids can be further divided into guilds depending on the life stage of the host they attack, whether they are endo- or ecto- parasitoids and whether development is continuous or extended (Mills 1992). Eleven different guilds of parasitoid, which attack all life stages of Tortricid hosts have been identified (Mills 1993).

The host's vulnerability to attack by parasitoids is influenced by its feeding sites. An insect housed within a gall is vulnerable to fewer parasitoids than one exposed on a leaf surface (Hawkins 1994). Although OBLR make shelters by rolling leaves, they are highly vulnerable to parasitoid attack when they leave this shelter to feed. Even within the confines of their leaf rolls OBLR are vulnerable to attack by parasitoids adapted to ovipositing through the leafroll structure. Host density also can increase the chance of parasitoid attack. Mills (1993) noted that host density may increase the amount of volatile cues given off by a plant subjected to feeding damage thereby attracting a searching parasitoid.

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Knowing the relative densities of parasitoid populations compared with host populations is important for an IPM approach that is parasitoid friendly. During an outbreak of a pest species the number of individual parasitoids and their species richness increases (Balazs 1997). Subsequently, as the pest population declines these parasitoids help establish and maintain the pest under economic threshold (Balazs 1997). The majority of parasitoids found in orchards are native species (Viggiani 2000). Surveys of the parasitoids attacking OBLR have been conducted in apple orchards, raspberry fields, and in wild host vegetation in Canada (Donganlar and Beirne 1978, Hagley and Barber 1991, Li et al. 1999, Vakenti et al. 2001) and the United States (Pogue 1985, Biddinger et al. 1994, Brunner 1996, Ho 1996). OBLR is attacked by several guilds of parasitoids including the egg parasitoid *Trichogramma* spp., which is being monitored for their level of success in suppressing leafroller populations in raspberries (McGregor et al. 1997). Li et al. (1999) collected OBLR from raspberry fields in British Columbia, Canada, and recovered 13 Hymenopteran species, 11 from larvae and 2 from pupae, and 1 Dipteran species from pupae. Vakenti et al. (2001) recovered 18 parasitoid species from OBLR collected from wild host plants. OBLR collected from unmanaged apple orchards in Ontario, Canada, were parasitized by 16 parasitoid species, 14 Hymenoptera and 2 Diptera (Hagley and Barber 1991). Donganlar and Beirne (1978) recovered 9 species of parasitoid from OBLR in apple orchards in the Vancouver district of British Columbia, Canada. OBLR are also routinely collected and assessed for parasitism in the state of Washington (Brunner 1996). Pogue (1985) reared 11 hymenopterous parasitoids from OBLR and two other leafroller pests, *Archips argyrospilus* (Walker) and *Anacampsis innocuella* (Zeller), collected from shelterbelts in Wyoming, United States. Biddinger et

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al. (1994) found that OBLR may be an alternate host for parasitoids that attack the tufted apple bud moth, *Platynota idaeusalis* (Walker), in Pennsylvania, United States, orchards.

Parasitoids of OBLR in Michigan

Ho (1996) conducted a survey of the parasitoids attacking OBLR in Michigan apple orchards during 1995 but recovered small numbers of parasitoids from three main families. This was the first parasitoid survey of this kind conducted in Michigan. During the spring and summer of 1999 a second survey of parasitoids attacking OBLR in Michigan apple orchards was initiated (Chapter 2). This survey resulted in the discovery of two important parasitoids, *Bassus dimidiator* (Nees.) and *Macrocentrus linearis* (Nees.) (Hymenoptera: Braconidae).

Bassus dimidiator

When considering the usefulness of a parasitoid species as an effective biological control agent in an orchard ecosystem it is vital to understand its life cycle and biology so that the appropriate timing of insecticides can be determined in order not to limit the fecundity and success of the parasitoid. *Bassus dimidiator* (Nees.) is a solitary endoparasitoid previously only collected from the eye-spotted bud moth, *Spilonota ocellana* (Denis and Schiffermuller) (Krombein et al.1979). The eye-spotted bud moth was introduced from Europe via nursery stock in the 1800's (Howitt 1993).

Dondale (1954) has reported the complete biology of this parasitoid as it occurs within the eye-spotted bud moth, which should be similar to its biology in OBLR. Dondale (1954) found that *B. dimidiator* overwinters within the host and resumes its development in the spring with the emergence of the host. This parasitoid lays its egg in the ganglion of the ventral nerve cord (Dondale 1954). The host larva is able to feed and develop normally as *B. dimidiator* feeds on "non-vital" structures. As the host nears the pupal stage and ends feeding, *B. dimidiator* consumes the host's entrails at a faster pace, exits the hosts body and finishes its meal externally, then pupates (Dondale 1954). Female *B. dimidiator* lays one egg per host and may parasitize between 15 and 20 hosts (Dondale 1954). Dondale (1954) observed this parasitoid visiting wild carrot, suggesting that it served as a possible source of nectar, and noted that *B. dimidiator* was able to live for greater than a week when supplied a 25% sugar cane solution. Asman and Lee (unpublished data) found that the days of survival of both *B. dimidiator* and *Macrocentrus linearis* were significantly lengthened when supplied a 50% honey solution compared to those parasitoids given water only. Although food resources are a possible limiting factor for these parasitoids given current weed control methods (mowing and herbicide use) within orchards, the more prevalent limitation to this parasitoids success may be the effects of broadly toxic insecticides.

The vulnerability of *B. dimidiator* to insecticides appears to depend on the chemical and the length of time that chemical has been in use. Stultz (1954) found that in areas where DDT was used for more than a year *Agathis laticinctus* (Cresson) or *B. dimidiator* (Nees.) was able to parastitize up to 90% of the bud moth population. At this time bud moths were becoming resistant to DDT. The possibility of resistance to DDT by the parasitoid is unknown however, *B. dimidiator* populations were not affected by other chemicals, such as nicotine sulphate, which had a great impact on several other important parasitoids (Stultz 1955).

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Macrocentrus linearis

No literature was found describing the biology of *Macrocentrus linearis* (Nees.) specifically, although my observations indicate it is a solitary polyembyronic endoparasitoid. Li et al. (1999) described the biology of the polyembryonic, *Macrocentrus nigridorsis* Viereck, a parasitoid of OBLR in raspberries. Polyembryony refers to the ability of a single egg laid by a female to multiply into multiple embryos. Parasitoids can be either haploid (males) or diploid (females). In the polyembryonic species that yield males and females it is likely that two eggs were laid; one fertilized (females) and one unfertilized (males) (Li et al. 1999). The OBLR that were parasitized by *M. nigridorsis* had 36 parasitoids emerge from each individual host on average (Li et al. 1999). This is an endoparasitoid that feeds on the host internally and emerges from the host as the host nears its final instar. The parasitoids at this time begin to feed externally until parasitoid pupation. These parasitoids "spin up" their cocoons simultaneously, binding together with silk and cocoons oriented parallel to one another (Li et al. 1999). Adults emerge at approximately the same time.

Conclusions

The obliquebanded leafroller's resistance to organophosphate insecticides has caused significant economic concerns in Michigan apple production. Alternative forms of control are being assessed and biological control along with pheromone mating disruption show promise as sustainable and long-term solutions for this problem. The potential of a parasitoid as an effective biological control agent in an orchard ecosystem is greatly limited by the use of insecticides. The effects of the most widely used chemicals for control of OBLR on the survivability of parasitoids present in the orchard ecosystem are unknown and need to be investigated. A current survey of the parasitoids attacking OBLR in Michigan apple orchards could reveal which parasitoids may be the most effective control agents for this pest. Phenology of these parasitoids should be studied as they develop on OBLR in Michigan apple orchards. Recommendations could then be made to Michigan apple growers that could increase the success of an integrated pest management program for OBLR that utilizes biological control agents. Reducing the use of insecticides and allowing for OBLR control by natural enemies will lead to a more economical and sustainable orchard ecosystem.

Objectives of Study

1. Complete a two year survey of the parasitoids attacking OBLR in commercially sprayed Michigan apple orchards in two important apple producing regions.
2. Test the direct effects of five of the principal insecticides currently used in Michigan apple orchards for control of OBLR or other fruit pests on the survival and longevity of adult *B. dimidiator* and *M. linearis*.
3. Determine the time at which adult parasitoids *B. dimidiator* and *M. linearis* are present in commercially sprayed apple orchards in Michigan.

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Chapter 2

Parasitism of Larval Obliquebanded Leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), in Commercially Sprayed Michigan Apple Orchards.

Abstract

The obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris), is one of the major arthropod pests in Michigan apple production. In 1999 and 2000 a survey of the parasitoid community of OBLR in commercially sprayed apple orchards in Michigan was conducted to determine the species present and their importance to OBLR population management. A total of 9,044 OBLR larvae were collected of which 2,229 were parasitized. Parasitism of OBLR was found to increase from the overwintering generation to the first generation for both regions and both years. In 1999 11% of the 1,126 overwintering OBLR collected were parasitized, while 29% of the 3,749 first generation OBLR collected were parasitized. In 2000 8% of the 489 overwintering OBLR collected were parasitized, while 26% of the 3,680 first generation OBLR collected were parasitized. A total of approximately 21 species of parasitoids from 9 families were recovered from OBLR, composed of Hymenopteran and Dipteran parasitoids. The most abundant Hymenopteran parasitoids were *Bassus dimidiator* (Braconidae) comprising 47% of the parasitism, followed by *Colpoclypeus florus* (8% of the total) (Eulophidae) and *Macrocentrus linearis* (2% of the total) (Braconidae). Dipteran parasitoids (Tachinidae) accounted for 37% of the parasitism, and were largely comprised of *Nilea erecta* (30%) and *Actia interrupta* (22%). These collections include new host records for *B. dimidiator* (Braconidae) and *Hyphantrophaga blanda* and *Comsilura concinnata* (Tachinidae). The parasitoid *C. florus* (Eulophidae) was also reported from Michigan for the first time.

Introduction

The obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris), is a Tortricid moth native to North America (Reissig 1978). In Michigan, OBLR has two generations per year, overwintering as second or third instar larvae and emerging from overwintering hibernaculae in late April to early May (Howitt 1993). First adult flight of OBLR occurs in late June or early July and second adult flight at the end of August (Howitt 1993). OBLR larvae can be easily spotted in the apple tree canopy by the presence of their leaf shelters. Larvae create shelters by folding over a leaf and binding it with silk. Larvae will also bind several leaves together or to nearby fruit and feed within the safety of the shelter (Howitt 1993, Ohlendorf 1999). OBLR larvae are phytophagous, commonly feeding on plants in the Rosaceae family, including apple, pear, cherry, peach, plum, rose, raspberry, gooseberry, currant, strawberry, blueberry, and various weeds (Reissig 1978, Howitt 1993, Ohlendorf 1999). In apple, OBLR larvae feed on flower buds, leaves, and developing fruit (Reissig 1978, Howitt 1993, Ohlendorf 1999). The greatest damage to fruit occurs after petal fall as fruit increases in size (Reissig 1978, Howitt 1993). Fruit injury caused by overwintering larvae early in the season is characterized by deep scars while injury caused during the summer can be recognized by shallow feeding scars (Madsen et al. 1984, Howitt 1993, Ohlendorf 1999). As a result of its damage to fruit and resistance to pesticides, OBLR is one of the most economically important pests of apple causing more than 15% damage to harvested fruit in some orchards (Ho 1996).

In the past, organophosphate (OP) insecticides such as azinphos-methyl, have been widely used for control of OBLR. As a result OBLR has developed resistance to OP

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insecticides in Canada, New York and Michigan (Reissig et al. 1986, Smirle et al. 1998, Gut et al. 1998). Alternative methods for OBLR control are actively being developed as OP's are being slowly phased out as a result of the Environmental Protection Agency's Food Quality Protection Act (1996). These methods include pheromone mating disruption (Gut et al. 1999), microbial insecticides (*Bacillus thuringiensis* or Bt) (Li et al. 1995), and insect growth regulators (Sun et al. 2000). In addition, summer pruning and thinning of apples (Lawson et al. 1998) as well as biological control (Viggiani 2000) have been explored.

Reduction of broad-spectrum insecticide use in orchards should result in an increase in natural enemy populations. In the absence of these toxic materials, parasitoids are known to cause significant mortality in OBLR populations (Pfannenstiel et al. 1998) and have the potential to become a significant form of control of OBLR in Michigan apple orchards. Ho (1996) was the first to survey the parasitoids attacking OBLR in Michigan. However, he was unable to collect large numbers of OBLR and therefore recovered only parasitoids from two Hymenopteran families, Braconidae and Ichnuemonidae, and one Dipteran family, Tachinidae. None of the parasitoids recovered were identified to species. More fruitful surveys of the parasitoids attacking OBLR have been conducted in apple orchards, raspberry fields, and in wild host vegetation in Canada (Donganlar and Beirne 1978, Hagley and Barber 1991, Li et al. 1999, Vakenti et al. 2001) and production areas in the U.S. (Pogue 1985, Biddinger et al. 1994, Brunner 1996, Ho 1996). Li et al. (1999) recovered 13 Hymenopteran species, 11 from larvae and 2 from pupae, and 1 Dipteran species from pupae of OBLR collected from raspberry fields in British Columbia, Canada. Vakenti et al. (2001) recovered 18 parasitoid species from

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OBLR collected from wild host plants. OBLR collected from unmanaged apple orchards in Ontario, Canada, were parasitized by 16 parasitoid species, 14 Hymenoptera and 2 Diptera (Hagley and Barber 1991). Donganlar and Beirne (1978) recovered 9 species of parasitoid from OBLR in apple orchards in the Vancouver district of British Columbia, Canada. OBLR are also routinely collected and assessed for parasitism in the state of Washington (Brunner 1996). Pogue (1985) reared 11 hymenopterous parasitoids from OBLR and two other leafroller pests, *Archips argyrospilus* (Walker) and *Anacampsis innocuella* (Zeller), collected from shelterbelts in Wyoming. Biddinger et al. (1994) found that OBLR maybe an alternate host for parasitoids that attack the tufted apple bud moth, *Platynota idaeusalis* (Walker) in Pennsylvania orchards. Though many of the same parasitoids were recovered from OBLR in the surveys cited above there were differences in the species that made up the largest percentage of the parasitoid complex as well as in numbers recovered from OBLR.

The objective of this study was to complete a two year survey of the parasitoids attacking OBLR in commercially sprayed Michigan apple orchards in two of the largest apple producing regions of the state. The results of the survey will be used to determine species occurrence, abundance, and impact of OBLR parasitoids in these regions and to assess the potential for biological control of OBLR in Michigan.

Methods

During the 1999 and 2000 growing seasons, parasitoids attacking OBLR were surveyed in commercially sprayed apple orchards located in two main apple producing regions of Michigan (Fruit Ridge and Southwest). The Fruit Ridge region is comprised of ca. 15,000 acres (Kleweno and Matthews 2001) of orchard in Kent, Ottawa, and

Montcalm counties (Figure 2.1). The Southwest region refers to suite of ca. 11,000 acres (Kleweno and Matthews 2001) of orchards located in Van Buren and Berrien counties (Figure 2.1). Data from the Allegan County orchard was included with that from orchards located in the Fruit Ridge region during 1999 (Figure 2.1). In all locations, parasitoids were surveyed by collecting overwintering generation and first generation OBLR larvae from orchards once per week and rearing on artificial diet until an adult OBLR or parasitoid emerged, or the host died.

OBLR were collected from a total of 15 orchards in 5 counties during 1999 and 10 orchards in 4 counties during 2000 (Table 2.1). Orchard blocks within each orchard where OBLR were collected were chosen based on OBLR population pressure the previous year as well as by preliminary observation of large numbers of OBLR shelters in the collection year. Blocks in each orchard, therefore, varied in size, variety, and management strategy. The number of blocks sampled per orchard for both the overwintering and first generations of OBLR in 1999 and 2000 are given in Table 2.1. Overwintering generation OBLR were sampled until the beginning of the first flight of adult OBLR and first generation OBLR were sampled until the second flight of adult OBLR. During 1999 the overwintering and first generation OBLR larvae were sampled from May 21 until June 10 and from July 6 until August 12 respectively (Table 2.1). Overwintering and first generation OBLR larvae were sampled during 2000 from May 24 until June 8 and from July 12 until August 17 respectively (Table 2.1).

OBLR were sampled each week by a crew of four to five individuals. Each person was assigned a row according to the size of the block being surveyed and was equipped with a pole pruner that extended to 3.048 m (ARS Company, Japan), 1 oz. diet cups with

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lids, and a zip lock bag. Rows were selected to space sampling over the entire block. Crew members walked slowly while visually searching for OBLR shelters in the interior and exterior of the apple canopy, searching both the upper and lower half of the tree. Pole pruners were used to clip branches and remove shelters that were high in the tree canopy. OBLR shelters were individually examined and those containing larvae were placed individually into cups and the lids were marked with the row number and height (upper or the lower half of the tree canopy). Cups were then placed within a zip lock bag, which was labeled with the orchard name, block name, and date. The length of time spent searching for OBLR depended on the number of samplers and the abundance of OBLR within a block. In order to ensure a uniform sampling effort, all samples were based on 2 person hours/block; i.e. the total time spent searching in each block by individual samplers equaled two hours. In addition, the sampling method sought to ensure that at least 30 larvae were collected per sample. Blocks that were unlikely to yield this minimum sample size were quickly eliminated from sampling by the following procedure. Each block was initially sampled for 10 minutes at which point the crew leader would determine the total number of shelters collected. If the rate of shelter collection fell below the minimum required to achieve a total sample size of 30 shelters in 2 person hours further collection from the block was terminated. In this way a decision to terminate or continue the search could be made within the first 10 minutes, increasing the numbers of productive blocks sampled. Bags containing the cups with larvae were placed in a cooler with ice packs and taken to the USDA APHIS Niles Plant Protection Center, Niles, Michigan where APHIS staff reared OBLR on a modified pinto bean diet

(Shorey and Hale 1965) in the laboratory at 26°C, 60% RH, and 16 Light (L): 8 Dark (D) until the emergence of an adult OBLR or parasitoid.

Parasitoid Identification

Parasitoids were identified to species by comparison with specimens in the A.J. Cook Arthropod Museum at Michigan State University, East Lansing, Michigan and continued by specialists in particular taxa. Host and hymenopteran parasitoid records were found in Krombein et al. (1979) and updated species names in Poole (1996). Unknown specimens were sent to K. Ahlstrom (Braconidae), NCDA & CS Plant Protection Section, Raleigh, North Carolina; J. O'Hara (Tachinidae) ECORC, Systematic Entomology Section, Ontario, Canada; M.J. Sharkey (Braconidae), University of Kentucky, Lexington, Kentucky; M. E. Schauff (Eulophidae), Systematic Entomology Laboratory, Washington D.C., Maryland; and P.M. Marsh (Braconidae), Systematic Entomology Laboratory, Washington D.C., Maryland. Voucher specimens have been deposited in the A.J. Cook Arthropod Museum at Michigan State University, East Lansing, Michigan and at the Niles Plant Protection Center, Niles, Michigan. Specimens were also left with each of the systematists that made the identifications. Specimens that are in the process of being identified by specialists will be deposited as vouchers in the A.J. Cook Arthropod Museum at Michigan State University at a later date. Specimens that were lost or damaged have either been identified to family or have been described as unknowns, parasitoids that never developed into adults are also described as unknowns.

Statistical Analysis

To test the hypothesis that percent parasitism was independent of the position in the tree where larvae were collected, I compared the number of parasitized and unparasitiz

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unparasitized OBLR that were collected either high or low in the tree canopy by Pearson Chi-Square Test (SAS 2000). The exact P-value for each comparison is reported.

Results

During the course of this two-year study a total of 9,044 OBLR larvae were collected of which 2,229 were parasitized (Table 2.2). The number of OBLR collected and the percent parasitism increased from the overwintering to first generations in both the Southwest and Fruit Ridge/Allegan regions during 1999 and 2000 (Table 2.2). In 1999 11% of the 1,126 overwintering generation OBLR collected were parasitized, while 29% of the 3,749 first generation OBLR collected were parasitized. In 2000 8% of the 489 overwintering generation OBLR collected were parasitized, while 26% of the 3,680 first generation OBLR collected were parasitized.

Percent parasitism of overwintering OBLR ranged from 4% to 23% for orchards in the Southwest and from 1% to 11% in the Fruit Ridge/Allegan region during 1999 (Table 2.3). Percent parasitism of first generation OBLR ranged from 8% to 52% for orchards in the Southwest and from 10% to 81% in the Fruit Ridge/Allegan region during 1999 (Table 2.3). Percent parasitism of overwintering OBLR ranged from 0% to 25% for orchards in the Southwest and from 0% to 29% in the Fruit Ridge region during 2000 (Table 2.4). Percent parasitism of first generation OBLR ranged from 5% to 29% for orchards in the Southwest and from 24% to 52% in the Fruit Ridge region during 2000 (Table 2.4).

Numbers of parasitoid species attacking OBLR also varied considerably between orchards in both regions and years (Tables 2.5 – 2.8). For example in the Southwest region in 1999 a single species was recovered from overwintering OBLR larvae in Kugel orchard wi

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orchard while approximately 9 species were recovered from larvae in Calderwood orchard (Table 2.5). Approximately 2 species of parasitoid were recovered from first generation OBLR in Pagel orchard while approximately 6 species were recovered from R. Winkel orchard during 1999 (Table 2.5). Numbers of parasitoid species recovered from overwintering and first generation OBLR also varied between orchards in the Southwest during 2000 (Table 2.6). The greatest number of parasitoid species recovered in a single year, 12, was recorded for first generation collections of OBLR in R. Winkle orchards in 2000 (Table 2.6).

Approximately 14 species of parasitoids from 6 families were recovered from overwintering and first generation OBLR during 1999 (Table 2.9) and 19 species from seven families were recovered from OBLR during 2000 one of which was a Tachinid hyperparasitoid (Table 2.10). The parasitoid community was composed of Dipteran and Hymenopteran parasitoids.

During 1999, *Bassus dimidiator* (Nees.) made up the largest percentage (53%) of the total parasitoid complex attacking overwintering generation OBLR in the Southwest region followed by the Tachinidae (23%), *Macrocentrus linearis* (Nees.) (12%), *Itoplectis conquisitor* (Say) (6%) and the unknowns (6%) (Figure 2.2). In the Fruit Ridge region during 1999 the Tachinidae made up 75% of the total parasitoid complex attacking overwintering generation OBLR, *B. dimidiator* made up 20% and *I. conquisitor* made up 5% of the total (Figure 2.2). During 1999 in both the Southwest and Fruit Ridge/Allegan regions *B. dimidiator* made up 65% and 46% respectively of the parasitoid complex attacking first generation OBLR, Tachinidae made up 25% and 46% respectively, while the unknov

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the unknowns made up 9% in the Southwest and *Colpoclypeus florus* Walker made up 6% of the parasitoid complex in the Fruit Ridge region (Figure 2.3).

