XENOTEMNA PALLORANA (LEPIDOPTERA: TORTRICIDAE), A POSSIBLE ALTERNATIVE HOST FOR *COLPOCLYPEUS FLORUS* (HYMENOPTERA: EULOPHIDAE) USING ALFALFA GROUND COVER IN ORCHARD SYSTEMS

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of CHRISTOPHER ANDREW NOBBS find it satisfactory and recommend that it be accepted.

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Abstract

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The leafroller *Xenotemna pallorana* Robinson was reared on apple, cherry, pear, and alfalfa foliage. All of these host plants were suitable, however, development time and pupal weights were in some cases found to be significantly different. Adult females of this species were exposed to apple foliage in a no-choice situation and found to oviposit on the upper portion of the leaves. In a similar experiment, given the choice of apple or ground cover foliage including alfalfa, *X. pallorana* females preferentially selected alfalfa over the others.

Xenotemna pallorana was found to be a suitable host for the parasitoid *Colpoclypeus florus* Walker, when compared to a preferred host *Choristaneura rosaceana* (Harris) in both laboratory and field studies. There was found to be no parasitism preference between *X. pallorana* and *C. rosaceana* larvae by *C. florus* females. In both caged and open studies, *C. florus* preferred apple to ground cover habitats, although parasitism did occur in both. From

these studies, it seems that *X. pallorana* could serve as an alternative host for *C. florus* in orchards without increasing the risk of crop loss. At the very least, *X. pallorana* and an alfalfa cover crop could be used as a model to study the potential of enhancing leafroller biological control in orchards by augmenting populations of an alternative host for a parasite instead of the parasite population.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

CHAPTER 1

CHAPTER 2

LIST OF TABLES

Page

INTRODUCTION

Leafrollers in Tree Fruits

Once considered secondary or minor pests, leafrollers (Lepidoptera: Tortricidae) have become key pests in Washington pome fruit orchards (Brunner 1988, Brunner and Beers 1990, Brunner 1994, Brunner 1996b). This is especially true in orchards that are using pheromone based mating disruption for the control of codling moth (*Cydia pomonella* L.) (Lepidoptera: Tortricidae) (Gut and Brunner 1994a, Gut and Brunner 1994b, Gut et al. 1996, Brunner et al. 1996c). Leafrollers are widespread throughout the Northwest tree fruit growing regions; the larvae roll and skeletonize leaves (Beers et al. 1993, Smirle 1993). However, when populations are high and larvae come in contact with fruit, they can cause serious injury. Overwintering larvae can also cause significant damage (Reissig 1978) at, or just after, bloom by feeding on buds and webbing developing flower parts together.

Leafrollers are recognized as key pests in many parts of the world. In Europe, the summer fruit tortrix, *Adoxophyes orana* (Fischer von Röslerstamm), feeds on all types of pome and stone fruits. This is probably the most widespread and harmful leafroller pest in Europe (Dickler 1991). Another leafroller, *Pandemis heperana* (Denis and Schiffermüller), commonly found in European orchards, was once thought to be only a pest of the Mediterranean areas, but its range now extends as far north as the Netherlands (Dickler 1991). *Pandemis heperana* was first observed on the American continent in 1978 (Matuura 1980) and is now known in western Washington (La Gasa 1995). Many other species of leafroller are reported as pests from Europe but most are of minor importance in commercial fruit orchards (Alford 1984).

The orchard leafroller complex of eastern North America differs from that of the west. Twenty-five years ago, the redbanded leafroller, *Argyrotaenia velutinana* (Walker), was considered the most important tortricine pest of the eastern United States (Weires and Riedl 1991). It is widely distributed throughout the U.S. and Canada. It has been reported as far west as British Columbia, but in the U.S. it has never been reported west of the 100th meridian. Two other leafrollers that have been historically important in the eastern United States are the fruittree leafroller, *Archips argyrospila* (Walker), and the European leafroller, *Archips rosana* (Linnaeus) (Weires and Riedl 1991). In recent years none of these leafroller species have been important in commercial pome fruit orchards, but two other species that have risen to pest, even key pest status are the obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris), and the tufted apple bud moth (TABM), *Platynota idaeusalis* (Walker). The tufted apple budmoth is widely distributed in the northern U.S. and in southern Canada. It is polyphagous; feeding on a variety of ground cover plants (Weires and Riedl 1991). Both OBLR and the TABM have become important because populations have developed tolerance to organophosphate and carbamate insecticides used in orchards (Weires and Riedl 1991).