During 2000 in the Southwest and Fruit Ridge regions *B. dimidiator* made up the largest percentage (38% and 96% respectively) of species attacking overwintering OBLR (Figure 2.4). In the Southwest region during 2000, 23% of the total complex attacking overwintering generation OBLR was composed of *Enytus sp.*, 15% were unknown, 8% were *M. linearis*, 8% *Apanteles polychrosidis* Viereck, and 8% Tachinidae (Figure 2.4). In the Fruit Ridge region during 2000, 4% of the complex attacking overwintering OBLR was unknown (Figure 2.4). The parasitoid complex attacking first generation OBLR in the Southwest region during 2000 was composed of 51% *B. dimidiator*, 20% Tachinidae, 17% *C. florus*, and 12% unknown (Figure 2.5). The parasitoid complex attacking first generation OBLR in the Fruit Ridge region in 2000 was composed of 70% Tachinidae, 15% *B. dimidiator*, 10% *C. florus*, and 5% unknown (Figure 2.5). The unknowns are composed of parasitoids that made up less than 5% of the parasitoid complex (species ID and % of the total complex are given for these parasitoids for both, generations, regions and years in Table 2.11), and those that were nonviable or unidentifiable.

There were a total of 5 identified species of Tachinidae attacking overwintering and first generation OBLR during 1999 and 2000 in the Southwest and FruitRidge/ Allegan regions of Michigan (Table 2.12). The Tachinids *Nilea erecta* (Coquillet), *Actia interrupta* Curran, *Hemistrumia parva* (Bigot), *Hyphantrophaga blanda* (Osten Sacken), and *Compsilura concinnata* (Meigen) ranged between 30% of the Tachinidae total complex and 0.32% during 1999 and 2000 (Table 2.12). Unknown Tachinids made up between 22% and 100% of the total Tachinidae complex attacking overwintering and first generati

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generation OBLR during 1999 and 2000 in the Southwest and Fruit Ridge/Allegan regions (Table 2.12). Unknown Tachinidae were specimens that had been lost, damaged, or were non-viable.

Figure 2.6 shows the percent parasitism of OBLR that were collected from either high or low in the apple tree canopy. Numbers of un-parasitized and parasitized overwintering and first generation OBLR collected high and low in the tree canopy from orchards located in the Fruit Ridge/Allegan) and Southwest regions of Michigan during 1999 and 2000 can be seen in Table 2.13. Comparisons of parasitized and un-parasitized larvae in high vs. low samples reveled significant differences in these positions in the tree canopy for both overwintering and first generation OBLR. During 1999 in the Southwest greater parasitism of overwintering generation OBLR was found high in the tree (χ^2=7.5928, df=1, P=0.0067) and greater parasitism of first generation OBLR was also found to occur high in the tree (χ^2=5.8694, df=1, P=0.0170) (Figure 2.6 and Table 2.13). In the Fruit Ridge/Allegan region during 1999 there were no significant differences in the overwintering generation of OBLR parasitized high or low in the tree (χ^2=0.1813, df=1, P=0.8168) however, there were significantly more first generation OBLR parasitized low in the tree canopy (χ^2=4.5996, df=1, P=0.0326) (Figure 2.6 and Table 2.13). There were no significant differences in parasitized and un-parasitized overwintering and first generation OBLR collected either high or low in apple trees during 2000 from orchards in the Southwest region of Michigan (χ^2=0.0822, df=1, P=0.7826, and χ^2=0.0625, df=1, P=0.8070 respectively) (Figure 2.6 and Table 2.13). There were no significant differences in parasitized and un-parasitized overwintering and first generation OBLR collected either high or low in apple trees during 2000 from orchards in the Fruit Ridge region of

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Michigan (χ^2=0.0044, df=1, P=1.0000, and χ^2=0.000005, df=1, P=1.0000 respectively) (Figure 2.6 and Table 2.13).

Discussion

Percent parasitism was lower for the overwintering generation than the first generation in both the Southwest and Fruit Ridge/Allegan regions in 1999 and 2000. Biological factors that could account for the differences in numbers of OBLR collected and the percent parasitism between the overwintering and first generation are seasonal differences in temperature, natural overwintering mortality of host and parasitoid, and an increase in activity as temperatures increase. Insecticide use patterns may have impacted parasitoids as well. In most locations, broadly toxic materials such as chlorpyrifos and esfenvalerate were applied early in the season for control of aphids, leafminers, and leafrollers. More selective insecticides such as tebufenozide and spinosad were used for leafroller control beginning at petal fall. The organophosphate, azinphos-methyl, was used routinely throughout the season for control of codling moth, *Cydia pomonella* (Linnaeus), or apple maggot, *Rhagoletis pomonella* (Walsh), but tapered off later in the summer as harvest approached. Also during 2000 there was a severe fire blight, *Erwinia amylovora*, outbreak in Michigan apple orchards resulting in the loss of thousands of acres of apple and many growers discontinued spraying for the season. Differences in the amount of parasitism between orchards could also have been due to the differences in the surrounding landscapes where some orchards may have been bordered by woods, abandoned orchard, or another working orchard. Orchards that have a more diverse landscape should have a greater number of natural enemies (Root 1973) as a result of a greater an

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greater amount of non- crop vegetation supporting alternate hosts, and providing floral resources for parasitoids to feed upon.

Many of the parasitoid species recovered from OBLR collected in Michigan apple orchards were the same as those found to parasitize OBLR throughout North America (Pogue 1985, Hagley and Barber 1991, Biddinger et al. 1994, Li et al. 1999). However the major exception was that, *B. dimidiator* was the most consistently abundant Hymenopteran parasitoid attacking overwintering and first generation OBLR in both the Southwest and Fruit Ridge/Allegan regions of the state during 1999 and 2000. *Bassus dimidiator* made up 15% to 96% of the parasitoids attacking OBLR (Figure 2.2 – Figure 2.5). This OBLR survey represents a new host record for this highly abundant species. *Bassus dimidiator* is a solitary endoparasitoid that had previously been only reported to attack the eye-spotted bud moth, *Spilonota ocellana* (Denis and Schiffermuller) (Krombein et al. 1979). The complete biology of *B. dimidiator* has been described by Dondale (1954) under the name *Agathis laticinctus* (Cresson).

Tachinids were the second most consistently abundant parasitoids attacking OBLR in Michigan apple orchards. *Nilea erecta* and *A. interrupta* followed by *H. parva* being the major species recovered (Table 2.12). The Tachinidae *N. erecta* and *A. interrupta* were also recovered from OBLR in other parasitoid surveys (Hagley and Barber 1991, Biddinger et al. 1994). Two specimens of the Tachinid *Hyphantrophaga blanda* (Osten Sacken) were reared each from a first generation OBLR collected in the Southwest and Fruit Ridge regions during 2000 (Table 2.12). *Hyphantrophaga blanda* has been reported only one other time to have been reared in OBLR in British Columbia, Canada (Personal Communication Dr. J. O'Hara). Two specimens of the widely known generalis

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generalist Tachinidae, *Compsilura concinnata* (Meigen) were also recovered from overwintering and first generation OBLR collected from the Southwest region of Michigan during 1999 and 2000. *Compsilura concinnata* was introduced into the United States as a biological control of various Lepidopteran pests from 1906 to 1986 especially the gypsy moth, *Lymantria dispar* (L.), and has since been found to be a major mortality factor from many non-target Lepidopteran communities (Boettner et al. 2000). It was not previously known to attack OBLR (Arnaud 1978)

The gregarious ectoparasitoid, *Colpoclypeus florus* Walker (Eulophidae) was also found in Michigan apple orchards for the first time. *Colpoclypeus florus* was released into Ontario Canada in the 1960's and was found for the first time in Washington orchards in 1992 (Brunner 1996). *Colpoclypeus florus* has contributed to over 90% parasitism of leafrollers in Washington (Pfannenstiel et al. 2000). *Colpoclypeus florus* was absent in both the Southwest and Fruit Ridge regions of Michigan during the overwintering generation but was present during the first generation. The reason for *C. florus's* absence during the overwintering generation is probably due to the lack of a suitable overwintering host within the orchard. Because *C. florus* requires a late instar host for overwintering while OBLR overwinters as a 2^{nd} or 3^{rd} instar, *C. florus* probably leaves the orchard in search of a suitable overwintering host and does not return until later in the season (Gruys and Vaal 1984, Dijkstra 1986). The percent composition of the parasitoid complex composed of *C. florus* increased from 2% (Table 2.11) in the Southwest in 1999 to 17 % in 2000 (Figure 2.5), and increased from 6% (Figure 2.3) in the Fruit Ridge/Allegan region in 1999 to 10% in 2000 (Figure 2.5).

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A polyembryonic endoparasitoid, *Macrocentrus linearis* (Nees.) was also recovered from overwintering and first generation OBLR in the Southwest region in 1999 and 2000. In the Southwest *M. linearis* made up 12% of the total parasitoid complex attacking overwintering generation OBLR in 1999 (Figure 2.2) and 8% in 2000 (Figure 2.4). *Macrocentrus linearis* made up 4% of the parasitoids complex attacking first generation OBLR in the Southwest in 1999 and 0.56% in 2000 (Table 2.11). *Macrocentrus linearis* were found to emerge from two first generation OBLR in the Fruit Ridge/Allegan region (Table 2.9) making up only 0.43% of the total parasitoid complex during 1999 (Table 2.11).

The differences in the number of parasitized and un-parasitized OBLR collected high or low in the apple tree could possibly be due to the differences in parasitoid searching behavior or the pattern of OBLR dispersal into the tree. OBLR prefer to feed on new growth and move throughout the tree during the season. Waldstein et.al. (2001) found high rates of larval movement which may have been influenced by foliage availability. Orchards where fire blight was present in 2000 may have influenced the numbers of OBLR that were collected from high or low in the tree during our study due to a reduction in suitable foliage for feeding.

Conclusion

A complex of parasitoids are contributing to the control of overwintering and first generation OBLR in apple orchards in the Southwest and Fruit Ridge regions of Michigan. The parasitoids *B. dimidiator* and the Tachinids were the most abundant parasitoids attacking OBLR in Michigan apple orchards, followed by *C. florus*, and *M. linearis*. There appears to be considerable potential for biological control agents to contribute to

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contribute to the control of OBLR in Michigan however, studies have shown that both *B. dimidiator* and *M. linearis* are susceptible to many of the common insecticide chemistries used for OBLR control in Michigan (Chapter 3). Reducing the use of harmful insecticides could allow parasitoids such as *B. dimidiator* and the Tachinidae to become a major form of controlling OBLR populations in commercial apple orchards. The parasitoid *C. florus* should be monitored further to see if *C. florus* will become the most abundant parasitoid attacking first generation OBLR. The effects of the increases in the population of *C. florus* on the populations of *B. dimidiator* and the Tachinidae *Compsilura concinnata* should also be monitored to determine if this generalist parasitoid will become more abundant than the major Hymenopteran parasitoids currently attacking OBLR in Michigan orchards. The effects of the new more selective insecticides used for OBLR control in Michigan apple orchards should be tested on natural enemies to ensure that their use will not affect biological control of OBLR in the apple orchards managed with these materials.

Table 2.1. *Orchard location and number of blocks sampled for overwintering and first generation OBLR during 1999 and 2000.*

Table 2.1. Orchard location and number of blocks sampled for overwintering and first generation OBLR during 1999 and 2000.

				Overwintering Generation	First Generation
Year	**Region**	**County**	**Orchard**	**Number of Blocks Sampled May 21 - June 10**	**Number of Blocks Sampled July 6 – August 12**
1999	Southwest	Berrien	Calderwood	5	4
		Berrien	Kugel	2	3
		Berrien	Pagel	2	3
		Berrien	R. Winkel	3	4
		Van Buren	K. Winkel	3	3
		Van Buren	Hill	-	4
	Fruit Ridge/ Allegan	Kent	Beuschel	-	5
		Kent	Rasch	4	8
		Kent	Wittenbach	2	2
		Kent	Nyblad	2	2
		Kent	Kraft	3	4
		Kent	Succop	-	1
		Kent	Steffen	-	2
		Montcalm	Klackle	2	2
		Allegan	TNRC	-	2
	Total	5 counties	15 orchards	28 blocks	49 blocks

Table 2.1. (continued)

Table 2.1. (continued)

Year	Region	County	Orchard	Overwintering Generation Number of Blocks Sampled May 24 - June 8	First Generation Number of Blocks Sampled July 12 – August 17
2000	Southwest	Berrien	Calderwood	1	2
		Berrien	Kugel	1	1
		Berrien	R. Winkel	2	3
		Van Buren	K. Winkel	3	3
		Van Buren	Hill	1	1
	Fruit Ridge	Kent	Beuschel	1	2
		Kent	Rasch	-	4
		Kent	Wittenbach	2	6
		Kent	Kraft	-	4
		Ottawa	Wells	-	7
	Total	4 counties	10 orchards	11 blocks	33 blocks

Table 2.2. *Percent parasitism and total overwintering and first generation* **OBLR** collected from apple orchards in the Southwest and *Fruit Ridge/Allegan regions of Michigan during 1999* and the Southwest and Fruit Ridge regions during 2000.

Table 2.2. Percent parasitism and total overwintering and first generation OBLR collected from apple orchards in the Southwest and Fruit Ridge/Allegan regions of Michigan during 1999 and the Southwest and Fruit Ridge regions during 2000.

		Overwintering Generation			First Generation		
Year	**Region**	**Total Parasitoids**	**Total Parasitism**	**% OBLR**	**Total Parasitoids**	**Total Parasitism**	**% OBLR**
1999	Southwest	623	101	16	1916	628	33
	Fruit Ridge/ Allegan	503	20	4	1833	469	26
	Total	**1126**	**121**	**11**	**3749**	**1097**	**29**
2000	Southwest	402	13	3	2495	532	21
	Fruit Ridge	87	24	28	1185	442	37
	Total	**489**	**37**	**8**	**3680**	**974**	**26**
Overall Total		**1615**	**158**	**10**	**7429**	**2071**	**28**

Table 2.3. *Percent parasitism of overwintering and first generation OBLR for each apple orchard located in the Southwest and Fruit Ridge/Allegan regions of Michigan during 1999.*

Table 2.3. Percent parasitism of overwintering and first generation OBLR for each apple orchard located in the Southwest and Fruit Ridge/Allegan regions of Michigan during 1999.

		Overwintering Generation				First Generation			
Year	**Region**	**Orchard**	**Total OBLR**	**Total Parasitoids**	**% Parasitism**	**Orchard**	**Total OBLR**	**Total Parasitoids**	**% Parasitism**
1999	Southwest	Calderwood	294	35	12	Calderwood	159	74	47
		R. Winkel	68	14	21	R. Winkel	789	414	52
		Pagel	21	2	10	Pagel	30	10	33
		Kugel	26	1	4	Kugel	124	10	8
		K. Winkel	214	49	23	K. Winkel	347	67	19
						Hill	470	53	11
	Fruit Ridge/ Allegan	Wittenbach	18	2	11	Wittenbach	48	39	81
		Nyblad	86	2	2	Nyblad	262	71	27
		Klackle	148	2	1	Klackle	143	15	10
		Kraft	69	7	10	Kraft	261	64	25
		Rasch	182	7	4	Rasch	169	21	12
						Steffen	498	48	10
						Succop	24	9	38
						TNRC	61	37	61
						Beuschel	367	165	45

Table 2.4. *Percent parasitism of overwintering and first generation OBLR for each apple orchard located in the Southwest and Fruit Ridge regions of Michigan during 2000.*

Table 2.4. Percent parasitism of overwintering and first generation OBLR for each apple orchard located in the Southwest and Fruit Ridge regions of Michigan during 2000.

		Overwintering Generation				First Generation			
Year	**Region**	**Orchard**	**Total OBLR**	**Total Parasitiods**	**% Parasitism**	**Orchard**	**Total OBLR**	**Total Parasitiods**	**% Parasitism**
2000	Southwest	Calderwood	46	3	7	Calderwood	436	100	23
		R. Winkel	16	4	25	R. Winkel	816	246	30
		Kugel	31	84	1	Kugel	685	31	5
		K. Winke	19	2	11	K. Winkel	279	82	29
		Hill	3	0	0	Hill	279	73	26
	Fruit Ridge	Wittenbach	4	0	0	Wittenbach	216	112	52
		Beuschel	83	24	29	Beuschel	212	103	49
						Kraft	447	141	32
						Wells	214	52	24
						Rasch	97	34	35

Table 2.5. *Parasitoids recovered from overwintering and first generation OBLR collected from apple orchards in the Southwest region of Michigan during 1999.*

Table 2.5. Parasitoids recovered from overwintering and first generation OBLR collected from apple orchards in the Southwest region of Michigan during 1999.

			Overwintering Generation				
Year	**Region**	**Parasitoid Identification**	**Calderwood**	**R. Winkel**	**Pagel**	**Kugel**	**K. Winkel**
1999	Southwest	**Tachinidae**					
		Nilea erecta (Coquillett)	3	1	1	-	2
		Actia interrupta Curran	2	-	-	-	3
		Hemisturmia parva (Bigot)	2	3	-	-	-
		Compsilura concinnata (Meigen)	-	1	-	-	-
		Tachinidae Unknown	5	-	-	-	-
		Braconidae					
		Bassus dimidiator (Nees)	4	5	1	1	42
		Macrocentrus linearis (Nees)	11	1	-	-	-
		Apanteles polychrosidis Viereck	1	-	-	-	-
		Ichnuemonidae					
		Itoplectis conquisitor (Say)	6	-	-	-	-
		Enytus sp.	-	2	-	-	-
		Bethylidae					
		Bethylinae	1	-	-	-	-
		Unknown	-	-	-	-	2

Table 2.5. *(Continued)*

Table 2.5. (Continued)

			First Generation					
Year	**Region**	**Parasitoid Identification**	**Calderwood**	**R. Winkel**	**Pagel**	**Kugel**	**K. Winkel**	**Hill**
1999	Southwest	**Tachinidae**						
		Nilea erecta (Coquillett)	-	2	-	1	1	-
		Actia interrupta Curran	-	2	-	-	-	1
		Tachinidae Unknown	47	42	8	3	15	33
		Braconidae						
		Bassus dimidiator (Nees)	12	349	-	1	46	5
		Macrocentrus linearis (Nees)	11	1	2	3	5	-
		Apanteles polychrosidis Viereck	1	-	-	-	-	-
		Ichnuemonidae						
		Itoplectis conquisitor (Say)	1	-	-	-	-	-
		Labeninae	1	-	-	-	-	-
		Eulophidae						
		Colpoclypeus florus Walker	-	1	-	-	-	10
		Ceraphronidae	-	4	-	-	-	-
		Unknown	1	13	-	2	-	4

Table 2.6. *Parasitoids recovered from overwintering and first generation OBLR collected from apple orchards in the Southwest region of Michigan during 2000.*

Table 2.6. Parasitoids recovered from overwintering and first generation OBLR collected from apple orchards in the Southwest region of Michigan during 2000.

			Overwintering Generation			
Year	**Region**	**Parasitoid Identification**	**Calderwood**	**R. Winkel**	**Kugel**	**K. Winkel**
2000	Southwest	**Tachinidae**				
		Nilea erecta (Coquillett)	1	-	-	-
		Braconidae				
		Bassus dimidiator (Nees)	1	2	-	2
		Macrocentrus linearis (Nees)	-	1	-	-
		Apanteles polychrosidis Viereck	-	1	-	-
		Ichnuemonidae				
		Enytus sp.	-	-	3	-
		Unknown	1	-	1	-

Table 2.6. (continued)

Year	Region	Parasitoid Identification	First Generation				
			Calderwood	R. Winkel	Kugel	K. Winkel	Hill
2000	Southwest	**Tachinidae**					
		Nilea erecta (Coquillett)	1	11	10	4	2
		Hemisturmia parva (Bigot)	-	4	1	-	-
		Actia interrupta Curran	1	5	-	4	-
		Compsilura concinnata Meigen	-	-	-	-	1
		Hyphantrophaga blanda (Osten Sacken)	-	1	-	-	-
		Tachinidae Unknown	10	22	3	23	4
		Tachinidae Unknown hyperparasite	1	-	-	-	-
		Braconidae					
		Bassus dimidiator (Nees)	57	159	-	35	20
		Macrocentrus linearis (Nees)	3	-	-	-	-
		Oncophanes americanus (Weed)	-	2	-	1	-
		Ichnuemonidae					
		Itoplectis conquisitor (Say)	-	13	4	-	-
		Enytus sp.	4	-	-	-	1
		Phygadeuontinae	-	1	1	2	-
		Eulophidae					
		Colpoclypeus florus (Walker)	10	19	9	10	41
		Eulophinae	2	-	-	-	-
		Entedoninae	-	1	-	-	-
		Chalcididae					
		Chalcidinae	2	-	-	-	-
		Pteromalidae					
		Pteromalinae	1	-	-	-	-

Table 2.6 *(continued)*

Table 2.6 (continued)

			First Generation				
Year	**Region**	**Parasitoid Identification**	**Calderwood**	**R. Winkel**	**Kugel**	**K. Winkel**	**Hill**
2000	Southwest	**Eurytomidae**					
		Eurytominae	-	1	-	-	-
		Unknown	7	6	3	3	4

Table 2.7. Parasitoids recovered from overwintering and first generation OBLR collected from apple orchards in the Fruit Ridge/Allegan region of Michigan during 1999.

			Overwintering Generation				
Year	**Region**	**Parasitoid Identification**	**Wittenbach**	**Nyblad**	**Klackle**	**Kraft**	**Rasch**
1999	Fruit Ridge/ Allegan	**Tachinidae**					
		Actia interrupta Curran	1	1	-	1	-
		Nilea erecta (Coquillett)	-	-	1	-	-
		Hemistrumia parva (Bigot)	-	1	-	-	-
		Tachinidae Unknown	1	-	1	1	7
		Braconidae					
		Bassus dimidiator (Nees)	-	-	-	4	-
		Ichnuemonidae					
		Itoplectis conquisitor (Say)	-	-	-	1	-

Table 2.7 (continued)

Year	Region	Parasitoid Identification	First Generation				
			Wittenbach	Nyblad	Klackle	Kraft	Rasch
1999	Fruit Ridge/	**Tachinidae**					
	Allegan	Tachinidae Unknown	36	54	11	24	17
		Actia interrupta Curran	-	-	-	-	-
		Nilea erecta (Coquillett)	-	-	-	-	-
		Braconidae					
		Bassus dimidiator (Nees)	-	3	-	35	1
		Macrocentrus linearis (Nees)	2-		-	-	-
		Apanteles polychrosidis Viereck	-	-	-	-	-
		Oncophanes americanus (Weed)	-	1	-	-	-
		Ichnuemonidae					
		Itoplectis conquisitor (Say)	-	-	-	-	-
		Eulophidae					
		Colpoclypeus florus Walker	-	12	4	5	3
		Unknown	1	1	-	-	-

Table 2.7 (continued)

Year	Region	Parasitoid Identification	First Generation			
			Steffen	**Succop**	**TNRC**	**Beuschel**
1999	Fruit Ridge/ Allegan	**Tachinidae**				
		Tachinidae Unknown	19	7	32	12
		Actia interrupta Curran -	-	-	1	
		Nilea erecta (Coquillett)	-	-	-	2
		Braconidae				
		Bassus dimidiator (Nees)	26	2	-	147
		Macrocentrus linearis (Nees)	-	-	-	-
		Apanteles polychrosidis Viereck	-	-	1	-
		Oncophanes americanus (Weed)	-	-	-	-
		Ichnuemonidae				
		Itoplectis conquisitor (Say)	2	-	1	1
		Eulophidae				
		Colpoclypeus florus Walker	-	-	3	-
		Unknown	1	-	-	2

Table 2.8. Parasitoids recovered from overwintering and first generation OBLR collected from apple orchards in the Fruit Ridge region of Michigan during 2000.

			Overwintering Generation
Year	**Region**	**Parasitoid Identification**	**Beuschel**
2000	Fruit Ridge	**Braconidae**	
		Bassus dimidiator (Nees)	23
		Unknown	1

			First Generation				
Year	**Region**	**Parasitoid Identification**	**Beuschel**	**Wittenbach**	**Kraft**	**Wells**	**Rasch**
2000	Fruit Ridge	**Tachinidae**					
		Tachinidae Unknown	68	64	61	13	25
		Actia interrupta Curran	20	21	25	4	7
		Hyphantrophaga blanda (Osten Sacken)	1	-	-	-	-
		Braconidae					
		Bassus dimidiator (Nees)	9	5	46	6	-
		Apanteles polychrosidis Viereck	-	-	-	1	-
		Oncophanes americanus (Weed)	-	1	-	1	-
		Eulophidae					
		Colpoclypeus florus Walker	3	14	4	18	2
		Unknown	2	4	6	8	-

Table 2.9. Parasitoids attacking overwintering and first generation OBLR in apple orchards from the Southwest and Fruit Ridge/Allegan regions of Michigan during 1999.

			Overwintering Generation	First Generation
Year	**Region**	**Parasitoid Identification**	**Number of Parasitoids**	**Number of Parasitoids**
1999	Southwest	**Tachinidae**		
		Nilea erecta (Coquillett)	7	4
		Actia interrupta Curran	5	3
		Hemisturmia parva (Bigot)	5	-
		Compsilura concinnata (Meigen)	1	-
		Tachinidae Unknown	5	153
		Braconidae		
		Bassus dimidiator (Nees)	54	411
		Macrocentrus linearis (Nees)	12	22
		Apanteles polychrosidis Viereck	1	1
		Eulophidae		
		Colpoclypeus florus Walker	-	11
		Ichnuemonidae		
		Itoplectis conquisitor (Say)	6	1
		Enytus sp.	2	-
		Labeninae	-	1
		Bethylidae		
		Bethylinae	1	-
		Ceraphronidae	-	4
		Unknown	2	17

Table 2.9. (continued)

			Overwintering Generation	First Generation
Year	**Region**	**Parasitoid Identification**	**Number of Parasitoids**	**Number of Parasitoids**
1999	Fruit Ridge/ Allegan	**Tachinidae**		
		Actia interrupta Curran	3	1
		Nilea erecta (Coquillett)	1	1
		Hemisturmia parva (Bigot)	1	-
		Tachinidae Unknown	10	212
		Braconidae		
		Bassus dimidiator (Nees)	4	214
		Macrocentrus linearis (Nees)	-	2
		Oncophanes americanus (Weed)	-	1
		Apanteles polychrosidis Viereck	-	1
		Eulophidae		
		Colpoclypeus florus Walker	-	27
		Ichnuemonidae		
		Itoplectis conquisitor (Say)	1	4
		Unknown	-	5

Table 2.10. Parasitoids attacking overwintering and first generation OBLR in apple orchards from the Southwest and Fruit Ridge regions of Michigan during 2000.

			Overwintering Generation	First Generation
Year	**Region**	**Parasitoid Identification**	**Number of Parasitoids**	**Number of Parasitoids**
2000	Southwest	**Tachinidae**		
		Nilea erecta (Coquillett)	1	28
		Hemisturmia parva (Bigot)	-	5
		Hyphantophaga blanda (Osten Sacken)	-	1
		Actia interrupta Curran	-	10
		Compsilura concinnata (Meigen)	-	1
		Tachinidae Unknown	-	63
		Tachinidae Unknown Hyperparasitoid	-	1
		Braconidae		
		Bassus dimidiator (Nees)	5	271
		Macrocentrus linearis (Nees)	1	3
		Oncophanes americanus (Weed)	-	3
		Apanteles polychrosidis Viereck	1	-

Table 2.10. (continued)

			Overwintering Generation	First Generation
Year	**Region**	**Parasitoid Identification**	**Number of Parasitoids**	**Number of Parasitoids**
2000	Southwest	**Eulophidae**		
		Colpoclypeus florus Walker	-	89
		Eulophinae	-	2
		Entedoninae	-	1
		Ichnuemonidae		
		Itoplectis conquisitor (Say)	-	17
		Enytus sp.	3	5
		Phygadeuontinae	-	5
		Pteromalidae		
		Pteromalinae	-	1
		Eurytomidae		
		Eurytominae	-	1
		Chalcidae		
		Chalcidinae	-	2
		Unknown	2	23

Table 2.10. (continued)

			Overwintering Generation	First Generation
Year	**Region**	**Parasitoid Identification**	**Number of Parasitoids**	**Number of Parasitoids**
2000	Fruit Ridge	**Tachinidae**		
		Actia interrupta Curran	-	77
		Hyphantrophaga blanda (Osten Sacken)	-	1
		Tachinidae Unknown	-	232
		Braconidae		
		Bassus dimidiator (Nees)	23	66
		Oncophanes americanus (Weed)	-	2
		Apanteles polychrosidis Viereck	-	1
		Eulophidae		
		Colpoclypeus florus Walker	-	42
		Unknown	1	21

Table 2.11. Hymenopteran parasitoids that comprised less than 5% of the total parasitoid complex attacking overwintering and first generation OBLR from apple orchards located in the Southwest and Fruit Ridge regions of Michigan during 1999 and 2000.

		Overwintering Generation		**First Generation**	
Year	**Region**	**Parasitoid**	**% of Total**	**Parasitoid**	**% of Total**
1999	Southwest	*Apanteles polchrosidis*	0.99	*Macrocentrus linearis*	3.50
		Enytus sp.	1.98	*Apanteles polchrosidis*	0.16
		Bethylinae	0.99	*Colpoclypeus florus*	1.75
		Unknown	1.98	*Itoplectis conquisitor*	0.16
				Labeninae	0.16
				Cerphronidae	0.64
				Unknown	2.71
	Fruit Ridge/ Allegan			*Macrocentrus linearis*	0.43
				Oncophanes americanus	0.21
				Apanteles polchrosidis	0.21
				Itoplectis conquisitor	0.85
				Unknown	1.07
2000	Southwest			*Macrocentrus linearis*	0.56
				Oncophanes americanus	0.56
				Itoplectis conquisitor	3.20
				Enytus sp.	0.94
				Eulopinae	0.38
				Entedininae	0.19
				Phygadeuontinae	0.94
				Pteromalinae	0.19

Table 2.11. (continued)

		Overwintering Generation		First Generation	
Year	**Region**	**Parasitoid**	**% of Total**	**Parasitoid**	**% of Total**
2000	Southwest			Eurytominae	0.19
				Chalcidinae	0.38
				Unknown	4.32
	Fruit Ridge	Unknown	4.17	*Oncophanes americanus*	0.45
				Apanteles polchrosidis	0.23
				Unknown	4.75

Table 2.12. Percentage of species comprising the complex of Tachinidae attacking overwintering and first generation OBLR from apple orchards located in the Southwest and Fruit Ridge regions of Michigan during 1999 and 2000.

		Overwintering Generation		First Generation	
Year	**Region**	**Tachinid**	**% of Total**	**Tachinid**	**% of Total**
1999	Southwest	*Nilea erecta*	30	*Nilea erecta*	3
		Actia interrupta	22	*Actia interrupta*	2
		Hemistrumia parva	22	Unknown	96
		Compsilura concinnata	4		
		Unknown	22		
	Fruit Ridge/ Allegan	*Actia interrupta*	20	*Actia interrupta*	0.47
		Nilea erecta	7	*Nilea erecta*	1
		Hemistrumia parva	7	Unknown	99
		Unknown	67		
2000	Southwest	*Nilea erecta*	100	*Nilea erecta*	26
				Hemistrumia parva	5
				Actia interrupta	9
				Hyphantrophaga blanda	1
				Compsilura concinnata	1
				unknown	58
				unknown hyperparasitoid	1

Table 2.12. (continued)

		First Generation	
Year	**Region**	**Tachinid**	**% of Total**
2000	Fruit Ridge	*Actia interrupta*	25
		Hyphantrophaga blanda	0.32
		Unknown	75

Table 2.13. Number of overwintering and first generation OBLR that were parasitized or un-parasitized from apple orchards in the Southwest and Fruit Ridge/Allegan regions of Michigan during 1999 and 2000.

			Overwintering Generation			First Generation		
Year	**Region**	**Height**	**Number Un-parasitized**	**Number Parasitized**		**Number Un-parasitized**	**Number Parasitized**	
1999	Southwest	high	178	49	*	605	332	*
		low	344	52		683	296	
	Fruit Ridge/ Allegan	high	192	7	ns	496	144	*
		low	291	13		867	322	
2000	Southwest	high	225	7	ns	940	258	ns
		low	164	6		1023	274	
	Fruit Ridge	high	31	12	ns	385	229	ns
		low	32	12		358	213	

[1]Pearson Chi-Square Test: ns = $p > 0.05$
* = $p \leq 0.05$

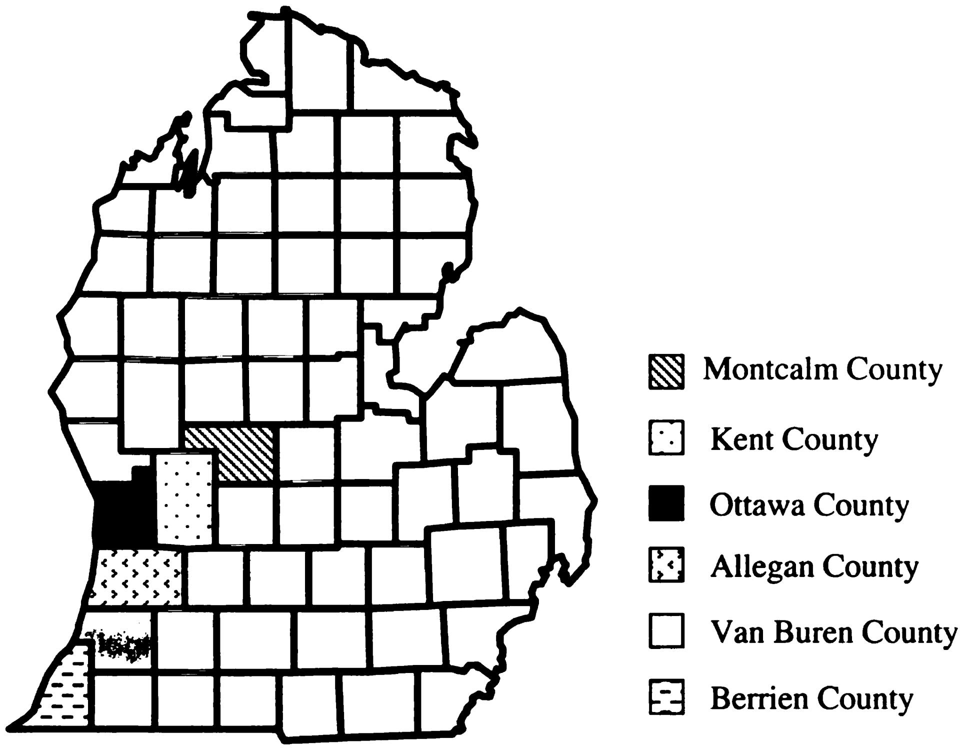

Figure 2.1. Michigan counties where survey of parasitoids attacking OBLR in apple orchards was conducted during 1999 and 2000. Where Montcalm, Kent, and Ottawa counties are located in the Fruit Ridge region and Van Buren and Berrien counties are located in the Southwest region of the state. The Trevor Nichols Research Center is located in Allegan County.

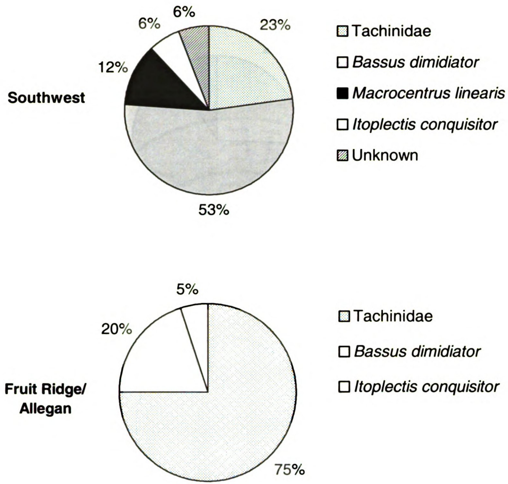

Figure 2.2. Percentage of parasitoid species attacking overwintering generation OBLR in apple orchards located in the Southwest and Fruit Ridge/Allegan regions of Michigan during 1999.

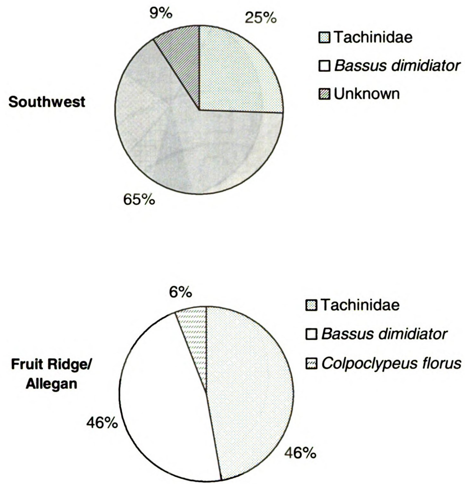

Figure 2.3. Percentage of parasitoid species attacking first generation OBLR in apple orchards located in the Southwest and Fruit Ridge/Allegan regions of Michigan during 1999.

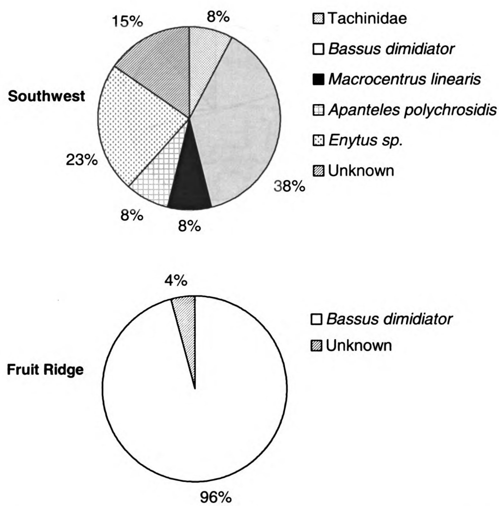

Figure 2.4. Percentage of parasitoid species attacking overwintering generation OBLR in apple orchards located in the Southwest and Fruit Ridge regions of Michigan during 2000.

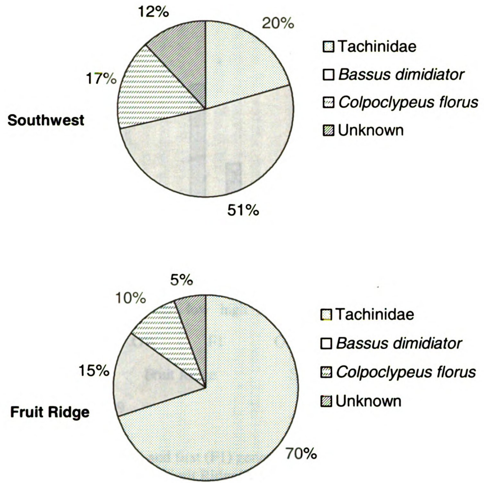

Figure 2.5. Percentage of parasitoid species attacking first generation OBLR in apple orchards located in the Southwest and Fruit Ridge regions of Michigan during 2000.

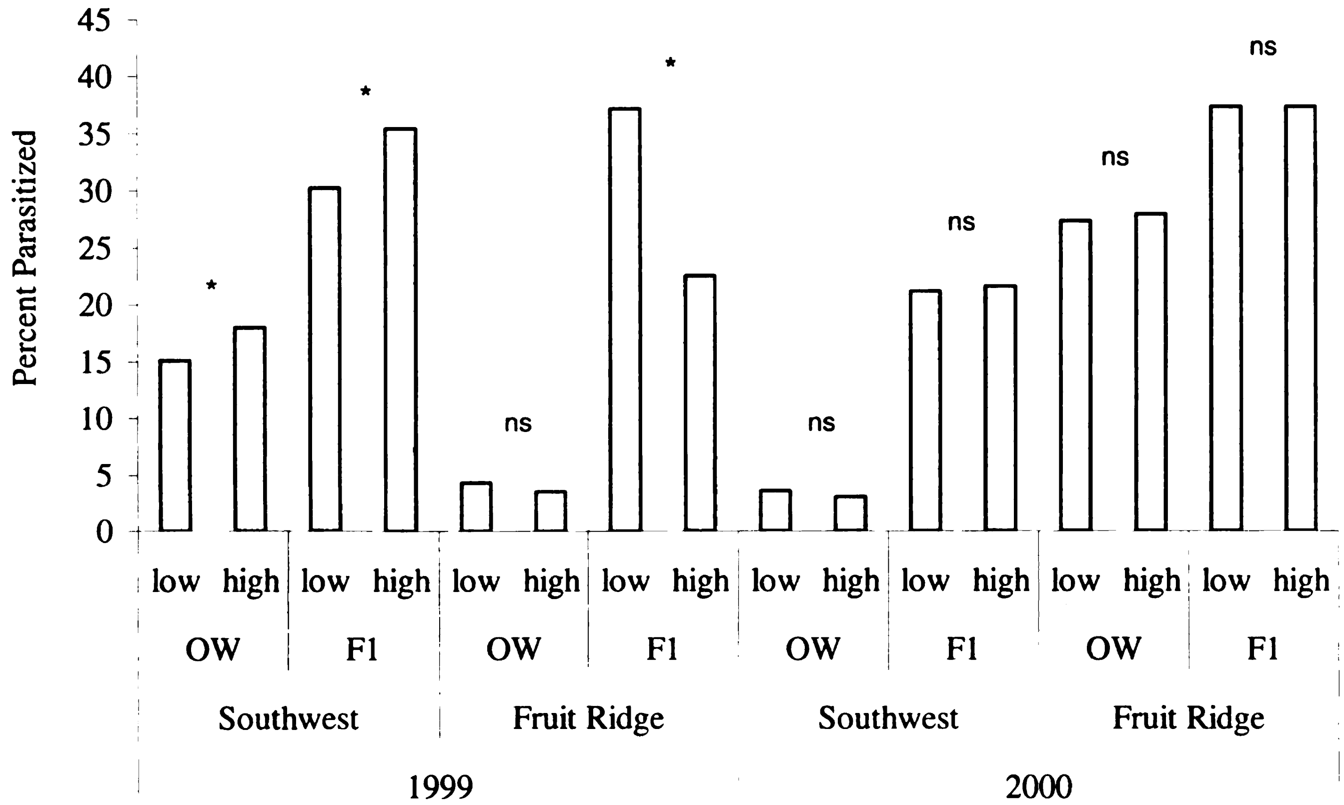

Figure 2.6. Percent of overwintering (OW) and first (F1) generation OBLR parasitized either high or low in the apple tree canopy in the Southwest and Fruit Ridge (Fruit Ridge/Allegan) regions of Michigan during 1999 and 2000.

[1]Pearson Chi-Square Test: ns = $p > 0.05$
* = $p \leq 0.05$

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Chapter 3

The direct effects of five insecticides on survival of *Bassus dimidiator* (Nees.) and *Macrocentrus linearis* (Nees.) (Hymenoptera: Braconidae), parasitoids of the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae).

Abstract

The obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris), has become a major pest in Michigan apple production due to its resistance to organophosphate insecticides. New alternative insecticides are being developed for OBLR control, influenced by the development of resistance and the increasing restriction being implemented as a result of the Environmental Protection Agency's Food Quality Protection Act. Many, but not all of the new insecticides are selective and less harmful for natural enemies. If a grower wants to conserve natural enemies present in the orchard it is important to know which insecticides should be avoided when parasitoids are present. The International Organization of Biological Control (IOBC) has developed standard methods for testing the effects of insecticides on natural enemies. Insecticide bioassays based on IOBC standards were conducted to test the effects of the residues of formulated product of five insecticides on two important Braconid parasitoids of OBLR in Michigan apple orchards, *Bassus dimidiator* (Nees.) and *Macrocentrus linearis* (Nees.). The insecticides methoxyfenozide (Intrepid™), esfenvalerate (Asana®), imidacloprid (Provado®), spinosad (SpinTor™), and azinphos-methyl (Guthion®) were chosen to represent a range of broad-spectrum and selective chemistries. Insecticides and a water control were sprayed onto Petri dishes or apple leaves. Methoxyfenozide caused no change in survival of both parasitoids, while azinphos-methyl was highly toxic to

both species. Based on the level of toxicity azinphos-methyl should not be used in an IPM program that integrates biological control. Imidacloprid, esfenvalerate, and spinosad were moderately to highly toxic depending on the surface that was sprayed. The utility of moderately toxic insecticides for control of OBLR in times of parasitoid inactivity should be studied further.

Introduction

The obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris), is one of the most serious pests in Michigan apple production (Gut et al. 1999). Larvae feed on leaves, the developing fruit, flower buds, and water sprouts (Reissig 1978, Howitt 1993, Ohlendorf 1999). Fruit injury caused by overwintering larvae early in the season is characterized by deep scars while injury caused during the summer can be recognized by shallow feeding scars (Madsen et al. 1984, Howitt 1993, Ohlendorf 1999). .Much of the young developing fruit that is damaged by overwintering OBLR drops from the tree as larval feeding interferes with normal fruit development (Reissig 1978, Howitt 1993, Ohlendorf 1999). The greatest damage to fruit occurs after petal fall as fruit increases in size (Reissig 1978, Howitt 1993). Larval feeding by first generation OBLR has been known to cause more than 15% damage to harvested fruit (Ho 1996).

The presence of OBLR larvae in the apple tree canopy can be easily detected by the presence of their leaf shelters. Shelters are created by the larvae folding over a leaf and binding it with silk. Larvae will also bind several leaves together or to nearby fruit and feed within the safety of the shelter (Howitt 1993, Ohlendorf 1999). OBLR completes two generations per year in Michigan with first adult flight occurring in late June to early July and second adult flight occurring late August (Howitt 1993). Second generation OBLR larvae overwinter as 2nd or 3rd instars emerging from their overwintering hibernacula in late April to early May (Howitt 1993).

For more than 40 years OBLR had been controlled using broad-spectrum organophosphate (OPs) and carbamate insecticides in United States apple production

(Gut et al. 1999). However, OBLR resistance to OPs became evident in Michigan during the 1970's (Howitt 1993). More recent bioassay experiments with populations of OBLR from Michigan apple orchards have determined that OBLR is 21x resistant to Guthion® (azinphos-methyl) and 7x resistant to Lorsban® (chlorpyrifos) (Gut et al. 1998). Ahmad et al. (2002) found 27x resistance at LC_{50} for azinphos-methyl and 26x resistance at LC_{50} for chlorpyrifos. OBLR resistance to OPs along with the growing restrictions on use or the total loss of registered OPs as a result of the Environmental Protection Agency's Food Quality Protection Act (1996) has led to the development of alternative methods for controlling OBLR. New methods include the use of insect growth regulators (Sun et al. 2000), microbial insecticides (*Bacillus thuringiensis* or Bt) (Li et al. 1995), pheromone mating disruption (Gut et al. 1999), and biological control (Vigianni 2000).