In Washington State there are two leafroller species that cause significant damage to tree fruits. The obliquebanded leafroller is named for the obliquely directed median band on the forewing of the adult. The larvae of this species are readily identified in the field with their characteristic black head capsules and green bodies. The pandemis leafroller (PLR), *Pandemis pyrusana* Kearfott, looks much like the obliquebanded leafroller in the adult stage but, in contrast, the larva has a green head capsule. The seasonal life histories of both species are similar and fairly synchronous. Within an orchard only one species is usually present.

Leafroller larvae tie leaves together, either singularly or in a group, using silk produced by glands near their mouthparts. These leaf refugia serve as retreats from which larvae feed and in which they develop. Larvae sometimes build retreats by tying leaves to adjacent developing fruit. When a site becomes unsuitable for feeding, they move to another site within the canopy and build a new retreat. This movement can occur several times before the leafroller completes its larval development. Initial feeding sites by larvae up to the third instar are usually along the midrib of the leaf under a protective layer of silk (Chapman and Lienk 1971). Fourth and fifth instars exhibit the typical "rolled" damage we see most often with this group. As with other Lepidoptera, the penultimate immature stage causes the most serious defoliation.

Both OBLR and PLR are polyphagous feeders but prefer hosts in the family Rosaceae (Onstad et al. 1985). They are bivoltine in Washington and overwinter as second or third instars within a close fitting hibernaculum (Beers et al. 1993, Brunner 1991). This hibernaculum is constructed under bark on or around the base of the tree and is largely indistinguishable due to the deposition of fecal material on the silk of the structure (Chapman and Lienk 1971).

OBLR and PLR eggs are typically yellow to green and are deposited on the upper surfaces of leaves. Eggs overlap one another and are deposited in masses ranging from 50 to 200 (Chapman and Lienk 1971, Beers et al. 1993). Just before eclosion, the head capsules of both leafroller species melanize and can be seen through the shell of the egg chorion. This gives an overall dark color to the egg mass just before hatching.

Leafroller management in Washington orchards has become more difficult as conventional methods begin to fail due to pesticide resistance (Brunner 1994, 1996b) and as new methods, e.g. softer insecticides and mating disruption, are implemented for control of

other pests. Several tactics for leafroller management are being developed, including microbial insecticides, mating disruption, attract and kill, and biological controls (Brunner, personal communication). Softer programs do allow for more predators and parasites to inhabit orchards and may help reduce the overall number of leafrollers and other pests. These biological control agents include many species of parasitic wasps, flies, and other generalized predators that may not otherwise be found in orchards where conventional methods, i.e. broad-spectrum insecticides, are the dominant tactic employed.

Colpoclypeus florus Walker

Colpoclypeus florus Walker is a gregarious ectoparasitic eulophid that is known to attack over 30 species of tortricid larvae in Europe (Dijkstra 1986). It has successfully suppressed populations of leafrollers on both apple and strawberries to an economically acceptable level (Gruys and Vaal 1984). *Colpoclypeus florus* was first discovered in Washington State in 1992 when it parasitized approximately 80 percent of the leafrollers in an unsprayed apple orchard (Brunner 1996a). This parasitoid attacks third to fifth instar leafroller larvae but prefers fourth and fifth instars (Gruys and Vaal 1984). The wasp uses olfactory cues produced by the host's silk to locate them (van Veen and Wijk 1987). When *C. florus* finds a host of suitable size it stings the larva several times in or near the head capsule. The venom produced by this wasp does not paralyze the host but instead elicits a behavioral response that causes the host to tightly enclose itself in silk within its retreat and also arrests development (van Veen and Wijk 1987). The wasp then lays eggs in and around the silk of the host.

Female wasps spend a long time (2 to 26 hr) within the host's retreat and can vary the number of eggs laid per retreat according to host size (van Veen and Wijk 1987). Therefore, *C. florus* females probably parasitize only one or two hosts during their lifetime. Clutch size has been observed to be as low as one and as high as 100. This high number, however, may be due to superparasitism (Dijkstra 1986). Clutches tend to be protandic (males emerge first), and spanandrous (female biased), with the average number of males being 2 to 3. Host larvae that are stung, but where no *C. florus* develop, die as larval/pupal intermediates. *Colpoclypeus florus* is arrhenotokous, meaning that fertilized eggs produce females, while unfertilized eggs produce males. Females control the number and order of fertile and infertile eggs, and Dijkstra (1986) notes that males are usually produced toward the end of egg deposition.

Colpoclypeus florus is susceptible to both temperature extremes and pesticides (Brunner, personal communication). It has been shown that *C. florus* fail to pupate after being sprayed with fenoxycarb (de Reede et al. 1984). Insect growth regulators, however, seem to have little or no effect on *C. florus* populations (de Reede et al. 1984). Certain areas of The Netherlands have recorded up to 90% parasitism of leafrollers in the field during the months of July and August (van Veen and Wijk 1987), while laboratory studies averaged 85% parasitism.