Gut et al. (1999) also discuss some newer insecticides available for OBLR control and their effectiveness. Azinphos-methyl (Guthion®) is a broad-spectrum organophosphate insecticide with strong contact activity that acts via cholinesterase inhibition causing insects to lose control of their nervous function (Ware 1994). Unlike azinphos-methyl, and esfenvaletrate (Asana®), that are strong contact poisons, many of the new selective insecticides including methoxyfenozide (Intrepid™), spinosad (SpinTor™), and imidacloprid (Provado®) must be ingested to kill the target insect. Esfenvalerate, is a pyrethroid insecticide and also affects insect nervous function , but in a different manner than azinphos-methyl (Ware 1994). Methoxyfenozide, is an ecdysteroid antagonist which interferes with the molting process of insect larvae and has been found to be an effective control for Lepidoteran larvae but has low impact on non-target organisms (Carlson et al. 2001). Spinosad, is a metabolite of the soil actinomycete, *Saccharopolyspora spinosa* (Mertz and Yoa), which when ingested by the target insect cause the loss of nervous function (Thompson et al. 2000). Imidacloprid, is a neonicotinoid insecticide that is absorbed into the plant it is sprayed onto (Wise et al. 2002) and also interferes with insect's nervous function once its ingested (Gut et al. 1999). Imidacloprid is generally targeted at Homoptera.

With a reduction in use of broad-spectrum insecticides and greater reliance on newer more selective insecticides for orchard pest control, natural enemy populations may increase and become a more significant mortality factor for OBLR populations. However, some insecticides that are promoted based on apparent selectivity, such as spinosad, have in some cases been found to be harmful to some natural enemies (Hill and Foster 2000, Suh et al. 2000, Brunner et al. 2001, Cisneros et al. 2002, Mason et al. 2002). Successful biological control of OBLR in commercial apple orchards will therefore depend on when and how each insecticide is incorporated into an Integrated Pest Management (IPM) program.

The International Organization of Biological Control, West Palaearctic Regional Section (IOBC/WPRS) working group on Pesticides and Beneficial Organisms has developed standards for testing insecticide effects on natural enemies (Hassan 1998). The goal of the working group is to provide information on insecticides with reduced risks to natural enemies for use in IPM programs (Hassan 1998). The methods developed by the working group involve laboratory testing, semi-field testing, and field-testing of insecticides (Hassan 1998). Testing of insecticide effects on natural enemies is required in some European countries (Hassan 1998).

A survey of parasitoids attacking OBLR in Michigan apple orchards during 1999 and 2000 recovered approximately 21 species of parasitoids from 8 families of Hymenoptera and 1 family of Diptera from OBLR (Chapter 2). Parasitism of OBLR collected ranged from 3% up to 37% (Chapter 2). The most consistently abundant Hymenopteran parasitoid for both 1999 and 2000 was *Bassus dimidiator* (Nees.) (Braconidae) that constituted up to 96% of the total parasitoid complex attacking OBLR in Michigan apple orchards (Chapter 2). A second Braconid parasitoid recovered from OBLR during 1999 and 2000 was *Macrocentrus linearis* (Nees.) comprised up to 12% of the total parasitoid complex attacking OBLR in Michigan apple orchards (Chapter 2).

Bassus dimidiator (Nees.) is a solitary endoparasitoid, which had previously only been reported from the eye-spotted bud moth, *Spilonota ocellana* (Denis and Schiffermuller) (Krombein et al. 1979). A complete biology of *B. dimidiator* is given by Dondale (1954) under the name *Agathis laticinctus* (Cresson). By dissection of S. ocellana larvae, Dondale (1954) found that *B. dimidiator* lays a single egg in the ventral nerve ganglion of the host and is capable of parasitizing between 15 and 20 hosts. The larvae of *B. dimidiator* feed on the host internally and pupate outside the host's body (Dondale 1954). *Macrocentrus linearis* (Nees.) is a polyembryonic endoparasitoid that lays a single egg per host, however, that egg is able to divide into multiple embryos. Li et al. (1999) described the biology of *M. nigridorsis* Viereck which is similar to that of M. linearis and other species of *Macrocentrus*. Li et al. (1999) found that as many as 36 parasitoids could emerge from a single host. *Macrocentrus linearis* as with *M. nigridorsis* larvae feed inside the host's body and pupate on the outside of the hosts body (Li et al. 1999). The larvae of

M. linearis all pupate simultaneously and spin together into a silken football shaped cocoon as does *M. nigridorsis* (Li et al. 1999).

Maximizing the impacts of parasitoids like *B. dimidiator* and *M. linearis* has the potential to contribute to sustainable control of OBLR in Michigan apple orchards. Conservation of these parasitoids requires knowledge of the impact of insecticides used for OBLR control on these natural enemies. The objective of this study was to test the direct effects of five of the principal insecticides currently used in Michigan apple orchards for control of OBLR or other fruit pests on the survival and longevity of adult *B. dimidiator* and *M. linearis*. This information could then be used to make recommendations to improved integrated control of OBLR.

Methods

The effects of the residues from the formulated product of five insecticides (azinphos-methyl; Guthion®, esfenvalerate; Asana®, methoxyfenozide; Intrepid™, imidacloprid; Provado®, and spinosad; SpinTor™) currently used for control of OBLR and other pests in apple orchards were tested on the adult parasitoids *B. dimidiator* and *M. linearis*. Insecticides were tested at the highest recommended field rate in order to give the worst-case scenario on a weekly basis for 5 weeks. The methods chosen for this study were adapted from the standard methods developed by the IOBC/ WPRS working group, Pesticides and Beneficial Organisms (Hassan 1998).

The parasitoids (*B. dimidiator* and *M. linearis*) were initially obtained from field collected OBLR and then reared on a laboratory colony of OBLR at the Niles,USDA, APHIS, Plant Protection Center in Niles, Michigan. Parasitoids were reared at 26°C, 40-60% RH, 16:8 L:D. Parasitoid cocoons were placed individually into 1 oz diet cups with lids and then cups were placed into Ziploc bags and sent to Michigan State University via overnight mail. Upon arrival cocoons were then placed into a growth chamber set at 26°C, ~ 60% RH, 16:8 L:D until adult emergence. Adults were then placed into a screened cage and given a 25% honey solution in a 1 oz diet cup with lid, honey water could be freely accessed by parasitoids from a cotton dental wick placed into the lid of the diet cup. Males and females were placed into the same cage (*B. dimidiator* and *M. linearis* were in separate cages).

Bassus dimidiator Exposed to Residues on Petri Dishes

Insecticides were mixed with distilled water and applied at their highest recommended field rates (Wise et al. 2002) using an auto-load Potter Spray Tower (Burkard Scientific). Table 3.1 lists the common name, class, chemical name, and rates for each insecticide used. Insecticides were sprayed onto the outer top and bottom of 60 x 15mm polystyrene Petri dishes (Falcon®) and were allowed to dry for 1h. Controls were Petri dishes sprayed in the same manner with water only. A 1-1.5cm wide stainless steel mesh ring (McMaster-Carr, Aurora) was sandwiched between the two Petri dishes in order to create a ventilated space to enclose the parasitoids. The Petri dishes and ring were held together using two thin strips of packaging tape on either side of the arena and a cotton dental wick soaked in 25% honey solution was placed through a hole in the top Petri dish (Figure 3.1). Before taping the arena together a male and female parasitoid were placed into the arena.

The *B.dimidiator* adults used in the study ranged in age from a few hours to a maximum of 72h old. All parasitoids were given the opportunity to mate and feed before being exposed to the insecticide treatments. The number of parasitoids available for the experiment each week varied and resulted in the need to block the experiment by weeks. For arena a single male and female pair were aspirated from the larger cage and placed into the arena containing the insecticide residue. The honey water in the dental wick was replenished each day by pipeting new solution onto the dental wick. Mortality was assessed at 4h, 24h, 48h, and 120h. Parasitoids were reported as being alive (actively moving) or dead (lying on their side or back and unresponsive to touch) in order to fit the assumptions of the statistical model used.

***Bassus dimidiator* Exposed to Residues on Leaves**

To test the effect of drying time and exposure on a natural substrate, insecticides were applied to apple leaves and allowed to dry for either 1h or 24h before parasitoids were exposed to the residues. Insecticides and water controls were applied using the same methods when *B. dimidiator* was exposed to residues on Petri dishes only. The upper surface of an entire apple leaf was sprayed. Leaf petioles were placed in water contained in a 1 oz diet cup with each individual leaf stem placed through a hole in the lid of a 1 oz diet cup, the water contained four drops of Floralife® Crystal Clear™ (Floralife Inc., Walterboro) fresh flower food in order to extend the life of the leaf. Leaves were collected from unsprayed antique and scab resistant varieties of apple trees on the Michigan State University Collins Road Entomology Farm in East Lansing, Michigan. Leaves were provided water throughout the entire experiment and cups were filled as necessary. After residues were dried, leaves were fixed to the bottom of a Petri dish (60 x 15mm polystyrene Falcon®) using double sided tape with the sprayed surface of the leaf facing upwards. The stainless steel metal ring was then set on top of the leaf and a second Petri dish was then placed on top of the ring to complete the enclosure after a male and female pair of *B. dimidiator* was put into the arena. Securing of the arena and parasitoid feeding was as previously described (Figure 3.2). Parasitoids ranged in age from a few hours old up to a maximum of 48h old. The numbers of male and female parasitoids available varied and resulted in the need to block the experiment by weeks. Mortality was assessed at the same time intervals as in the previously described *B. dimidiator* experiment however, additional observations were made at 72h and 96h. Parasitoids were reported as being either alive or dead as previously explained.

For each treatment containing a leaf with insecticide residue a single Petri dish arena was sprayed and allowed to dry either 1h or 24h. Petri dishes were sprayed the same as previously described (Figure 3.1). A single male and female pair were aspirated from the larger cage and placed into the arena with insecticide residue. The number of parasitoids available varied and the experiment was blocked by week. Mortality was assessed at the same time intervals as those exposed to residues on the leaves. A 25% honey solution was provided via a dental wick and was replenished each day.

***Macrocentrus linearis* Exposed to Residues on Petri Dishes and Leaves**

Leaves and Petri dishes (Figure 3.1 and Figure 3.2) were sprayed using the same methods as in the *B. dimidiator* experiments and were allowed to dry for 1h or 24h before parasitoid exposure. Parasitoids ranged in age from a few hours old up to a maximum of 48h old. A single male and female pair were aspirated from a larger cage and placed into the arenas with leaves or those without leaves. The number of male and female *M. linearis* available for the experiments varied each week resulting in blocking the experiments by week. Mortality was assessed at the same time intervals described for the *B. dimidiator* experiment with leaves. *Macrocentrus linearis* parasitoids were also provided a 25% honey solution via a dental wick with the solution being replenished daily.

Statistical Analysis

Data were analyzed using survival analysis assuming Cox's proportional hazards model with a complementary log-log function in SAS® with the GENMOD procedure (Allison 1995). Data had a binomial distribution with parasitoids either alive or dead at specified time periods. The effects of chemical, gender, the time interval in which the chemicals were allowed to dry, and the presence or absence of leaves were included in the model and their significance was tested using likelihood ratio tests. Week was included as a blocking factor. For the trials where both leaves and Petri dishes were sprayed, Least Squares Means were used to compare pairwise significant effects. Parasitoids that had escaped from the arena or where discrepancies occurred in the data due to observational error were omitted from all analyses. For the *B. dimidiator* experiment where insecticides were sprayed onto Petri dishes only, few to no parasitoids survived to 120h, that time period was omitted from the survival analysis in order to fit the assumptions of the model. For *M. linearis* few to no parasitoids lived past 4h for Guthion and that chemical therefore was omitted from the survival analysis.

Results

***Bassus dimidiator* Exposed to Residues on Petri Dishes**

Survival analysis for female and male *B. dimidiator* parasitoids that had been exposed to insecticides sprayed onto Petri dishes and allowed to dry for 1h (Table 3.2) showed significant effects for the time interval (χ^2=178.02, df=2, P<0.0001) at which data was recorded, the chemicals used (χ^2=267.10, df=5, P<0.0001), parasitoid gender

(χ^2=6.52, df=1, P=0.0107), a chemical and gender interaction (χ^2=18.82, df=5, P=0.0021), and week or blocking (χ^2=28.26, df=4, P<0.0001).

Females: residues dried 1h

The mean proportion of females surviving that were exposed to insecticides dried 1h on Petri dishes were calculated over each of the 5 weeks in which the experiment was conducted (Table 3.3). At 4h female *B. dimidiator* exposed to the methoxyfenozide treatment had a mean proportion of 1.00 surviving. The mean proportion of female parasitoids surviving in the control and spinosad treatments at 4h was 0.98. Mean proportions of females surviving in the imidacloprid, esfenvalerate, and azinphos-methyl treatments at 4h were 0.95, 0.41, and 0.24 respectively. At 24h female *B. dimidiator* in the methoxyfenozide treatments continued to have the greatest mean proportion surviving (0.90) followed by the control (0.75), esfenvalerate (0.31), imidacloprid (0.26), spinosad (0.21), and no females exposed to azinphos-methyl surviving. At 48h female *B. dimidiator* in the control treatments had the greatest mean proportion surviving (0.50) followed by methoxyfenozide (0.46), esfenvalerate (0.26), and imidacloprid and spinosad which both had 0.03. The mean proportion of females surviving at 120h was greatest for those on the controls (0.04) followed by methoxyfenozide and esfenvalerate which both had 0.03 the rest had no survivors.

Pairwise comparisons (using contrast estimate) of the six treatments for *B. dimidiator* females exposed to insecticide residues dried 1h onto Petri dishes only, showed that there were no significant differences in survival between the control and methoxyfenozide treatments (P=0.8262), esfenvalerate and imidacloprid (P=0.1479), esfenvalerate and spinosad (P=0.1786), and spinosad and imidacloprid (P=0.9082). All other combinations of treatments were significantly different from one another ($P \leq 0.05$). Treatments can be ranked from the least toxic to the most toxic for *B. dimidiator* females when exposed to residues dried 1h on Petri dishes where control= methoxyfenozide < esfenvalerate = imidacloprid = spinosad < azinphos-methyl.

Males: residues dried 1h

The mean proportions of males surviving that were exposed to insecticides dried 1h on Petri dishes were calculated over the 5 weeks in which the experiment was conducted (Table 3.3). At 4h all of the male *B. dimidiator* in the control, methoxyfenozide, and spinosad treatments were surviving. The mean proportion of male parasitoids surviving in the imidacloprid treatments at 4h was 0.84. Mean proportions of males surviving in the esfenvalerate, and azinphos-methyl treatments at 4h were 0.26 and 0.25 respectively. At 24h male *B. dimidiator* in the methoxyfenozide treatments continued to have the greatest mean proportion surviving (0.90) followed by the control (0.84), imidacloprid (0.34), spinosad (0.13), esfenvalerate (0.10), and no males exposed to azinphos-methyl surviving. At 48h male *B. dimidiator* in the control treatments had the greatest mean proportion surviving (0.44) followed by methoxyfenozide (0.27), esfenvalerate (0.05), imidacloprid (0.03), and spinosad and azinphos-methyl had no survivors. The mean proportion of males surviving at 120h was 0.03 for the controls and all other insecticides had no survivors.

Pairwise comparisons (using contrast estimate) of the six treatments for *B. dimidiator* males exposed to insecticide residues dried 1h onto Petri dishes only, showed that there were no significant differences between the control and methoxyfenozide treatments ($P=0.4827$), and spinosad and imidacloprid ($P=0.1812$). All other combinations of treatments were significantly different from one another ($p \leq 0.05$). Treatments can be ranked from the least toxic to the most toxic for *B. dimidiator* males when exposed to residues dried 1h on Petri dishes where control= methoxyfenozide <esfenvalerate<imidacloprid=spinosad<azinphos-methyl.

Bassus dimidiator Exposed to Residues on Petri Dishes and Leaves

Survival analysis for female and male *B. dimidiator* parasitoids that had been exposed to insecticides sprayed onto Petri dishes or apple leaves and allowed to dry for 1h or 24h (Table 3.4) showed significant effects for week (blocking) (χ^2=31.00, df=4, P <0.0001), the chemicals used (χ^2=242.61, df=5, P<0.0001), parasitoid gender (χ^2=11.69, df=1, P=0.0006), the presence of a leaf (χ^2=19.33, df=1, P <0.0001), time interval (χ^2=139.56, df=5, P <0.0001) at which data was recorded, and a chemical and leaf interaction (χ^2=26.80, df=5, P <0.0001). There were no significant effects of the time in which insecticides were allowed to dry (χ^2=0.92, df=1, P=0.3374), a chemical and gender interaction (χ^2=6.22, df=5, P=0.2857), and a chemical and leaf and gender interaction (χ^2=4.80, df=6, P=0.5700).

Females: residues on Petri dishes dried 1h

The sample size of *B. dimidiator* females available for treatments without leaves was small and the number of parasitoids surviving was totaled for all five weeks and then the mean proportion surviving was calculated (Table 3.5). The total mean proportion of females surviving at 4h was 1.00 for parasitoids in the control, methoxyfenozide, imidacloprid, and spinosad treatments. The total mean proportion of females surviving at 4h for the esfenvalerate and azinphos-methyl treatments was 0.60 and 0.20 respectively. At 24h the control and methoxyfenozide treatments had a total mean proportion of 1.00

females surviving. Females in the spinosad treatment had the second highest total mean proportion surviving (0.80) at 24h followed by esfenvalerate (0.60), imidacloprid (0.50), and no surviving females in the azinphos-methyl treatments. At 48h 0.80 females survived in the controls and intrepid treatments, 0.40 surviving in the esfenvalerate and spinosad treatments, and no females surviving in the imidacloprid and azinphos-methyl treatments. The total mean proportion of females surviving at 72h in the control, methoxyfenozide, and esfenvalerate treatments was the same as at 48h the proportion surviving in the spinosad treatments was 0.20. At 96h and 120h the control had the greatest survival (0.80) followed by methoxyfenozide (0.60), esfenvalerate (0.40), and all others had no surviving females.

Pairwise comparisons (using least squares means) of the six treatments for *B. dimidiator* females exposed to insecticide residues dried 1h onto Petri dishes, showed that there were no significant differences between the control and methoxyfenozide treatments (P=0.1278), esfenvalerate and control (P=0.0797), esfenvalerate and methoxyfenozide (P=0.7925), esfenvalerate and imidacloprid (P=0.0833), methoxyfenozide and imidacloprid (P=0.0539), spinosad and imidacloprid (P=0.8776), esfenvalerate and spinosad (P=0.0902) and spinosad and methoxyfenozide (0.0580). All other treatments were significantly different from one another ($p \leq 0.05$). Treatments ranked from the least toxic to the most toxic for *B. dimidiator* females when exposed to residues dried 1h on Petri dishes where control= methoxyfenozide =esfenvalerate= spinosad = imidacloprid <azinphos-methyl.

Males: residues on Petri dishes dried 1h

The mean proportion surviving was calculated for males in treatments without leaves as previously described for females (Table 3.5). The total mean proportion of males surviving at 4h was 1.00 for parasitoids in the control, methoxyfenozide, imidacloprid, and spinosad treatments. The total mean proportion of males surviving at 4h for the esfenvalerate and azinphos-methyl treatments was 0.60. At 24h the control had a total mean proportion of 1.00 males surviving. Males in the methoxyfenozide and spinosad treatment had the second highest total mean proportion survived (0.60) at 24h followed by imidacloprid (0.50), esfenvalerate (0.40), and no surviving males in the azinphos-methyl treatments. At 48h 0.80 males survived in the controls, 0.60 males in the methoxyfenozide treatments survived, 0.20 surviving in the esfenvalerate treatments, and no males surviving in the imidacloprid, spinosad, and azinphos-methyl treatments. The total mean proportion of males surviving at 72h in the control and esfenvalerate treatments were the same as at 48h the proportion surviving in the methoxyfenozide treatments was 0.20. At 96h the control had the greatest survival (0.80) followed by methoxyfenozide (0.20), and all others had no surviving males. At 120h the total mean proportion of males surviving in the control was 0.60, and 0.20 surviving for methoxyfenozide.

Pairwise comparisons (using least squares means) of the six treatments for *B. dimidiator* males exposed to insecticide residues dried 1h onto Petri dishes, showed that there were no significant differences between the esfenvalerate and spinosad treatments (P=0.4065), esfenvalerate and methoxyfenozide (P=0.3810), spinosad and imidacloprid (P=0.1488), and spinosad and methoxyfenozide (P=0.0952). All other treatments are significantly different from one another ($P \leq 0.05$). Treatments ranked from the least toxic to the most toxic for *B. dimidiator* males when exposed to residues dried 1h on Petri dishes where control< methoxyfenozide =esfenvalerate= spinosad = imidacloprid <azinphos-methyl.

Females: residues on Petri dishes dried 24h

The mean proportion surviving was calculated for females in treatments without leaves dried 24h as previously described for females and males in treatments without leaves dried 1h (Table 3.6). At 4h females in the control, methoxyfenozide, imidacloprid, and spinosad treatments had a total mean proportion of 1.00 surviving. Females in the esfenvalerate and azinphos-methyl treatments had a total mean proportion of 0.75 and 0.25 respectively surviving at 4h. Females in the control and methoxyfenozide treatments had a total mean proportion of 1.00 surviving at 24h, 0.75 surviving for females in the esfenvalerate, imidacloprid, spinosad treatments, and no females in the azinphos-methyl treatments were surviving. At 48h the control had the greatest total mean proportion of females surviving (0.80), followed by methoxyfenozide (0.75), esfenvalerate and imidacloprid both had 0.50 surviving, and no females in apinosad surviving. At 72h the same proportion surviving in the control as at 48h. Methoxyfenozide and esfenvalerate both had a total mean survival of 0.50 at 72h and 0.25 surviving in the imidacloprid treatments. At 96h and 120h the total mean proportion of surviving females in the control was 0.80, in methoxyfenozide 0.50, and esfenvalerate and imidacloprid had 0.25 surviving.

Survival analysis showed no significant differences for the time in which the insecticides were allowed to dry therefore the least squares means results for treatments allowed to dry 24h are the same as those dried 1h and so are the toxicity rankings.

Males: residues on Petri dishes dried 24h

The mean proportion surviving was calculated for males in treatments without leaves dried 24h as previously described for females (Table 3.6). At 4h and 24h all males were surviving in the control, methoxyfenozide, and spinosad treatments a mean proportion of 1.00. At 4h a mean proportion of 0.60 males were surviving in the esfenvalerate treatment, 0.75 in the imidacloprid treatment, and 0.25 in the azinphos-methyl treatment. At 24h 0.40 males in the esfenvalerate treatment was surviving and no males were surviving in the imidacloprid and azinphos-methyl treatments. The control had the greatest total mean proportion surviving (0.80) at 48h followed by methoxyfenozide (0.75), esfenvalerate (0.40), and spinosad (0.50). The mean proportion of males in the controls stayed at 0.80 for 72, 96, and 120h. The mean proportion of males in the methoxyfenozide treatment decreased from 0.50 at 72h to 0.25 at 96 and 120h. The mean proportion of males surviving in the esfenvalerate treatments remained at 0.20 for 72, 96, and 120h. No males were surviving in the spinosad treatment at 72h.

Survival analysis showed no significant differences for the time in which the insecticides were allowed to dry therefore the least squares means results for treatments allowed to dry 24h are the same as those dried 1h and so are the toxicity rankings.

Females: residues on apple leaves dried 1h

Parasitoid sample size was larger than in the *B. dimidiator* treatments without leaves dried 1 or 24h and the mean proportion of surviving females was calculated over the five weeks in which the experiment was conducted (Table 3.7). At 4h females in all treatments except for in esfenvalerate (0.90) and azinphos-methyl (0.70) had a mean proportion of 1.00 surviving. The control had a mean proportion of 0.90 females surviving at 24h followed by methoxyfenozide with a mean proportion of 0.95 surviving, 0.80 in imidacloprid, 0.75 in esfenvalerate, 0.50 in spinosad, and no survivors in azinphos-methyl. At 48h the control had the greatest mean proportion of females surviving (0.90) followed by imidacloprid (0.80), methoxyfenozide (0.75), esfenvalerate (0.65), and spinosad (0.25). At 72 and 96h the control had the greatest mean proportion of females surviving (0.80) followed by methoxyfenozide (0.75). Females in the imidacloprid treatment had a mean proportion of 0.65 surviving at 72h decreasing to 0.45 at 96h and 0.40 at 120h. Females in the esfenvalerate treatment had a mean proportion of 0.50 surviving at 72, 96, and 120h. A mean proportion of 0.25 females were surviving at 72h decreasing to 0.05 at 96 and 120h.

Pairwise comparisons (using least squares means) of the six treatments for *B. dimidiator* females exposed to insecticide residues dried 1h onto apple leaves, showed that there were no significant differences between the control and methoxyfenozide treatments (P=0.9742), esfenvalerate and methoxyfenozide (P=0.0865), esfenvalerate and control (P=0.0609), and esfenvalerate and imidacloprid (P=0.1930). All other treatments are significantly different from one another ($P \leq 0.05$). Treatments ranked from the least toxic to the most toxic for *B. dimidiator* females when exposed to residues dried 1h on apple leaves where control= methoxyfenozide =esfenvalerate= imidacloprid<spinosad <azinphos-methyl.

Males: residues on apple leaves dried 1h

The mean proportion of males surviving with leaves dried 1h was calculated as described for females with apple leaves dried 1h (Table 3.7). At 4h males in the control, methoxyfenozide, imidacloprid, and spinosad treatments had a mean proportion of 1.00

surviving. The mean proportion of males surviving at 4h in the azinphos-methyl and esfenvalerate treatments was 0.65 and 0.60 respectively. At 24h males in the control, methoxyfenozide, and imidacloprid treatments had a mean proportion of 1.00 surviving. A mean proportion of 0.40 males were surviving in the esfenvalerate treatments, 0.33 in the spinosad treatments and 0.05 in the azinphos-methyl treatments at 24h. The control and methoxyfenozide treatments had the greatest mean proportion (0.80) of males surviving at 48h followed by imidacloprid (0.75), esfenvalerate (0.30), spinosad (0.05), and no survivors in the azinphos-methyl treatment. At 72h the control, azinphos-methyl, and esfenvalerate treatments all had the same mean proportion of males surviving as at 48h. At 72h the mean proportion of males surviving in the imidacloprid treatment was 0.60 and no males were surviving in spinosad treatments. At 96h methoxyfenozide had the greatest mean proportion of males surviving (0.80) followed by the control (0.70), imidacloprid (0.50), and esfenvalerate (0.30). At 120h methoxyfenozide had the greatest mean proportion of males surviving (0.73) followed by the control (0.65), imidacloprid (0.40), and esfenvalerate (0.30).

Pairwise comparisons (using least squares means) of the six treatments for *B. dimidiator* males exposed to insecticide residues dried 1h onto apple leaves, showed that there were no significant differences between the control and methoxyfenozide treatments ($P=0.7422$), methoxyfenozide and imidacloprid ($P=0.1174$), and imidacloprid and control ($P=0.0521$). All other treatments are significantly different from one another ($P\leq0.05$). Treatments ranked from the least toxic to the most toxic for *B. dimidiator* males when exposed to residues dried 1h on apple leaves where control= methoxyfenozide = imidacloprid< esfenvalerate <spinosad <azinphos-methyl.

Females: residues on apple leaves dried 24h

The mean proportion of females surviving with leaves dried 24h was calculated as described for females and males with apple leaves dried 1h (Table 3.8). At 4h females in the control, methoxyfenozide, imidacloprid, and spinosad treatments had a mean proportion of 1.00 surviving. The mean proportion of females surviving at 4h in the esfenvalerate and azinphos-methyl treatments was 0.95 and 0.94 respectively. At 24h females in the methoxyfenozide had a mean proportion of 1.00 surviving while 0.93 surviving in the control, 0.88 in spinosad, 0.85 in esfenvalerate, 0.81 in imidacloprid, and 0.19 in the azinphos-methyl treatments. The methoxyfenozide and control treatments had the greatest mean proportion 1.00 and 0.93 respectively of females surviving at 48h followed by esfenvalerate (0.80), imidacloprid (0.67), spinosad (0.44), and azinphos-methyl (0.06). At 72h methoxyfenozide treatments had the greatest mean proportion of females surviving (0.87) followed by esfenvalerate (0.80), controls (0.79), imidacloprid (0.67), spinosad (0.38), and no survivors in the azinphos-methyl treatment. At 96h methoxyfenozide had the greatest mean proportion of females surviving (0.79) followed by the control (0.67), esfenvalerate (0.65), imidacloprid (0.54), and spinosad (0.13). At 120h methoxyfenozide had the greatest mean proportion of females surviving (0.79) followed by the control (0.67), esfenvalerate (0.65), imidacloprid (0.48), and spinosad (0.13).

Survival analysis showed no significant differences for the time in which the insecticides were allowed to dry therefore the least squares means results for treatments allowed to dry 24h are the same as those dried 1h and so are the toxicity rankings.

Males: residues on apple leaves dried 24h

The mean proportion of males surviving with leaves dried 24h was calculated as described for females with apple leaves dried 24h (Table 3.8). At 4h males in the control, methoxyfenozide, imidacloprid, and spinosad treatments had a mean proportion of 1.00 surviving. The mean proportion of males surviving at 4h in the azinphos-methyl and esfenvalerate treatments was 0.75 and 0.65 respectively. At 24h males in the control, methoxyfenozide, and imidacloprid treatments had a mean proportion of 1.00 surviving. A mean proportion of 0.60 males were surviving in the esfenvalerate treatments, 0.52 in the spinosad treatments and 0.08 in the azinphos-methyl treatments at 24h. The control had the greatest mean proportion (0.80) of males surviving at 48h followed by Imidacloprid (0.85), methoxyfenozide (0.75), esfenvalerate (0.45), azinphos-methyl (0.08), and no survivors in the spinosad treatment. At 72h the control had the greatest mean proportion of males surviving (0.73) followed by imidacloprid (0.71), methoxyfenozide (0.69), esfenvalerate (0.40), and no survivors in the azinphos-methyl treatment. At 96h the control had the greatest mean proportion of males surviving (0.73) followed by imidacloprid (0.58), methoxyfenozide (0.56), and esfenvalerate (0.40). At 120h the control had the greatest mean proportion of males surviving (0.63) followed by methoxyfenozide (0.56), imidacloprid (0.52), and esfenvalerate (0.35).

Survival analysis showed no significant differences for the time in which the insecticides were allowed to dry therefore the least squares means results for treatments allowed to dry 24h are the same as those dried 1h and so are the toxicity rankings.

***Macrocentrus linearis* Exposed to Residues on Petri Dishes and Leaves**

Survival analysis for female and male *M. linearis* parasitoids that had been exposed to insecticides sprayed onto Petri dishes or apple leaves and allowed to dry for

1h or 24h (Table 3.9) showed significant effects for week (blocking) (χ^2=29.89, df=4, P<0.0001), the chemicals used (χ^2=278.64, df=4, P <0.0001), the presence of a leaf (χ^2=103.08, df=1, P <0.0001), time interval (χ^2=105.67, df=5, P <0.0001) at which data was recorded, a chemical and leaf interaction (χ^2=39.84, df=4, P <0.0001), and a chemical and gender interaction (χ^2=36.04, df=4, P <0.0001) . There were no significant effects of parasitoid gender (χ^2=1.01, df=1, P=0.3157), the time in which insecticides were allowed to dry (χ^2=0.00, df=1, P=0.9637), and a chemical and leaf and gender interaction (χ^2=3.25, df=5, P=0.6613).

Females: residues on Petri dishes dried 1h

The mean proportion of *M. linearis* females surviving when exposed to insecticides dried 1h on Petri dishes was calculated over the five weeks in which the experiment took place (Table 3.10). At 4h the methoxyfenozide treatment had the greatest mean proportion of females surviving (1.00) followed by the control (0.90), spinosad (0.88), imidacloprid (0.80), esfenvalerate (0.42), and no survivors in the azinphos-methyl treatment. At 24h methoxyfenozide again had the greatest mean proportion of females surviving (0.95) followed by the control (0.90), spinosad (0.45), imidacloprid (0.40), and esfenvalerate (0.10). The mean proportion surviving in the control and methoxyfenozide at 48h was the same as at 24h. The mean proportion of females surviving at 48h for imidacloprid, spinosad, and esfenvalerate was 0.20, 0.17, and 0.05 respectively. At 72h the greatest mean proportion of females surviving was the control (0.90) followed by methoxyfenozide (0.88), imidacloprid (0.05), and no survivors in esfenvalerate and spinosad treatments. At 96h the mean proportion of females surviving in the control and imidacloprid treatments were the same as at 72h and the mean proportion of females surviving in the methoxyfenozide treatment decreased to 0.78. At 120h the control had the greatest mean proportion of females surviving (0.70) followed by methoxyfenozide (0.33) and imidacloprid (0.05).

Pairwise comparisons (using least squares means) of the six treatments for *M. linearis* females exposed to insecticide residues dried 1h onto Petri dishes, showed that there were no significant differences between the control and methoxyfenozide treatments (P=0.2319), and spinosad and imidacloprid (P=0.5773). All other treatments are significantly different from one another ($p \leq 0.05$). Treatments ranked from the least toxic to the most toxic for *M. linearis* females when exposed to residues dried 1h on Petri dishes where control= methoxyfenozide <imidacloprid= spinosad < esfenvalerate <azinphos-methyl.

Males: residues on Petri dishes dried 1h

The mean proportion of *M. linearis* males surviving when exposed to insecticides dried 1h on Petri dishes was calculated as described for females (Table 3.10). At 4h the control and spinosad treatments had the greatest mean proportion of males surviving (0.95), followed by methoxyfenozide (0.93), imidacloprid (0.80), esfenvalerate (0.63), and azinphos-methyl (0.05). At 24h the control had a mean proportion of 0.88 males surviving; methoxyfenozide had 0.83, esfenvalerate 0.22, spinosad 0.20, imidacloprid 0.10, and no survivors in the azinphos-methyl treatment. At 48h methoxyfenozide, control, esfenvalerate, and imidacloprid treatments had a mean proportion of 0.78, 0.77, 0.10, and 0.10 males surviving respectively and no males were surviving in the spinosad treatment. At 72h the control had the greatest mean proportion of males surviving (0.67) followed by methoxyfenozide (0.52), esfenvalerate (0.10), and imidacloprid (0.05). The mean proportion for the control, methoxyfenozide, and esfenvalerate treatments at 96h was the same as at 72h and no males were surviving in the imidacloprid treatments. At 120h the control had a mean proportion of 0.55 males surviving, 0.32 males were surviving in the methoxyfenozide treatments and 0.10 males were surviving in the esfenvalerate treatments.

Pairwise comparisons (using least squares means) of the six treatments for *M. linearis* males exposed to insecticide residues dried 1h onto Petri dishes, showed that there were no significant differences between the control and methoxyfenozide treatments (P=0.2673), spinosad and imidacloprid (P=0.6860), esfenvalerate and imidacloprid (P=0.4079), and esfenvalerate and spinosad (P=0.6719). All other treatments were significantly different from one another ($P \leq 0.05$). Treatments ranked from the least toxic to the most toxic for *M. linearis* males when exposed to residues dried 1h on Petri dishes where control= methoxyfenozide < esfenvalerate= imidacloprid= spinosad<azinphos-methyl.

Females: residues on Petri dishes dried 24h

The mean proportion of *M. linearis* females surviving when exposed to insecticides dried 24h on Petri dishes was calculated over the five weeks in which the experiment took place (Table 3.11). At 4h the control, methoxyfenozide, and imidacloprid treatments had the greatest mean proportion of females surviving (1.00) followed by the spinosad (0.90), esfenvalerate (0.35), and no survivors in the azinphos-methyl treatment. At 24h the control had the greatest mean proportion of females surviving (1.00) followed by the methoxyfenozide (0.95), imidacloprid and spinosad (0.50), and esfenvalerate (0.15). The mean proportion surviving in the control and methoxyfenozide at 48h was 0.95 and 0.80 respectively. The mean proportion of females surviving at 48h for imidacloprid and spinosad was 0.15 and 0.10 respectively and no females were surviving in the esfenvalerate treatment. At 72h the greatest mean proportion of females surviving was in the methoxyfenozide treatment (0.70) followed by the control (0.67), spinosad (0.10), imidacloprid (0.05). At 96h the mean proportion of females surviving in the control was 0.57 decreasing to 0.52 surviving at 120h. The mean proportion of females surviving in the methoxyfenozide treatment at 96h and 120h was 0.55. No females were surviving at 96h in the esfenvalerate and imidacloprid treatments.

Survival analysis showed no significant differences for the time in which the insecticides were allowed to dry therefore the least squares means results for treatments allowed to dry 24h are the same as those dried 1h and so are the toxicity rankings.

Males: residues on Petri dishes dried 24h

The mean proportion of *M. linearis* males surviving when exposed to insecticides dried 24h on Petri dishes was calculated as described for females (Table 3.11). At 4h the control and spinosad had a mean proportion of 1.00 males surviving, 0.95 were surviving in the methoxyfenozide treatment, 0.80 in imidacloprid, 0.75 in esfenvalerate, and 0.05 azinphos-methyl. At 24h the control and methoxyfenozide had the greatest mean proportion of males surviving (0.75) followed by esfenvalerate (0.45), spinosad (0.35), imidacloprid (0.30), and no surviving males in the azinphos-methyl treatment. At 48h methoxyfenozide had the greatest mean proportion of males surviving (0.62) followed by the control (0.58), esfenvalerate (0.15), imidacloprid (0.05), and no surviving males in the spinosad treatment. At 72h methoxyfenozide had the greatest mean proportion of males surviving (0.57) followed by the control (0.53), esfenvalerate (0.05), and no surviving males in the imidacloprid treatment. At 96h the control had a mean proportion of 0.42 males surviving and 0.45 were surviving in the methoxyfenozide treatment and no surviving males in the esfenvalerate treatment. At 120h the control and Intrepid had a mean proportion surviving of 0.35 and 0.45 respectively.

Survival analysis showed no significant differences for the time in which the insecticides were allowed to dry therefore the least squares means results for treatments allowed to dry 24h are the same as those dried 1h and so are the toxicity rankings.

Females: residues on apple leaves dried 1h

The mean proportion of *M. linearis* females surviving when exposed to insecticides dried 1h on apple leaves was calculated over the five weeks in which the experiment took place (Table 3.12). At 4h the control and methoxyfenozide had a mean proportion of 1.00 females surviving and imidacloprid and spinosad both had 0.88 surviving, while esfenvalerate and azinphos-methyl had 0.52 and 0.45 surviving respectively. At 24h the control treatment had the greatest mean proportion of females surviving (1.00) followed by methoxyfenozide (0.95), imidacloprid (0.73), spinosad (0.65), esfenvalerate (0.42), and no females surviving in the azinphos-methyl treatment. At 48h the control had a mean proportion of 1.00 females surviving while methoxyfenozide had 0.75, imidacloprid 0.57, spinosad 0.43, and esfenvalerate 0.42. At 72h the control had the greatest mean proportion of females surviving (0.90) followed by methoxyfenozide (0.60), imidacloprid (0.50), esfenvalerate (0.37), and spinosad (0.22). At 96h the control had a mean proportion of 0.90 females surviving, methoxyfenozide and imidacloprid had 0.50, esfenvalerate had 0.32, and spinosad had 0.10. At 120h the control had a mean proportion of 0.90 females surviving; Intrepid had 0.45, esfenvalerate 0.32, imidacloprid 0.30, and spinosad 0.05.

Pairwise comparisons (using least squares means) of the six treatments for *M. linearis* females exposed to insecticide residues dried 1h onto apple leaves, showed that there were no significant differences between the methoxyfenozide and imidacloprid treatments (P=0.2246), and esfenvalerate and spinosad (P=0.4905). All other treatments are significantly different from one another ($P \leq 0.05$). Treatments ranked from the least toxic to the most toxic for *M. linearis* females when exposed to residues dried 1h on apple leaves where control<methoxyfenozide= imidacloprid < esfenvalerate = spinosad<azinphos-methyl.

Males: residues on apple leaves dried 1h

The mean proportion of *M. linearis* males surviving when exposed to insecticides dried 1h on apple leaves was calculated as described for females (Table 3.12). At 4h the control and methoxyfenozide had a mean proportion of 1.00 males surviving and esfenvalerate and imidacloprid both had 0.90 surviving, while spinosad and azinphos-methyl had 0.82 and 0.45 surviving respectively. At 24h the control and methoxyfenozide treatment had the greatest mean proportion of males (1.00) surviving followed by imidacloprid (0.80), esfenvalerate (0.75), spinosad (0.48), and no males surviving in the azinphos-methyl treatment. The control and methoxyfenozide treatments had a 0.87 mean proportion surviving at 48h while imidacloprid had 0.63, esfenvalerate 0.60, and spinosad 0.33 males surviving. At 72h the control had the greatest mean proportion of males surviving (0.87) followed by methoxyfenozide (0.77), esfenvalerate (0.60), imidacloprid (0.47), and spinosad (0.15). At 96h the control had a mean proportion of 0.87 males surviving; methoxyfenozide had 0.65, esfenvalerate 0.55, imidacloprid 0.32, and spinosad 0.05. At 120h the control had a mean proportion of 0.73 males surviving; methoxyfenozide had 0.60, esfenvalerate 0.55, imidacloprid 0.20, and spinosad 0.05.

Pairwise comparisons (using least squares means) of the six treatments for *M. linearis* males exposed to insecticide residues dried 1h onto apple leaves, showed that there were no significant differences between the control and methoxyfenozide treatments (P=0.5916), esfenvalerate and methoxyfenozide (P=0.5295), esfenvalerate and control (P=0.2506), and esfenvalerate and imidacloprid (P=0.1035). All other treatments are significantly different from one another (P≤0.05). Treatments ranked from the least toxic to the most toxic for *M. linearis* males when exposed to residues dried 1h on apple leaves where control=methoxyfenozide=esfenvalerate=imidacloprid<spinosad<azinphos-methyl.

Females: residues on apple leaves dried 24h

The mean proportion of *M. linearis* females surviving when exposed to insecticides dried 24h on apple leaves was calculated over the five weeks in which the experiment took place (Table 3.13). At 4h the imidacloprid treatment had the greatest mean proportion of females surviving (1.00) followed by the control (0.95), spinosad (0.90), methoxyfenozide (0.83), esfenvalerate (0.57), and azinphos-methyl (0.55). At 24h imidacloprid the control and methoxyfenozide had the same mean proportion of females surviving as at 4h. The treatments esfenvalerate and spinosad had a mean proportion of 0.30 and 0.65 females surviving respectively at 24h and no females surviving in the azinphos-methyl treatment. At 48h the control and imidacloprid treatments had the greatest mean proportion of females surviving (0.95), followed by methoxyfenozide

(0.83), spinosad (0.35), and esfenvalerate (0.30). At 72h the control, methoxyfenozide, imidacloprid, esfenvalerate, and spinosad had a mean proportion of 0.90, 0.83, 0.80, 0.30, and 0.25 females surviving respectively. At 96h the control had the greatest mean proportion of females surviving (0.85) followed by methoxyfenozide (0.77), imidacloprid (0.70), esfenvalerate (0.30), and spinosad (0.05). At 120h the control had the greatest mean proportion of females surviving (0.85) followed by methoxyfenozide (0.62), imidacloprid (0.40), esfenvalerate (0.30), and spinosad (0.05).

Survival analysis showed no significant differences for the time in which the insecticides were allowed to dry therefore the least squares means results for treatments allowed to dry 24h are the same as those dried 1h and so are the toxicity rankings.

Males: residues on apple leaves dried 24h

The mean proportion of *M. linearis* males surviving when exposed to insecticides dried 24h on apple leaves was calculated as described for females (Table 3.13). At 4h the control and methoxyfenozide had all males surviving with a mean proportion of 1.00. Spinosad, esfenvalerate, imidacloprid, and azinphos-methyl had a mean proportion of 0.93, 0.85, 0.75, and 0.50 males surviving respectively at 4h. At 24h the control had the greatest mean proportion of males surviving (0.85) followed by methoxyfenozide and imidacloprid (0.75), esfenvalerate (0.60), spinosad (0.57), and no survivors in the azinphos-methyl treatment. At 48h the control had the greatest mean proportion of males surviving (0.75) followed by methoxyfenozide (0.70), esfenvalerate and imidacloprid (0.60), and spinosad (0.07). At 72h the control had the greatest mean proportion of males surviving (0.65) followed by methoxyfenozide (0.60), esfenvalerate and imidacloprid (0.50), and spinosad (0.07). At 96h the control had the greatest mean proportion of males surviving (0.65) followed by methoxyfenozide (0.60), imidacloprid (0.50), esfenvalerate (0.45), and spinosad (0.07). At 120h the control had the greatest mean proportion of males surviving (0.60) followed by methoxyfenozide (0.55), esfenvalerate (0.45), imidacloprid (0.40), and spinosad (0.07).

Survival analysis showed no significant differences for the time in which the insecticides were allowed to dry therefore the least squares means results for treatments allowed to dry 24h are the same as those dried 1h and so are the toxicity rankings.

Discussion

A significant blocking effect (by week) occurred for both *B. dimidiator* experiments (Table 3.2 and 3.4) and for the *M. linearis* experiment (Table 3.9). A blocking effect was present in the Survival analysis due to individual parasitoids that would survive to 120h for certain insecticides in different weeks where other individuals exposed to the same insecticide would die before that time. A significant chemical effect (Tables 3.2, 3.4, 3.9) occurred because of the substantial differences in the toxicity of the insecticides. Fewer *B. dimidiator* and *M. linearis* would survive past 4h when exposed to azinphos-methyl while many individual parasitoids would survive to 120h for other insecticides such as methoxyfenozide. For *B. dimidiator* exposed to residues on leaves or Petri dishes, there were significant effects of gender (Table 3.2 and 3.4) where females appeared to survive longer than males (Table 3.3, 3.5, 3.6, 3.7, and 3.8). In the *B. dimidiator* experiment where insecticides were sprayed on Petri dishes or apple leaves and in the *M. linearis* experiment the 24h drying time period was added to determine if the age of the residues would increase the survival of the parasitoids. No differences between a 1h drying time and a 24h drying time occurred, however, the insecticides were not exposed to the same type of environment as in an orchard where rain, sun and other elements would degrade the toxicity of the insecticides over time. All experiments had a significant effect of the time interval in which the mortality of the parasitoids was assessed because as time progresses and parasitoids age there is a certain degree of natural mortality. The complementary log-log function of survival analysis also recognizes that parasitoids could have died at any point in time between when data was actually recorded.