In the Netherlands, *C. florus* has four to five generations per year (Gruys and Vaal 1984). Wasps are often difficult to find in spring, while populations can be large in late summer and early fall. This is due to the lack of synchrony between the life cycles of *C. florus* and the leafrollers in orchard systems (van Veen and Wijk 1987). In Europe, there has been difficulty finding alternative leafroller hosts to help maintain summer populations and provide overwintering hosts (Evenhuis and Vlug 1983). The same is true in Washington (Brunner

1996a). Oblique-banded and pandemis leafrollers overwinter in stages that do not support *C. florus* which overwinters as a mature larva within the silken retreat of the host (Gruys and Vaal 1984). This separation in generational sequence does suggest, however, that this parasitoid leaves the orchard in the fall and overwinters on some unknown hosts (van Veen and Wijk 1987). This has led to an investigation for alternative hosts of *C. florus* that could provide both a stable supply of hosts for spring and summer generations and hosts in the appropriate stages for overwintering.

Alternative Hosts

Alternative hosts are important in the life cycles of many generalized and specialized predators. Coccinellids often prey upon different hosts when preferred hosts are either not present or in small numbers. Alternative hosts sustain predatory mites in orchard systems during many occasions throughout the growing season (Beers et al.1993). The same is true for specialized predators and parasites such as wasps, although host interactions may be more difficult to observe.

Pavuk and Stinner (1991) found that lepidopterous larvae on broadleaf weeds in corn plantings may serve as alternative hosts for parasitoids that usually attack the European corn borer, *Ostrinia nubilalis* Hübner. A more classic example is that of *Anagrus epos* Girault, a mymarid parasitoid that attacks the egg of the grape leafhopper, *Erythroneura elagantula* Osborn (McKenzie and Beirne 1972). This parasitoid overwinters in the eggs of other leafhoppers because the grape leafhopper overwinters as an adult. It was found that in the Okanagan Valley of British Columbia *A. epos* moved to *Edwardsiana rosae* (Linnaeus), a

leafhopper associated with wild rose (McKenzie and Beirne 1972). In California, *A. epos* moves to the alternative host *Dikrella cruentata* Gillette on wild blackberry, *Rubus ursinus*. The wasp was also observed exploiting the eggs of the leafhopper *Edwardsiana prunicola* Edwards, on French prune trees (Wilson et al. 1989). Spring densities of *A. epos* were directly proportional to the proximity of these alternative hosts to vineyards (McKenzie and Beirne 1972).

Alternative hosts in association with *C. florus* have not been observed in the orchard system. The activity of *C. florus* in some orchards and not others suggests that it may be moving to an alternative host outside the orchard to sustain summer populations and to successfully overwinter. There has been an investigation into finding a suitable alternative leafroller species that could be introduced into the orchard ecosystem via ground cover management because, the search for alternative hosts near orchards has not been successful.

Ground cover manipulation can result in enhanced biological control of specific pests in orchards and vineyards (Altieri and Schmidt 1985). Mixed plantings can attract different insect groups, both beneficial and detrimental. Ground cover must be specifically evaluated for different crops and environmental conditions. Legumes have benefited orchard systems by increasing beneficial arthropod densities and by aiding in the supply of nitrogen to the soil (Smith et al. 1995). For these reasons, alfalfa was chosen as a potential host plant in which to search for leafrollers species that might provide an alternative host for *C. florus*.

Xenotemna pallorana Robinson

A leafroller that showed potential as an alternative host for *C. florus* was *Xenotemna pallorana* Robinson, a native leafroller species that typically feeds upon herbaceous perennials (Chapman and Lienk 1971). In the literature, *X. pallorana* was originally described as *Tortrix pallorana* and then changed to *Amelia (Tortrix) pallorana*. Chapman and Lienk (1971) refer to it as *Clepsis pallorana,* while later references call it *Xenotemna pallorana* (Hodges et al. 1983). The larva of *X. pallorana* is bright green with a similarly colored head. Adults are nondescript and straw colored. As with other leafrollers, they tie together terminal ends of host plants, build retreats, and feed within them.