For the *B. dimidiator* and *M. linearis* experiments where apple leaves were sprayed there was a significant leaf effect and a significant chemical leaf interaction (Table 3.4 and 3.9) where the presence of a leaf appeared to increase the length of survival for most insecticides, and especially for certain insecticides, such as with imidacloprid when compared to those with no leaf. One possible explanation for the differences in the presence or absence of leaves could be that parasitoids in arenas with leaves had more space to escape from the insecticide because only the upper leaf surface had been treated so the parasitoids could rest on the insecticide free meshed ring or on the top Petri dish that was also insecticide free, while parasitoids that were in Petri dish arenas had less insecticide free space to escape to because both the top and bottom Petri dishes contained insecticide. The arenas with leaves are closer to what would actually occur in the orchard where parasitoids are able to avoid insecticide residues. It is possible that parasitoids could be gaining some type of nutrient or water from the leaves that enhance their survival. In addition, leaves were also used to see if the Systemic properties of imidacloprid (Provado) (Wise et al. 2002) could result in an increase in survival of parasitoids when exposed to residues of this insecticide sprayed onto leaves. I did find that parasitoids exposed to imidacloprid sprayed on leaves had greater survival compared to those exposed to imidacloprid sprayed onto Petri dishes.

The insect growth regulator, methoxyfenozide (Intrepid) appears to be safe for *B. dimidiator* males and females when sprayed on Petri dishes or leaves. Survival of parasitoids exposed to this insecticide were similar to parasitoids in the control treatments where parasitoids in both treatments were able to survive to 120h and were not found to be significantly different from one another (Table 3.3, 3.5, 3.6, 3.7, and 3.8). Intrepid also appears to be safe for *M. linearis* males and females when sprayed on Petri dishes or leaves, where survival of parasitoids exposed to this insecticide is similar to parasitoids in the control and were found not to be significantly different from one another (Table 3.10, 3.11, 3.12, and 3.13). Methoxyfenozide was also found to be non-toxic to the parasitoid *Colpoclypeus florus* (Walker) in direct contact insecticide bioassay experiments (Brunner et al. 2001).

Azinphos-methyl (Guthion) was highly toxic to both male and female *B. dimidiator* and *M. linearis* when sprayed on leaves and Petri dishes (Table 3.3, 3.5, 3.6, 3.7, 3.8, 3.10, 3.11, 3.12, 3.13). Azinphos-methyl was also found to be highly toxic to parasitoids in contact and residual insecticide bioassays (Jones et al. 1995, Brunner et al. 2001) and to spiders when sprayed in orchards (Bajwa and Aliniazee 2001). In this experiment azinphos-methyl was so toxic to *M. linearis* that the insecticide could not be used in the survival analysis because it did not fit the assumptions of the model.

Spinosad (SpinTor) appears to be moderately too highly toxic for male and female *B. dimidiator* and *M. linearis* when sprayed on leaves and Petri dishes (Table 3.3, 3.5, 3.6, 3.7, 3.8, 3.10, 3.11, 3.12, and 3.13). Spinosad has been found to be toxic to parasitoids (Suh et al. 2000, Consoli et al. 2001, Mason et al. 2002) and predators (Cisneros et al. 2002) though it is touted as a "selective insecticide." Imidacloprid and esfenvalerate also appear to be moderately toxic to both *B. dimidiator* and *M. linearis* males and females when sprayed onto Petri dishes and leaves (Table 3.3, 3.5, 3.6, 3.7, 3.8, 3.10, 3.11, 3.12, and 3.13). The proportion of parasitoids surviving after exposure to imidacloprid also appears to have increased when this insecticide was sprayed onto leaves. Brunner et al. (2001) found that esfenvalerate (Asana) was toxic to parasitoids *C. florus* and *Trichogramma platneri* Nagarkatti, but could possibly be used at low rates at times of parasitoid inactivity without negative impact on parasitoid populations. Imidacloprid (Provado) was found to be toxic to *C. florus* and *T. platneri* in contact insecticide experiments (Brunner et al. 2001). Imidacloprid (Provado) has translaminar properties and is taken up by the leaves that it is sprayed on however there have been studies where imidacloprid has sublethal effects on parasitoids who feed on the nectar of plants sprayed with imidacloprid. Stapel et al.(2000) found that the longevity and fecundity of the parasitoid *Microplitis croceipes* Cresson, was negatively effected after parasitoids fed on nectar from cotton plants that had been sprayed with imidacloprid.

Conclusion

The insecticide methoxyfenozide appears to be safe for both *B. dimidiator* and *M. linearis*. The insecticide azinphos-methyl was highly toxic and should probably not be included in an apple pest management program that integrates biological control for OBLR into its management strategy. The insecticides imidacloprid, esfenvalerate, and spinosad appear to be moderately to highly toxic to these parasitoids depending method of application in this study (sprayed onto a leaf or onto a Petri dish) and could probably be used without interfering with biological control agents if applied with appropriate timing in regards to the presence of parasitoids. Further studies should be done with imidacloprid, esfenvalerate, and spinosad in an orchard setting in order to determine if field conditions make these insecticides less harmful to biological control agents than under laboratory conditions. Further study could also be done with these three insecticides applied at the lowest recommended field rates in the laboratory setting. The timing of adult parasitoid presence in the orchard should also be determined so that the moderately toxic insecticides can be used when parasitoids are least susceptible. Further tests should be conducted to determine if imidacloprid could adversely affect *B. dimidiator* and *M. linearis* through floral resources. The effects of the age of the residue should also be tested further, to determine how long residues are toxic in the field. Further studies should be conducted that look at the indirect effects, such as decreased fecundity, lost ability to parasitized or locate hosts, that these insecticides may have on parasitoids.

Table 3.1. The formulated product of five insecticides currently used for control of OBLR in apple orchards their class, chemical names, and highest recommended field rates (Wise et al. 2002).

Product Name	Class	Chemical Name	Max. Field Rate
Guthion 50 WP	organophosphate	azinphos-methyl	2 lb/100 gal. H_2O
Asana XL .66EC	pyrethroid	esfenvalerate	11.6 oz/100 gal.
Intrepid 2F	insect growth regulator	methoxyfenozide	2 lb/ gal.
Provado 1.6F	neonicotinoid	imidacloprid	8 oz/100 gal.
SpinTor 2SC	naturalyte	spinosad	10 oz/100 gal.

Table 3.2. Survival analysis results for *B. dimidiator* exposed to residues of five insecticides and water controls dried 1h on Petri dishes.

Source	df	χ^2	*p*-value
Time interval	2	178.02	<0.0001
Chemical	5	267.10	<0.0001
Gender	1	6.52	0.0107
Chemical x Gender	5	18.82	0.0021
Week	4	28.26	<0.0001

Table 3.3. Mean (± SE) proportion of *B. dimidiator* males and females surviving to 4, 24, 48, and 120h time after exposure to the residues of five insecticides dried 1h on Petri dishes. The total number of parasitoids (n) used at the start of each experiment over five weeks. Chemicals followed by different letters are significantly different ($p \leq 0.05$) determined by survival analysis.

				Mean Proportion Females Survived			
Treatment		**n**	**Time (hours)**	**4**	**24**	**48**	**120**
control	a	35		0.98 ± 0.03	0.75 ± 0.19	0.50 ± 0.21	0.04 ± 0.04
methoxyfenozide	a	34		1.00 ± 0.00	0.90 ± 0.10	0.46 ± 0.20	0.03 ± 0.03
esfenvalerate	bc	35		0.41 ± 0.11	0.31 ± 0.08	0.26 ± 0.07	0.03 ± 0.03
imidacloprid	cd	35		0.95 ± 0.03	0.26 ± 0.09	0.03 ± 0.03	0.00 ± 0.00
spinosad	bd	34		0.98 ± 0.03	0.21 ± 0.07	0.03 ± 0.03	0.00 ± 0.00
azinphos-methyl	e	35		0.24 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
				Mean Proportion Males Survived			
Treatment		**n**	**Time (hours)**	**4**	**24**	**48**	**120**
control	a	35		1.00 ± 0.00	0.84 ± 0.10	0.44 ± 0.19	0.03 ± 0.03
methoxyfenozide	a	34		1.00 ± 0.00	0.90 ± 0.07	0.27 ± 0.16	0.00 ± 0.00
esfenvalerate	b	35		0.26 ± 0.09	0.10 ± 0.05	0.05 ± 0.05	0.00 ± 0.00
imidacloprid	c	35		0.84 ± 0.05	0.34 ± 0.08	0.03 ± 0.03	0.00 ± 0.00
spinosad	c	34		1.00 ± 0.00	0.13 ± 0.07	0.00 ± 0.00	0.00 ± 0.00
azinphos-methyl	d	35		0.25 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.4. Survival analysis results for *B. dimidiator* exposed to residues of five insecticides and a water control on Petri dishes or apple leaves dried 1h or 24h.

Source	df	χ^2	p-value
Week	4	31.00	<0.0001
Chemical	5	242.61	<0.0001
Gender	1	11.69	0.0006
Drying Time	1	0.92	0.3374
Leaf	1	19.33	<0.0001
Time Interval	5	139.56	<0.0001
Chemical x Leaf	5	26.80	<0.0001
Chemical x Gender	5	6.22	0.2857
Chemical x Leaf x Gender	6	4.80	0.5700

Table 3.5. Total mean (± SE) proportion of *B. dimidiator* males and females surviving to 4, 24, 48, 72, 96 and 120h time after exposure to the residues of five insecticides dried for 1h on Petri dishes. The total number of parasitoids (n) used at the start of each experiment over five weeks. Chemicals followed by different letters are significantly different ($p \leq 0.05$) determined by survival analysis.

				Total Mean Proportion Females Survived					
Treatment		**n**	**Time (hours)**	**4**	**24**	**48**	**72**	**96**	**120**
control	ae	5		1.00 ± 0.00	1.00 ± 0.00	0.80 ± 0.18	0.80 ± 0.18	0.80 ± 0.18	0.80 ± 0.18
methoxyfenozide	abcf	5		1.00 ± 0.00	1.00 ± 0.00	0.80 ± 0.18	0.80 ± 0.18	0.60 ± 0.22	0.60 ± 0.22
esfenvalerate	cde	5		0.60 ± 0.22	0.60 ± 0.22	0.40 ± 0.22	0.40 ± 0.22	0.40 ± 0.22	0.40 ± 0.22
imidacloprid	bdg	5		1.00 ± 0.00	0.50 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
spinosad	fg	5		1.00 ± 0.00	0.80 ± 0.18	0.40 ± 0.22	0.20 ± 0.18	0.00 ± 0.00	0.00 ± 0.00
azinphos-methyl	h	5		0.20 ± 0.18	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
				Total Mean Proportion Males Survived					
Treatment		**n**	**Time (hours)**	**4**	**24**	**48**	**72**	**96**	**120**
control	a	5		1.00 ± 0.00	1.00 ± 0.00	0.80 ± 0.18	0.80 ± 0.18	0.80 ± 0.18	0.60 ± 0.22
methoxyfenozide	bd	5		1.00 ± 0.00	0.60 ± 0.22	0.60 ± 0.22	0.40 ± 0.22	0.20 ± 0.18	0.20 ± 0.18
esfenvalerate	bc	5		0.60 ± 0.22	0.40 ± 0.22	0.20 ± 0.18	0.20 ± 0.18	0.00 ± 0.00	0.00 ± 0.00
imidacloprid	e	5		1.00 ± 0.00	0.50 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
spinosad	cde	5		1.00 ± 0.00	0.60 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
azinphos-methyl	f	5		0.60 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.6. Total mean (± SE) proportion of *B. dimidiator* males and females surviving to 4, 24, 48, 72, 96 and 120h time after exposure to the residues of five insecticides dried for 24h on Petri dishes. The total number of parasitoids (n) used at the start of each experiment over five weeks.

		Total Mean Proportion Females Survived					
Treatment	**n**	**Time (hours) 4**	**24**	**48**	**72**	**96**	**120**
control	5	1.00 ± 0.00	1.00 ± 0.00	0.80 ± 0.18	0.80 ± 0.18	0.80 ± 0.18	0.80 ± 0.18
methoxyfenozide	4	1.00 ± 0.00	1.00 ± 0.00	0.75 ± 0.22	0.50 ± 0.25	0.50 ± 0.25	0.50 ± 0.25
esfenvalerate	5	0.75 ± 0.22	0.75 ± 0.22	0.50 ± 0.25	0.50 ± 0.25	0.25 ± 0.22	0.25 ± 0.22
imidacloprid	4	1.00 ± 0.00	0.75 ± 0.22	0.50 ± 0.25	0.25 ± 0.22	0.25 ± 0.22	0.25 ± 0.22
spinosad	4	1.00 ± 0.00	0.75 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
azinphos-methyl	4	0.25 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

		Total Mean Proportion Males Survived					
Treatment	**n**	**Time (hours) 4**	**24**	**48**	**72**	**96**	**120**
control	5	1.00 ± 0.00	1.00 ± 0.00	0.80 ± 0.18	0.80 ± 0.18	0.80 ± 0.18	0.80 ± 0.18
methoxyfenozide	4	1.00 ± 0.00	1.00 ± 0.00	0.75 ± 0.22	0.50 ± 0.25	0.25 ± 0.22	0.25 ± 0.22
esfenvalerate	5	0.60 ± 0.22	0.40 ± 0.22	0.40 ± 0.22	0.20 ± 0.18	0.20 ± 0.18	0.20 ± 0.18
imidacloprid	4	0.75 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
spinosad	4	1.00 ± 0.00	1.00 ± 0.00	0.50 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
azinphos-methyl	4	0.25 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.7. Mean (± SE) proportion of *B. dimidiator* males and females surviving to 4, 24, 48, 72, 96 and 120h time after exposure to the residues of five insecticides and a water control dried 1h on apple leaves. The total number of parasitoids (n) used at the start of each experiment over five weeks. Chemicals followed by different letters are significantly different ($p \leq 0.05$) determined by survival analysis.

			Mean Proportion Females Survived					
Treatment		**n**	**Time (hours) 4**	**24**	**48**	**72**	**96**	**120**
control	ab	18	1.00 ± 0.00	1.00 ± 0.00	0.90 ± 0.10	0.80 ± 0.20	0.80 ± 0.20	0.75 ± 0.19
methoxyfenozide	ac	18	1.00 ± 0.00	0.95 ± 0.05	0.75 ± 0.19	0.75 ± 0.19	0.75 ± 0.19	0.75 ± 0.19
esfenvalerate	bcd	18	0.90 ± 0.10	0.75 ± 0.16	0.65 ± 0.15	0.50 ± 0.18	0.50 ± 0.18	0.50 ± 0.18
imidacloprid	d	18	1.00 ± 0.00	0.80 ± 0.15	0.80 ± 0.15	0.65 ± 0.13	0.45 ± 0.15	0.40 ± 0.13
spinosad	e	18	1.00 ± 0.00	0.50 ± 0.18	0.25 ± 0.16	0.25 ± 0.16	0.05 ± 0.05	0.05 ± 0.05
azinphos-methyl	f	18	0.70 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

			Mean Proportion Males Survived					
Treatment		**n**	**Time (hours) 4**	**24**	**48**	**72**	**96**	**120**
control	ad	18	1.00 ± 0.00	1.00 ± 0.00	0.80 ± 0.20	0.80 ± 0.20	0.70 ± 0.20	0.65 ± 0.19
methoxyfenozide	ab	18	1.00 ± 0.00	1.00 ± 0.00	0.80 ± 0.20	0.80 ± 0.20	0.80 ± 0.20	0.73 ± 0.19
esfenvalerate	c	18	0.60 ± 0.13	0.40 ± 0.06	0.30 ± 0.09	0.30 ± 0.09	0.30 ± 0.09	0.30 ± 0.09
imidacloprid	bd	18	1.00 ± 0.00	1.00 ± 0.00	0.75 ± 0.16	0.60 ± 0.20	0.50 ± 0.18	0.40 ± 0.13
spinosad	e	18	1.00 ± 0.00	0.33 ± 0.14	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
azinphos-methyl	f	18	0.65 ± 0.19	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.8. Mean (± SE) proportion of *B. dimidiator* males and females surviving to 4, 24, 48, 72, 96 and 120h time after exposure to the residues of five insecticides and a water control dried for 24h on apple leaves. The total number of parasitoids (n) used at the start of each experiment over five weeks.

		Mean Proportion Females Survived					
Treatment	**n**	**Time (hours) 4**	**24**	**48**	**72**	**96**	**120**
control	16	1.00 ± 0.00	0.93 ± 0.07	0.93 ± 0.07	0.87 ± 0.13	0.67 ± 0.21	0.67 ± 0.21
methoxyfenozide	15	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.79 ± 0.13	0.79 ± 0.13	0.79 ± 0.13
esfenvalerate	19	0.95 ± 0.05	0.85 ± 0.15	0.80 ± 0.15	0.80 ± 0.15	0.65 ± 0.22	0.65 ± 0.22
imidacloprid	15	1.00 ± 0.00	0.81 ± 0.12	0.67 ± 0.16	0.67 ± 0.16	0.54 ± 0.21	0.48 ± 0.17
spinosad	15	1.00 ± 0.00	0.88 ± 0.13	0.44 ± 0.26	0.38 ± 0.24	0.13 ± 0.13	0.13 ± 0.13
azinphos-methyl	15	0.94 ± 0.06	0.19 ± 0.12	0.06 ± 0.06	0.06 ± 0.06	0.00 ± 0.00	0.00 ± 0.00

		Mean Proportion Males Survived					
Treatment	**n**	**Time (hours) 4**	**24**	**48**	**72**	**96**	**120**
control	16	1.00 ± 0.00	1.00 ± 0.00	0.80 ± 0.20	0.73 ± 0.19	0.73 ± 0.19	0.63 ± 0.19
methoxyfenozide	15	1.00 ± 0.00	1.00 ± 0.00	0.75 ± 0.10	0.69 ± 0.12	0.56 ± 0.16	0.56 ± 0.16
esfenvalerate	19	0.65 ± 0.22	0.60 ± 0.20	0.45 ± 0.17	0.40 ± 0.13	0.40 ± 0.13	0.35 ± 0.13
imidacloprid	15	1.00 ± 0.00	1.00 ± 0.00	0.85 ± 0.09	0.71 ± 0.14	0.58 ± 0.10	0.52 ± 0.13
spinosad	15	1.00 ± 0.00	0.52 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
azinphos-methyl	15	0.75 ± 0.10	0.08 ± 0.08	0.08 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.9. Results of survival analysis for *M. linearis* exposed to five insecticides and a water control dried 1h or 24h on Petri dishes or apple leaves.

Source	df	χ^2	p-value
Week	4	29.89	<0.0001
Chemical	4	278.64	<0.0001
Gender	1	1.01	0.3157
Drying Time	1	0.00	0.9637
Leaf	1	103.08	<0.0001
Time Interval	5	105.67	<0.0001
Chemical x Leaf	4	39.84	<0.0001
Chemical x Gender	4	36.04	<0.0001
Chemical x Leaf x Gender	5	3.25	0.6613

Table 3.10. Mean (± SE) proportion of *M. linearis* males and females surviving to 4, 24, 48, 72, 96 and 120h time after exposure to the residues of five insecticides and a water control dried for 1h on Petri dishes. The total number of parasitoids (n) used at the start of each experiment over five weeks. Chemicals followed by different letters are significantly different ($p \leq 0.05$) determined by survival analysis.

			Mean Proportion Females Survived					
Treatment		**n**	**Time (hours) 4**	**24**	**48**	**72**	**96**	**120**
control	a	20	0.90 ± 0.10	0.90 ± 0.10	0.90 ± 0.10	0.90 ± 0.10	0.90 ± 0.10	0.70 ± 0.09
methoxyfenozide	a	20	1.00 ± 0.00	0.95 ± 0.05	0.95 ± 0.05	0.88 ± 0.07	0.78 ± 0.10	0.33 ± 0.09
esfenvalerate	b	20	0.42 ± 0.10	0.10 ± 0.06	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
imidacloprid	c	20	0.80 ± 0.09	0.40 ± 0.10	0.20 ± 0.09	0.05 ± 0.05	0.05 ± 0.05	0.05 ± 0.05
spinosad	c	20	0.88 ± 0.07	0.45 ± 0.20	0.17 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
azinphos-methyl	d	20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
			Mean Proportion Males Survived					
Treatment		**n**	**Time (hours) 4**	**24**	**48**	**72**	**96**	**120**
control	a	20	0.95 ± 0.05	0.88 ± 0.07	0.77 ± 0.12	0.67 ± 0.11	0.62 ± 0.12	0.55 ± 0.17
methoxyfenozide	a	20	0.93 ± 0.07	0.83 ± 0.11	0.78 ± 0.10	0.52 ± 0.17	0.52 ± 0.17	0.32 ± 0.15
esfenvalerate	bc	20	0.63 ± 0.19	0.22 ± 0.15	0.10 ± 0.10	0.10 ± 0.10	0.10 ± 0.10	0.10 ± 0.10
imidacloprid	bd	20	0.80 ± 0.05	0.10 ± 0.06	0.10 ± 0.06	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
spinosad	cd	20	0.95 ± 0.05	0.20 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
azinphos-methyl	e	20	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.11. Mean (± SE) proportion of *M. linearis* males and females surviving to 4, 24, 48, 72, 96 and 120h time after exposure to the residues five insecticides and a water control dried for 24h on Petri dishes. The total number of parasitoids (n) used at the start of each experiment over five weeks.

		Mean Proportion Females Survived					
Treatment	**n**	**Time (hours) 4**	**24**	**48**	**72**	**96**	**120**
control	18	1.00 ± 0.00	1.00 ± 0.00	0.95 ± 0.05	0.67 ± 0.16	0.57 ± 0.14	0.52 ± 0.17
methoxyfenozide	18	1.00 ± 0.00	0.95 ± 0.05	0.80 ± 0.09	0.70 ± 0.09	0.55 ± 0.12	0.55 ± 0.12
esfenvalerate	18	0.35 ± 0.19	0.15 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
imidacloprid	18	1.00 ± 0.00	0.50 ± 0.18	0.15 ± 0.10	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
spinosad	18	0.90 ± 0.10	0.50 ± 0.08	0.10 ± 0.10	0.10 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
azinphos-methyl	18	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

		Mean Proportion Males Survived					
Treatment	**n**	**Time (hours) 4**	**24**	**48**	**72**	**96**	**120**
control	18	1.00 ± 0.00	0.75 ± 0.19	0.58 ± 0.17	0.53 ± 0.18	0.42 ± 0.13	0.35 ± 0.11
methoxyfenozide	18	0.95 ± 0.05	0.75 ± 0.11	0.62 ± 0.12	0.57 ± 0.14	0.45 ± 0.20	0.45 ± 0.20
esfenvalerate	18	0.75 ± 0.11	0.45 ± 0.15	0.15 ± 0.06	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
imidacloprid	18	0.80 ± 0.15	0.30 ± 0.18	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
spinosad	18	1.00 ± 0.00	0.35 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
azinphos-methyl	18	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.12. Mean (± SE) proportion of *M. linearis* males and females surviving to 4, 24, 48, 72, 96 and 120h time after exposure to the residues of five insecticides and a water control dried for 1h on apple leaves. The total number of parasitoids (n) used at the start of each experiment over five weeks. Chemicals followed by different letters are significantly different ($p \leq 0.05$) determined by survival analysis.

				Mean Proportion Females Survived					
Treatment		**n**	**Time (hours)**	**4**	**24**	**48**	**72**	**96**	**120**
control	a	20		1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.90 ± 0.06	0.90 ± 0.06	0.90 ± 0.06
methoxyfenozide	b	20		1.00 ± 0.00	0.95 ± 0.05	0.75 ± 0.16	0.60 ± 0.20	0.50 ± 0.18	0.45 ± 0.15
esfenvalerate	c	20		0.52 ± 0.10	0.42 ± 0.10	0.42 ± 0.10	0.37 ± 0.13	0.32 ± 0.12	0.32 ± 0.12
imidacloprid	b	20		0.88 ± 0.07	0.73 ± 0.14	0.57 ± 0.11	0.50 ± 0.16	0.50 ± 0.16	0.30 ± 0.09
spinosad	c	20		0.88 ± 0.07	0.65 ± 0.22	0.43 ± 0.18	0.22 ± 0.10	0.10 ± 0.10	0.05 ± 0.05
azinphos-methyl	d	20		0.45 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

				Mean Proportion Males Survived					
Treatment		**n**	**Time (hours)**	**4**	**24**	**48**	**72**	**96**	**120**
control	ab	20		1.00 ± 0.00	1.00 ± 0.00	0.87 ± 0.08	0.87 ± 0.08	0.87 ± 0.08	0.73 ± 0.12
methoxyfenozide	ac	20		1.00 ± 0.00	1.00 ± 0.00	0.87 ± 0.13	0.77 ± 0.12	0.65 ± 0.17	0.60 ± 0.15
esfenvalerate	bcd	20		0.90 ± 0.06	0.75 ± 0.08	0.60 ± 0.10	0.60 ± 0.10	0.55 ± 0.15	0.55 ± 0.15
imidacloprid	d	20		0.90 ± 0.10	0.80 ± 0.15	0.63 ± 0.13	0.47 ± 0.12	0.32 ± 0.05	0.20 ± 0.09
spinosad	e	20		0.82 ± 0.08	0.48 ± 0.14	0.33 ± 0.05	0.15 ± 0.10	0.05 ± 0.05	0.05 ± 0.05
azinphos-methyl	f	20		0.45 ± 0.12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3.13. Mean (± SE) proportion of *M. linearis* males and females surviving to 4, 24, 48, 72, 96 and 120h time after exposure to the residues five insecticides and a water control dried for 24h on apple leaves. The total number of parasitoids (n) used at the start of each experiment over five weeks.

			Mean Proportion Females Survived					
Treatment	**n**	**Time (hours)**	**4**	**24**	**48**	**72**	**96**	**120**
control	18		0.95 ± 0.05	0.95 ± 0.05	0.95 ± 0.05	0.90 ± 0.06	0.85 ± 0.06	0.85 ± 0.06
methoxyfenozide	18		0.83 ± 0.07	0.83 ± 0.07	0.83 ± 0.07	0.83 ± 0.07	0.77 ± 0.12	0.62 ± 0.20
esfenvalerate	18		0.57 ± 0.11	0.30 ± 0.12	0.30 ± 0.12	0.30 ± 0.12	0.30 ± 0.12	0.30 ± 0.12
imidacloprid	18		1.00 ± 0.00	1.00 ± 0.00	0.95 ± 0.05	0.80 ± 0.15	0.70 ± 0.15	0.40 ± 0.13
spinosad	18		0.90 ± 0.06	0.65 ± 0.19	0.35 ± 0.13	0.25 ± 0.16	0.05 ± 0.05	0.05 ± 0.05
azinphos-methyl	18		0.55 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

			Mean Proportion Males Survived					
Treatment	**n**	**Time (hours)**	**4**	**24**	**48**	**72**	**96**	**120**
control	18		1.00 ± 0.00	0.85 ± 0.10	0.75 ± 0.14	0.65 ± 0.13	0.65 ± 0.13	0.60 ± 0.17
methoxyfenozide	18		1.00 ± 0.00	0.75 ± 0.19	0.70 ± 0.18	0.60 ± 0.17	0.60 ± 0.17	0.55 ± 0.17
esfenvalerate	18		0.85 ± 0.10	0.60 ± 0.17	0.60 ± 0.17	0.50 ± 0.21	0.45 ± 0.20	0.45 ± 0.20
imidacloprid	18		0.75 ± 0.19	0.75 ± 0.19	0.60 ± 0.17	0.50 ± 0.18	0.50 ± 0.18	0.40 ± 0.17
spinosad	18		0.93 ± 0.07	0.57 ± 0.16	0.07 ± 0.07	0.07 ± 0.07	0.07 ± 0.07	0.07 ± 0.07
azinphos-methyl	18		0.50 ± 0.16	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

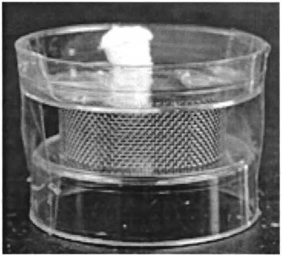

Figure3.1. Insecticide bioassay arena where both the top and bottom Petri dishes have been sprayed with insecticide and allowed to dry.

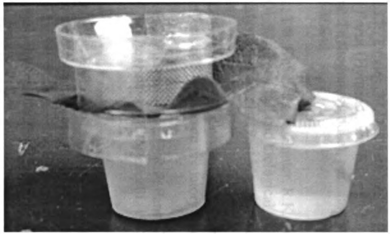

Figure 3.2. Insecticide bioassay arena where the leaf was sprayed with insecticide and allowed to dry.

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Chapter 4

Phenology of Adult *Bassus dimidiator* (Nees.) and *Macrocentrus linearis* (Nees.) (Hymenoptera: Braconidae), in commercially managed Michigan apple orchards.

Abstract

A final study was conducted to determine the activity periods of adult *B. dimidiator* and *M. linearis* in Michigan apple orchards. Yellow bucket traps and sentinel larvae were placed in two commercially managed apple orchards located in Kent, and Van Buren Counties, Michigan and in the Trevor Nichols Research Center orchard in Allegan County, Michigan. Parasitoid occurrence in the orchards was also compared to OBLR adult flight data obtained from pheromone trap catches. Out of a total of 2,790 OBLR sentinel larvae, thirteen parasitoids were recovered, two *M. linearis* and 11 *Enytus sp*. No *B. dimidiator* were recovered from the sentinel larvae. All parasitoids recovered emerged from sentinel larvae that had been placed in the orchard located in Allegan County. A total of 2,318 Hymenoptera and 2,987 Diptera were captured in the yellow bucket traps including three *B. dimidiator*. *Macrocentrus linearis* was not captured in bucket traps. All the *B. dimidiator* captured were from buckets placed in the Van Buren County orchard. *Macrocentrus linearis* was present in the orchards shortly after peak OBLR adult flight that occurred in mid June after codling moth and leafroller sprays are applied in the orchard. *Bassus dimidiator* was present in the orchards during peak OBLR flight at the time when codling moth and leafroller sprays are being applied. Recommendations can be made so that insecticides could be applied at times when these parasitoids are not active in Michigan apple orchards.

Introduction

A survey of the parasitoids attacking the obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris), an important pest in Michigan apple production, was conducted during 1999 and 2000 (Chapter 2). The survey resulted in the discovery of two important Braconid parasitoids, *Bassus dimidiator* (Nees.) and *Macrocentrus linearis* (Nees.) (Hymenoptera). *Bassus dimidiator* was the most abundant hymenopteran parasitoid comprising up to 96% of the parasitoid complex attacking OBLR while *M. linearis* made up to 12% of the parasitoid complex attacking OBLR (Chapter 2). The parasitoid survey conducted in Michigan apple orchards during 1999 and 2000 suggests that biological control could be a key component in an integrated pest management (IPM) program for OBLR in Michigan apple orchards.

The discovery of *B. dimidiator* developing on *C. rosaceana* constitutes a new host record for this parasitoid. Previously *B. dimidiator* had only been reported to attack the eye-spotted bud moth, *Spilonota ocellana* (Denis and Schiffermuller) (Krombein et al. 1979). The complete biology of *B. dimidiator*, a solitary endoparasitoid, has been described by Dondale (1954) under the name *Agathis laticinctus* (Cresson). In contrast, *M. linearis* is a known parasitoid of OBLR (Krombein et al. 1979). The biology of *M. linearis*, a polyembryonic endoparasitoid, is similar to that of *Macrocentrus nigridorsis* Viereck described by Li et al. (1999).

Organophosphate insecticides (OPs) have been the major form of control for OBLR for more than 40 years (Gut et al. 1999). However, the occurrence of up to 21-fold resistance to OPs by OBLR in Michigan apple orchards constitutes a major threat to this production system (Gut et al. 1998). Along with OBLR resistance, increasing restrictions on broad-spectrum insecticide use in agriculture resulting from the Environmental Protection Agency's Food Quality Protection Act (1996) has prompted the search for more sustainable and environmentally friendly forms of insect control. Reductions in the use of broad-spectrum insecticides and the development of pheromone mating disruption programs and more selective insecticides has left the door open for successful use of biological control agents as a major mortality factor for OBLR.

The selectivity of some of the newer insecticides however, is questionable and should be tested on natural enemies in the laboratory and in the orchard prior to wide scale commercial use. The direct effect of five insecticides currently used in Michigan apple orchards for control of OBLR was tested on the adult parasitoids *B. dimidiator* and *M. linearis* (Chapter 3). The insecticides ranged from traditional broad spectrum insecticides such as azinphos-methyl, to the newer more selective insecticides such as the insect growth regulator (IGR), methoxyfenozide. Azinphos-methyl was found to be highly toxic for both *B. dimidiator* and *M. linearis*, while methoxyfenozide appeared to be safe for both parasitoids (Chapter 3). The other insecticides tested (esfenvalerate, imidacloprid, and spinosad) were found to be highly to moderately toxic to the parasitoids depending on if the insecticide was sprayed onto Petri dishes or leaves (Chapter 3). Information on toxicity allows growers to avoid the use of broad-spectrum insecticides when *M. linearis* and *B. dimidiator* adults are present in the orchard.

The exact time in which *B. dimidiator* and *M. linearis* are present and attacking OBLR in Michigan apple orchards has not been determined. The abundance, diversity, and time in which parasitoids are present in an environment has been successfully delineated by others using yellow pan traps (Finnamore 1994, Purcell and Messing 1996) and

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and sentinel larvae (Marino and Landis 1996, Costamagna 2002). Yellow pan traps are shallow bowls or pans containing a mixture of water and soap, painted yellow to mimic the reflectance of leaves, which attract insects to their hosts. Sentinel larvae are larvae placed into an environment by the researcher for a predetermined amount of time that are later removed to determine if the larvae have been parasitized.

The objective of this study was to determine the time at which the adult parasitoids *B. dimidiator* and *M. linearis* are present in apple orchards in Michigan using two methods of sampling. The results of this study could then be combined with the information obtained from the insecticide bioassays and suggestions could be made to growers about the timing of insecticide applications that could minimize or avoid mortality of *B. dimidiator* and *M. linearis* in commercially managed orchards.

Methods

Two sampling methods (yellow bucket traps and sentinel larvae) were used together in the same orchard block in order to determine when the adult parasitoids, *B. dimidiator* and *M. linearis*, were present and parasitizing OBLR in Michigan apple orchards.

This study was conducted in two commercially managed apple orchards, and in a research orchard from late April to late July. Two of the orchards were located in the major apple producing regions of the state Kent County (Fruit Ridge), and Van Buren County (Southwest) (Figure 1). The third orchard was located at the Michigan State University Trevor Nichols Research Center (TNRC) in Allegan County, Michigan (Figure 4.1). Three blocks per orchard were chosen that were approximately 1.62 hectares in size, however, blocks varied in apple variety and tree size. Sampling did not occur within 9m of block edges so that edge effects could be avoided. Trees and rows within the sample area were then counted and a random number table was used to determine which rows and trees would be assigned to sentinel larvae or bucket traps.

In each block a total of ten sentinel larvae stations were placed onto apple tree branches. Stations consisted of a 2 L plastic pop bottle that had the bottom cut off and had a square cut out of the front half the length of the bottle so that a 32 oz cup could fit into it (Figure 4.2). Pop bottles were fixed to the trees with plastic cable ties placed around stable branches. Only five stations per block were used each week. The first five pop bottles were placed in randomly selected trees, and the other five bottles were placed in trees directly across the row (Figure 4.3). Trees containing sentinel larvae could then be alternated from week to week.

Apple shoots approximately 0.30m to 0.61m in length were cut each week from an abandoned apple orchard located in Allegan County, Michigan. A total of 450 shoots were cut and bundled into individual bouquets of 10 shoots each bound together with electrical tape. After three weeks into the study we switched to a total of 225 shoots cut each week and bundled into individual bouquets of five shoots each. Bouquets were each placed into 32oz. plastic cups containing water and Floralife® Crystal Clear™ (Floralife Inc., Walterboro) fresh flower food in order to extend the life of the bouquets. Melted paraffin wax was poured over the water in each cup to hold the bouquets in place and reduce evaporative water loss. In mid- summer we switched from paraffin wax to Parafilm M® (American National Can, Chicago) as high temperatures made the wax unstable.

OBLR egg masses were obtained from the TNRC laboratory colony, where the egg masses were laid onto pieces of wax paper. A single OBLR egg mass at black head or newly hatched stage was clipped to the bouquets with a small binder clip. Cups with bouquets were then placed into the sentinel larvae stations in the apple orchards where they remained for three days. Bouquets were disassembled in the orchard and leaves and stems were placed into Ziploc bags and put into a cooler with ice packs for transportation to the laboratory. OBLR larvae recovered from the bouquets were placed onto a modified pinto bean diet (Shorey and Hale 1965). The diet was contained in 4oz plastic soufflé cups with lid and approximately five larvae were placed into each cup. Larvae were reared on the laboratory bench at room temperature. Larvae were monitored weekly for mortality, pupation, adult OBLR emergence, and parasitism. Bouquets were replaced in the orchards the following week and placed into the station opposite from where the previous bouquet was placed. Parasitoids were identified from specimens collected in a previous survey of parasitoids attacking OBLR in Michigan apple orchards (Chapter 2).

Yellow bucket traps were constructed from half gallon plastic buckets painted yellow. Each bucket had four holes drilled into the sides covered with screen that were located half an inch from the bucket bottom to allow excess water to drain after rain. Buckets were filled just below the holes with a water and soap (Ivory liquid dish soap) mixture (four drops of soap/3.79L of H_2O). Buckets remained fixed to trees with plastic cable ties. Yellow bucket traps were placed on either side of one of the sentinel larvae stations (Figure 4.3) resulting in a total of ten-bucket traps per block. Bucket traps were checked twice per week. The contents of the bucket were emptied into a tea strainer and rinsed with water. Insects were placed onto a piece of paper towel and placed into a Ziploc bag for transport to the laboratory, and placed in the freezer for later examination.

Insects caught in the yellow bucket traps were sorted by order. For simplicity, insects in the orders Hymenoptera and Diptera were kept and counted while others were discarded. Hymenoptera were further divided into ants, bees, and wasps. Only *B. dimidiator* and *M. linearis* were identified to species.

Adult OBLR were monitored at the same time the sentinel larvae and yellow bucket traps were used. Moth captures in pheromone traps from each of the three orchards were provided by L. Stelinski, Tree Fruit Entomology Laboratory, Michigan State University, East Lansing, Michigan, and M. Haas, Trevor Nichols Research Center, Fennville, Michigan. With this additional data, OBLR adult flight and timing of leafroller insecticide applications could be compared to adult *B. dimidiator* and *M. linearis* occurrence in the orchards.

Results

A total of 2,790 OBLR sentinel larvae were recovered from the three apple orchards, of which 1,089 larvae died, and 1,688 emerged as adults (Table 4.1). The total percent mortality of the OBLR larvae recovered was 39.03%, and a total of 13 parasitoids emerged from the sentinel larvae (0.76% parasitism) (Table 4.1). The greatest number of larvae recovered, 1,087, came from the orchard located in Kent County, Michigan, which also had the highest percent mortality (44.16%) (Table 4.1). No parasitoids emerged from larvae collected from the Kent county orchard (Table 4.1). The second highest number of sentinel larvae, 880, was recovered from the orchard located in Allegan County (Table 4.1). All 13 parasitoids that had emerged from sentinel larvae also came from the Allegan county orchard (2.21% parasitism) (Table 4.1). Of the 13 OBLR that had parasitoids emerge from them 11 were parasitized by *Enytus sp.* (Ichnuemonidae) and two were parasitized by *M. linearis*. The fewest number of sentinel larvae, 823, were recovered from the orchard located in Van Buren County, but these larvae had the second highest percent mortality (38.07%) (Table 4.1). The parasitoid *B. dimidiator* had not parasitized any of the sentinel OBLR larvae recovered from the three orchards.

A total of 2,318 Hymenoptera and 2,987 Diptera were captured in the yellow bucket traps placed in the three apple orchards (Table 4.2). The greatest number of Hymenoptera, 931, was captured in the Kent County orchard followed by the Allegan County orchard with 814, and the Van Buren orchard with 573 (Table 4.2). The Hymenoptera captured in the yellow bucket traps were sorted into bees, wasps, and ants. A total of 342 bees were captured in traps located in the Allegan County orchard, while 80 and 47 bees were captured in the Kent and Van Buren orchards respectively (Table 4.2). A total of 371 wasps were collected from the Allegan orchard while 192 and 331 wasps were collected at the Kent and Van Buren orchards respectively (Table 4.2). No *M. linearis* were found in the bucket traps. A total of three *B. dimidiator* however, were found in bucket traps from the Van Buren orchard. No *B. dimidiator* were captured in bucket traps at the other two orchards (Table 4.2). A total of 659 ants were captured at the Kent county orchard, 195 were captured in buckets at the Van Buren orchard, and 101 were captured at the Allegan County orchard (Table 4.2). The greatest number of Diptera, a total of 1,233, was captured in bucket traps in the Van Buren orchard (Table 4.2). A total of 1,098 Diptera was captured in the Allegan county orchard and 656 were captures in the Kent county orchard (Table 4.2).

Data from the OBLR pheromone traps were not used for the Kent County orchard because no *B. dimidiator* or *M. linearis* were collected using either the yellow bucket traps or sentinel larvae methods of sampling. OBLR moth catches in pheromone traps were only collected for the two blocks where sentinel larvae and bucket traps were being used in the Allegan County orchard. The combined OBLR catches for the two blocks are presented in Figure 4.4. OBLR catches were also combined for the three blocks where sentinel larvae and bucket traps were being used in the Van Buren orchard (Figure 4.5). Peak adult OBLR flight for both the Allegan County and Van Buren orchards occurred around mid June (Figures 4.4 - 4.5). The *Enytus sp.* (Ichneumonidae) that were collected from sentinel larvae located in the Allegan County orchard were present in the orchard before peak adult OBLR flight from late May into early June during the time when coddling moth, *Cydia pomonella* (Linnaeus), insecticides are being applied in the orchards (Figure 4.4). The *M. linearis* (Braconidae) that were collected from the sentinel larvae that had been placed in the Allegan County orchard were present after peak adult OBLR flight from late June to the first of July after codling moth and leafroller insecticides are applied in the orchards (Figure 4.4). The *B. dimidiator* (Braconidae) that were captured in the yellow bucket traps that had been placed in the Van Buren County orchard were present during peak adult OBLR flight during mid June at the same time codling moth and leafroller insecticides are being applied (Figure 4.5).

Discussion and Conclusion

A large number of sentinel larvae were successfully recovered however, a large percentage of the larvae did not develop into adult moths. The level of mortality that had occurred in the sentinel larvae could possibly be due to injury to the larvae as they were being transferred from the apple branches to the diet. Insecticides that were applied when larvae were present in the orchard could have also killed many larvae. Other possible explanations for sentinel larvae mortality could be disease, and in some cases the diet became moldy. Though a reasonable number of OBLR larvae were recovered from the orchards, few parasitoids emerged from these larvae. Some possible reasons that could account for the low level of parasitism could be due to the searching habits of the parasitoids, where larger larvae are more apparent than early instar larvae. However, Dondale (1954) observed that *B. dimidiator* parasitized early instar (actual instar not specified) eye-spotted bud moth, *Spilonota ocellana* (Denis and Schiffermuller) in orchards.

A preliminary study that we conducted showed that *B. dimidiator* does parasitize first instar OBLR (n=14), however the OBLR were on artificial diet in plain sight of the parasitoid in a small, enclosed 4 oz. diet cup. Though *B. dimidiator* appears to be able to parasitize early instar OBLR in the laboratory it is possible that the parasitoid is unable to locate these larvae in the field where they are maybe concealed in leaf and flower bud terminals. The number of sentinel larvae placed into the field also may not have been numerous enough or spread widely enough throughout the orchard to enable parasitoids to easily locate them. Sentinel larvae were also left in the field for three days had they been left for a longer period of time there may possibly have been a higher incidence of parasitism.

The *Enytus sp.* (Ichneumonidae) that were recovered from the sentinel larvae were also found to have emerged from the OBLR that were collected during the 1999 and 2000 parasitoids survey that was previously conducted in Michigan apple orchards

(Chapter 2). The *Enytus sp.* recovered in the survey were not a consistently abundant parasitoid but made up to 23% of the total parasitoid complex attacking OBLR, while *M. linearis* which was also recovered in the sentinel larvae made up to 12% of the parasitoid complex attacking OBLR but was more consistently abundant (Chapter 2). A possible reason that these two parasitoids were recovered from the sentinel larvae when *B. dimidiator* was not could again be due to searching behavior of the parasitoid. There is also the possibility that the cues required for *B. dimidiator* to locate its host were not present or strong enough, where larger larvae would produce a grater amount of feeding damage.

The time (late May to early June) at which the *Enytus sp.* parasitized the sentinel OBLR larvae placed in the orchard coincided with the naturally occurring OBLR larvae that are in the later instars before peak adult OBLR flight. OBLR larvae are already in their 2-3rd instar when they emerge form their overwintering hibernaculum (Howitt 1993). There is the possibility that *Enytus sp.* could have an alternate host present in the orchard at the time when OBLR reaches its later instars and pupates. An additional possibility is that *Enytus sp.* is capable of parasitizing a range if instars. The *M. linearis* that were recovered from the sentinel OBLR were parasitizing these larvae at the same time (late June to early July, 2-3 weeks after peak adult flight) naturally occurring OBLR would be primarily in early to mid instar stages and egg hatch nearly complete.

The yellow bucket traps were successful in capturing a large number of beneficial and non-beneficial insects, although only three *B. dimidiator* and no *M. linearis* were captured. The *B. dimidiator* that were captured in the orchard occurred at the same time as peak adult OBLR flight. *Bassus dimidiator* was observed to have emerged from an overwintering OBLR larva (n=1) so it is possible that *B. dimidiator* overwinters in the 2-3rd instar OBLR and emerges before OBLR pupate and thus is present at the time when OBLR egg masses hatch. Had there been more yellow bucket traps placed throughout the orchard, more *B. dimidiator* may have been captured.

Though the sample sizes of the parasitoids recovered from the sentinel larvae and yellow bucket traps were too small to make firm conclusions the parasitoids that were recovered suggests that parasitoids are present in the orchard at times when major spray applications occur. Further studies should be done in order to determine if the times when parasitoid presence was detected are when these parasitoids are most abundant. Also it would be advantageous to determine how long parasitoids are present in the orchards. Once the timing of parasitoid presence is more thoroughly established then recommendations could be made to growers as to when to avoid insecticide applications, and thus decrease mortality of *B. dimidiator* and *M. linearis* in the orchard.

Table 4.1. Number of sentinel larvae and parasitoids recovered from three apple orchards located in Allegan, Kent, and Van Buren counties, Michigan.

Orchard	Total OBLR Larvae Recovered	Total OBLR Larvae Dead	Total OBLR Adults	% Mortality	Number of Parasitoids	% Parasitism
Allegan	880	291	576	33.07	13	2.21
Kent	1087	480	607	44.16	0	0.00
Van Buren	823	318	505	38.64	0	0.00
Total	2790	1089	1688	39.03	13	0.76

Table 4.2. Total number of Hymenoptera further broken down into the number of bees, wasps (including the number *B. dimidiator*), number of B. dimidiator, and ants, and the total number of Diptera collected in yellow bucket traps that had been placed into three apple orchards located in three Michigan counties (Allegan, Kent and Van Buren).

	Orchard		
	Allegan	**Kent**	**Van Buren**
Total number of Hymenoptera	814	931	573
Number of Bees	342	80	47
Number of Wasps (includes *B. dimidiator*)	371	192	331
Number of *B. dimidiator*	0	0	3
Number of Ants	101	659	195
Total number of Diptera	1098	656	1233

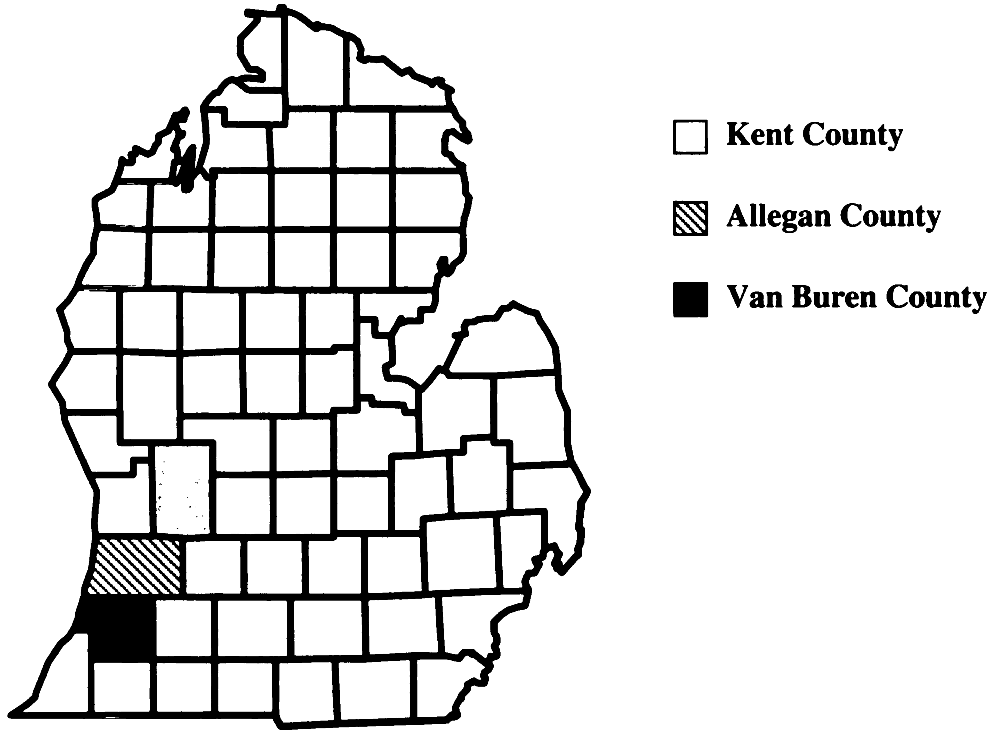

Figure 4.1. Michigan counties where sample orchards were located (n=1/county) in Michigan. Yellow bucket traps and sentinel OBLR larvae were used to identify the time when adult *B. dimidiator* and *M. linearis* are present in commercially sprayed apple orchards. Kent county is located in the "Fruit Ridge" region and Van Buren county is located in the "Southwest" region of the state, two of the major apple producing regions of Michigan.

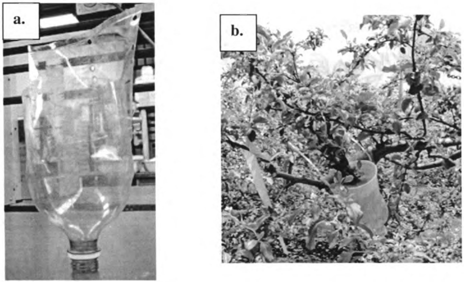

Figure 4.2. A sentinel larvae station consisting of (a.) 2L pop bottle support suspended from an apple branch with plastic cable ties and (b.) a station with cup containing cut apple branches and OBLR larvae.

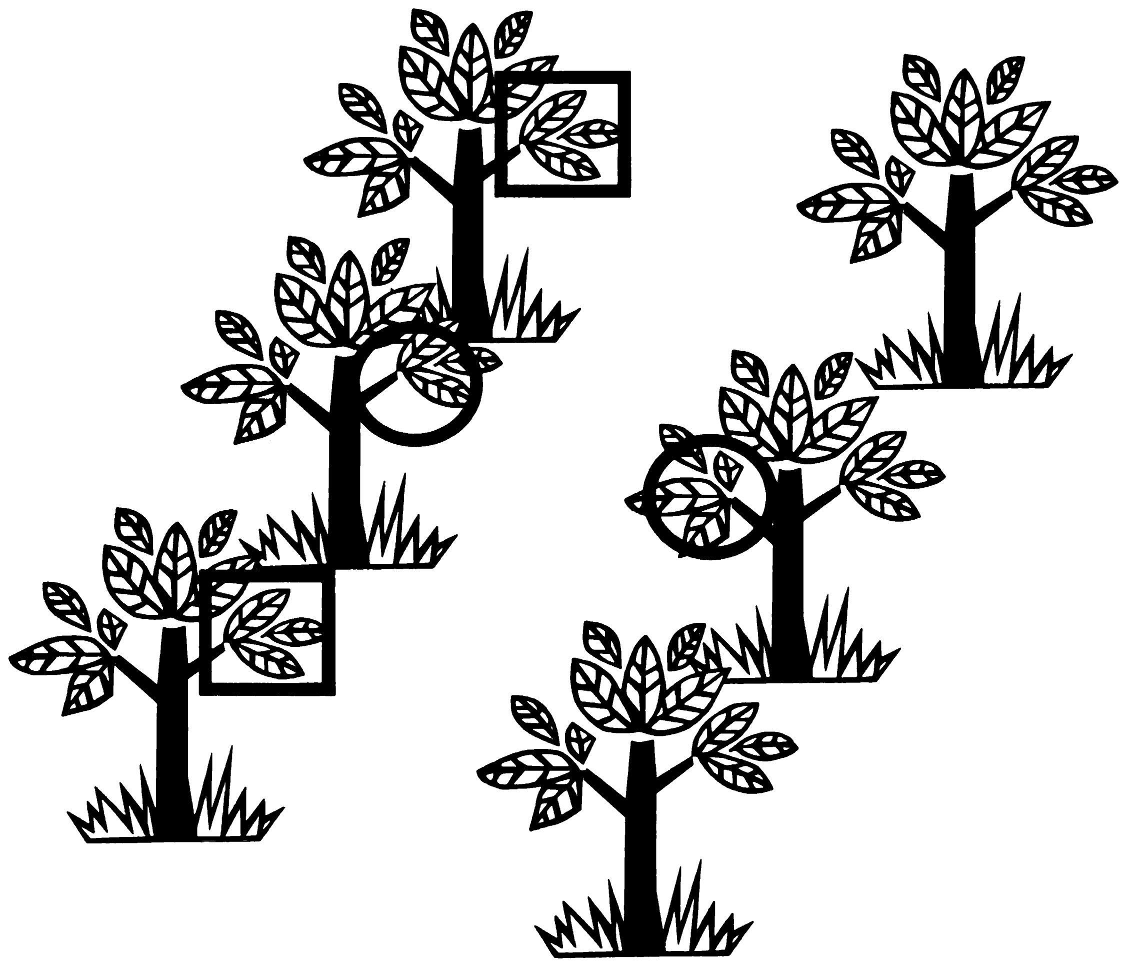

Figure 4.3. Placement of sentinel OBLR larvae stations and yellow bucket traps in apple trees. Sentinel larvae stations (circles) were located in trees opposite of one another in different rows and yellow bucket traps (squares) were located in a single row on either side of a sentinel larvae station.

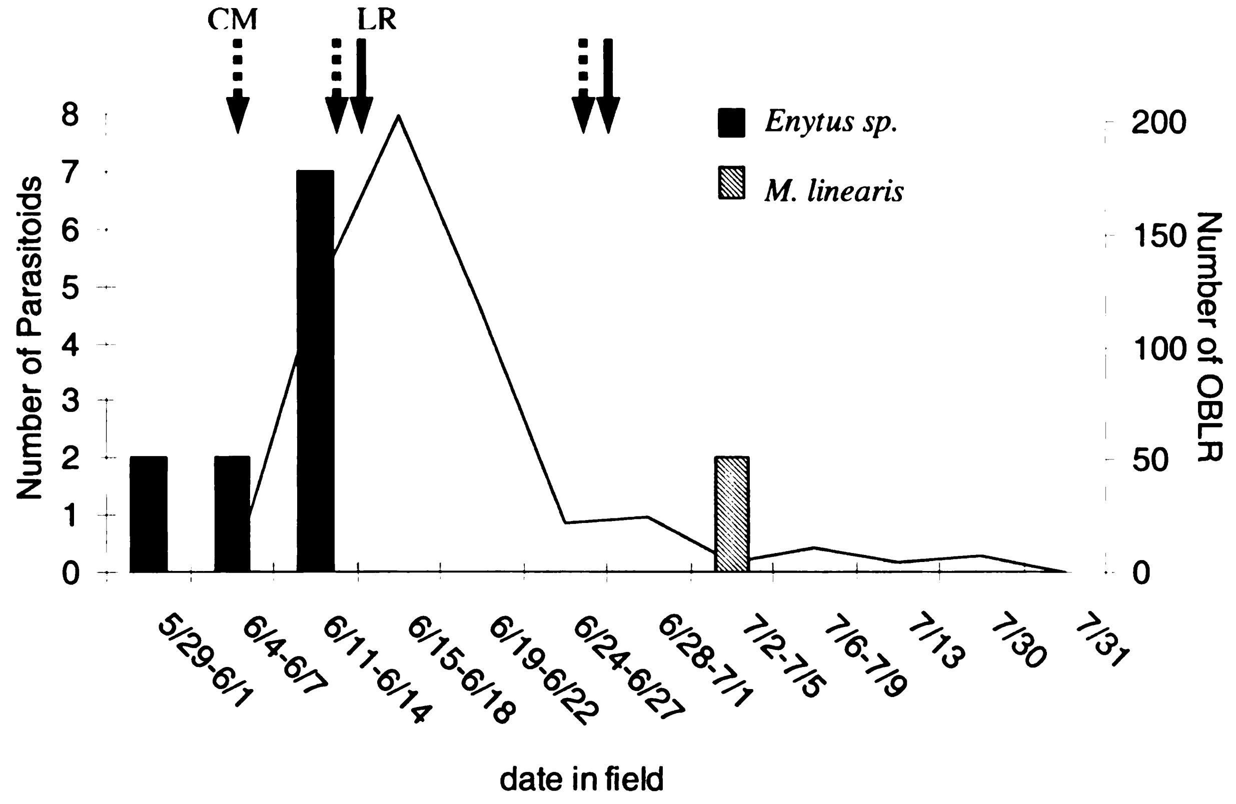

Figure 4.4. The number of *Enytus sp.* and *M. linearis* recovered from sentinel larvae and the dates that the larvae had been placed in the orchard located in Allegan county, Michigan, along with the number of adult OBLR caught in pheromone traps. The timing of codling moth (CM) spray and leafroller (LR) spray applications are indicated by dashed arrow and solid arrows respectively.

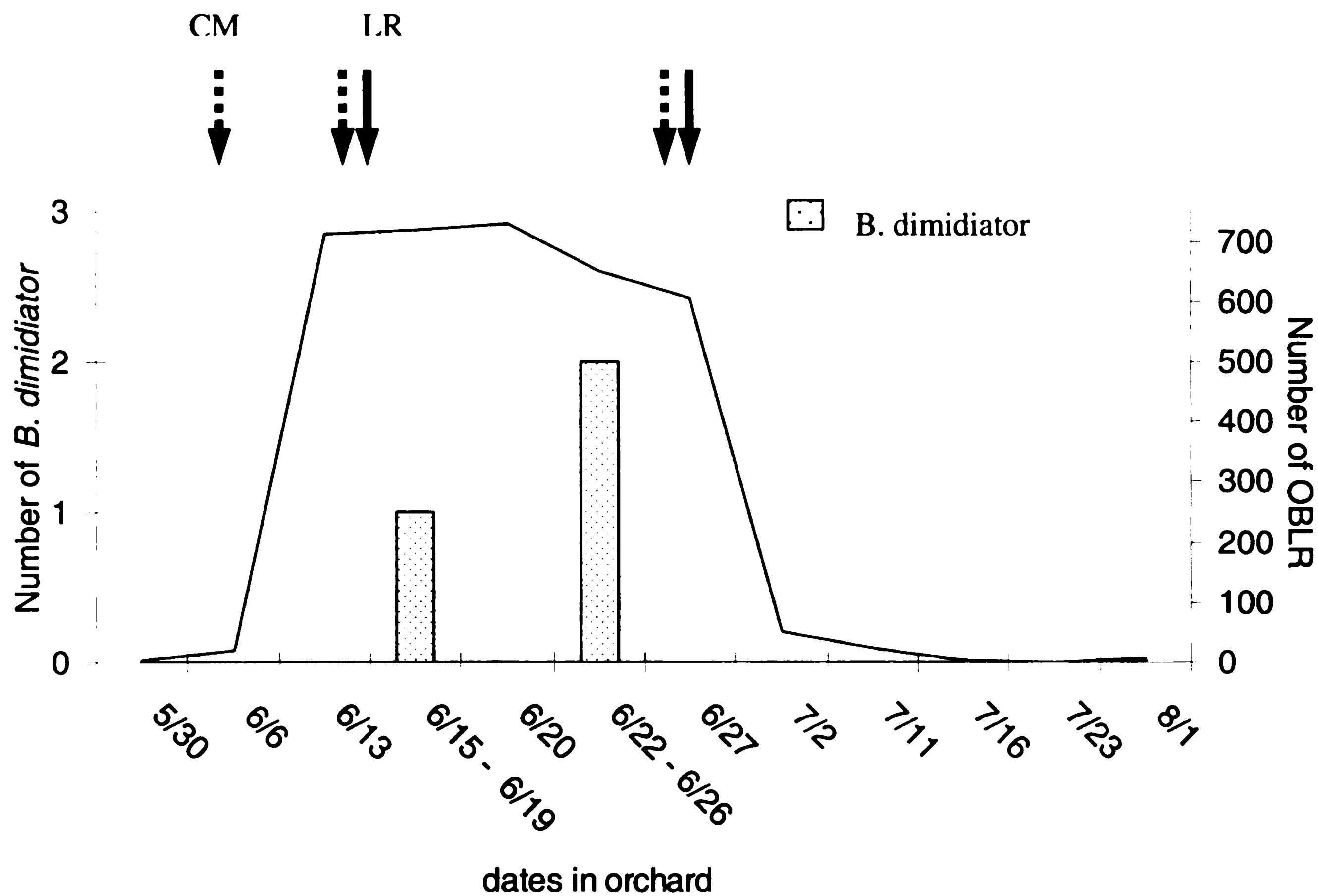

Figure 4.5. The number of *B. dimidiator* caught in yellow bucket traps located in an apple orchard in Van Buren County, Michigan, and the dates that the buckets were in left in the orchard, along with the number of OBLR adults caught in pheromone traps. The timing of codling moth (CM) spray and leafroller (LR) spray applications are indicated by dashed arrow and solid arrows respectively.

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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.: 2002-08

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

Biological Control of Obliquebanded Leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), in Michigan Apple Orchards.

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

Entomology Museum, Michigan State University (MSU)

Other Museums:

USDA-APHIS Niles Plant Protection Center, Niles, Michigan

K. Ahlstrom: NCDA & CS Plant Protection Section, Raleigh, North Carolina

J. O'Hara: ECORC, Systematic Entomology Section, Ontario, Canada

M.J. Sharkey: University of Kentucky, Lexington, Kentucky

M.E. Schauff: Systematic Entomology Laboratory, Washington D.C., Maryland

Investigator's Name(s) (typed)

Tammy K. Wilkinson

Date 11-19-02

*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.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 1.1

Voucher Specimen Data

Page 1 of 7 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Choristonuera rosaceana (Harris)	Michigan, Kalamazoo Co., Niles USDA-APHIS lab Sept. 2001 Lab culture begun at TNRC late summer 1998. 1st instar larvae.		10						MSU
Choristonuera rosaceana (Harris)	Michigan, Kalamazoo Co., Niles USDA-APHIS lab Sept. 2001 Lab culture begun at TNRC late summer 1998. 2nd instar larvae.		10						MSU
Choristonuera rosaceana (Harris)	Michigan, Kalamazoo Co., Niles USDA-APHIS lab Sept. 2001 Lab culture begun at TNRC late summer 1998. 3rd instar larvae.		10						MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Tammy K. Wilkinson

Date 11/19/02

Voucher No. 2002-08

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator 19 NOV 2002 Date

Appendix 1.1

Voucher Specimen Data

Page 2 of 7 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Choristonuera rosaceana (Harris)	Michigan, Kalamazoo Co., Niles USDA-APHIS lab Sept. 2001 Lab culture begun at TNRC late summer 1998. 4th instar larvae.		10						MSU
Choristonuera rosaceana (Harris)	Michigan, Van Buren Co., K. Winkle Farm 2Km S. Hartford 21-IV-00 Fuji Row 4							1	MSU
	Michigan, Berrien Co., Kugel Farm 4Km S. Berrien Springs 17-VIII-00 apple							8	MSU
	Michigan, Kent Co. Beuschel Farm 6Km W. Sparta 16-VIII-00 apple							1	MSU
Macrocentrus linearis (Nees.)	Michigan, Allegan Co., TNRC 3.5Km Fennville 2-VII-01 to 5-VII-01 Green, apple					5			MSU USDA

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Tammy K. Wilkinson

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Appendix 1.1

Voucher Specimen Data

Page 3 of 7 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Macrocentrus linearis (Nees.)	Michigan, Kent Co. Pagel Farm Sparta 11-VIII-99 apple Host: *Choristoneura rosaceana* (Harris)						5		MSU USDA
Bassus dimidiator (Nees.)	Michigan, Berrien Co. Niles 20-VII-01, 20-VI-01 lab colony, Calderwood 2-VI-99, R. Winkle 3-VI-99, 19-VII-99					5	1		MSU USDA
	Michigan, Van Buren Co. K. Winkle Farm 22-VI-01 to 26-VI-01, 15-VI-01 to 19-VI-01 3-VI-99						3		MSU USDA
	Michigan, Kent Co. Kraft Farm 26-VII-00						1		MSU USDA
Itoplectis conquisitor (Say)	Michigan, Berrien Co. Calderwood farm farm 2-VI-99, R. Winkle Farm 17-VIII-00, Kugel 17-VIII-00					9	1		MSU USDA

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

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Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Colpoclypeus florus Walker	Michigan, Ottawa Co. Wells Farm 16-VII-00							1	MSU USDA
	Michigan, Kent Co. Wittenbach Farm 16-VIII-00							2	MSU USDA
	Michigan, Berrien Co. Kugel Farm 17-VIII-00							2	MSU USDA
	Michigan, Ionia Co. Klackel Farm 13-VII-99							5	MSU USDA
Enytus sp.	Michigan, Berrien Co. R. Winkle Farm 3-VI-99 ,3-VIII-00 Calderwood Farm 8-VI-00, 10-VIII-00					4			MSU USDA
	Michigan, Allegan Co. TNRC 11-VI-01 to 14-VI-01, 4-VI-01 to 7-VI-01,					1	4		MSU USDA

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

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Curator Date

Appendix 1.1

Voucher Specimen Data

Page 5 of 7 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Enytus sp.	Michigan, Van Buren Co. K. Winkle Farm 3-VIII-00						1		MSU USDA
Apanteles polychrosidis Viereck	Michigan, Berrien Co. Calderwood Farm 21-V-99							1	MSU USDA
	Michigan, Van Buren Co. Hill Farms 10-VIII-00							1	MSU USDA
Oncophanes americanus (Weed)	Michigan, Ottawa Co. Wells Farm 16-VII-00							5	MSU USDA
Actia Interrupta Curran	Michigan, Kent Co. Beuschel Farm 16-VIII-00 12-VII-00, 21-VI-00, 16-VIII-00, Rasch Farm 12-VII-00, Wittenbach Farm 2-VIII-00							8	MSU USDA
	Michigan, Van Buren Co. k. Winkle Farm 21-VI-00							1	MSU USDA

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

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Appendix 1.1

Voucher Specimen Data

Page 6 of 7 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Actia Interrupta Curran	Michigan, Berrien Co. R. Winkle 17-VIII-00 Watervliet 17-VIII-0 Row5 Jonathon apple							1	MSU USDA
Nilea erecta (Coquillett)	Michigan, Van Buren Co. K. Winkel Farm 3-VI-99, 10-VIII-00							3	MSU USDA
	Michigan, Kent Co. Pagel Farm, 24-VI-99							1	MSU USDA
	Michigan, Berrien Co. Kugel 17-VIII-00 10-VIII-00, R. Winkle Farm 3-VIII-00							6	MSU USDA
Hemisturmia parva (Bigot)	Michigan, Berrien Co. R. Winkel Farm 17-VIII-00 ,3-VI-99 3-VI-99, Calderwood 2-VI-99							4	MSU USDA
	Michigan, Kent Co. Nyblad Farm, 24-VI-99							1	MSU USDA

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

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Appendix 1.1

Voucher Specimen Data

Page 7 of 7 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Hyphantrophaga blanda (Osten Sacken)	Michigan, Kent Co. Beuschel 11-VIII-00							1	ECORC
	Michigan, Berrien Co. R. Winkle 17-VIII-00							1	ECORC
Compsilura concinnata (Meigen)	Michigan, Berrien Co. R. Winkle 3-VI-99							1	ECORC
	Michigan, Van Buren Co. Hill 17-VIII-00							1	ECORC
Phygadueontinae	sent for ID to ECORC								USDA
Eulophinae	sent for ID to ECORC								USDA
Entedoninae	sent for ID to ECORC								USDA
Chalcidinae	sent for ID to ECORC								USDA
Pteromalinae	sent for ID to ECORC								USDA
Eurytominae	sent for ID to ECORC								USDA
Labeninae	specimen lost								USDA
Bethylinae	sent for ID to ECORC								USDA
Ceraphronidae	sent for ID to ECORC								USDA

(Use additional sheets if necessary)

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