Chapman and Lienk (1971) reported that the primary hosts of *X. pallorana* are alfalfa, *Medicago sative* L., and white sweet clover, *Melilotus alba* Desr.. Its original host plant is most likely a native legume, since neither alfalfa nor white sweet clover are endemic to North America. *Xenotemna pallorana* is recorded as a pest of rose (Schott 1925), lucerne (Anon. 1928), strawberries (Smith 1941), birdsfoot trefoil (Neunzig and Gyrisco 1955), seed alfalfa (Snow and McClellan 1951), and white pine (McDaniel 1936). In strawberries it did not cause significant damage, but it did mislead growers in timing of spraying for the strawberry leafroller, *Ancylis comptana* Frolich, due to misidentification (Smith 1941). In some white pine nurseries in Michigan, however, it has been known to damage up to 95% of all new growth (McDaniel 1936).

Xenotemna pallorana is widely distributed throughout North America. It has been observed as far north as Alaska and as far south as Texas (Chapman and Lienk 1971). According to Bennett (1961), the distribution is from Massachusetts to Illinois, Missouri and

Texas. In Washington, it is commonly found in alfalfa fields of the Yakima Valley and Columbia Basin (Newcomer and Carlson 1952).

This leafroller has been collected on apple, pear, and cherry, although it has not been reported as a pest, except on young trees where it was reported as a partial defoliator (Newcomer and Carlson 1952). Tree fruit crops are most likely secondary or incidental hosts used by *X. pallorana* to complete its life cycle when primary host plants are not present (Chapman and Lienk 1971). On these occasions, retreats are usually found on low growth adjacent to ground cover.

The life cycle of *X. pallorana* is similar to that of the OBLR and PLR, but Chapman and Lienk (1971) suggest that *X. pallorana* overwinters at a more advanced larval stage. The pupal stage generally lasts much longer than reports for other leafroller species, which suggests less likelihood of generation overlap with orchard leafroller pests. Lastly, oviposition is generally near the ground and essentially limited to primary host plants. These factors may make *X. pallorana* a potentially suitable alternative host for *C. florus* in orchards using alfalfa as ground cover.

This paper documents studies conducted to determine if *X. pallorana* is a suitable host for *C. florus* and how it compares as a host to other leafrollers found in the orchard ecosystem. The development time, number of progeny, and sex ratio of *C. florus* reared on *X. pallorana* were determined at constant temperature and compared with results from rearing on OBLR. The concern over *X. pallorana's* utilization of tree fruit plants as a host was investigated through a series of experiments where its development on apple, pear, and cherry was compared with alfalfa. In addition, oviposition behavior was investigated in choice and no-

choice tests, where adults were caged with host plant material. The behavior of *X. pallorana* in the field and its potential as a host for *C. florus* were investigated using caged and open small plot experiments.

CHAPTER ONE

THE BIOLOGY OF *XENOTEMNA PALLORANA* (LEPIDOPTERA: TORTRICIDAE) ON ORCHARD CROPS

INTRODUCTION

Xenotemna pallorana is a common leafroller (Lepidoptera: Tortricidae) found in alfalfa fields of Washington's Columbia Basin and Yakima Valley (Newcomer and Carlson 1952). This leafroller is native to North America, which means that its native host must have been some herbaceous legume other than alfalfa, *Medicago sative* L., and white sweet clover, *Melilotus alba* Desr. on which it is primarily found now because both of these plants were imported as forage crops for range animals. *Xenotemna pallorana* is bivoltine and has been reported to be a northern ranging species (Chapman and Lienk 1971). The larvae of *X. pallorana* have been reported as a pest of rose and birdsfoot trefoil in New York (Schott 1925; Neunzig and Gyrisco 1955), young white pine stands in Michigan (McDaniel 1936), strawberries in Ohio (Neiswander 1944), and seed alfalfa in Utah (Snow and McClellan 1951).

Like other leafrollers, *X. pallorana* larvae build retreats by rolling leaves together using silk produced by organs near their mouthparts. Larvae feed and develop within retreats that also serve to protect them from weather and natural enemies. In crops such as alfalfa, larvae can prevent pollination by rolling the leaves and racemes of developing foliage (Snow and McClellan 1951).

Females deposit overlapping egg masses on the tops of host leaves. Masses may contain only a few eggs, but usually have between 46 to 61 eggs (Snow and McClellan 1951). Egg masses are yellow in color but darken as larvae mature within them.

Shortly after eclosion, neonates find a place along the midrib of a leaf and hide themselves under a protective layer of silk. Larvae feed and develop in this manner until after the third stadium (Chapman and Lienk 1971). Fourth instars begin to display the typical

"rolling" behavior seen by most larvae in this group. Larvae of *X. pallorana* have a green body, head capsule, and thoracic shield. Adults are straw colored, lacking distinguishable marking on the wings, making them easy to tell apart from other common leafrollers found in orchards.

Xenotemna pallorana has been found to feed on apple leaves when native host foliage is depleted (Chapman and Lienk 1971), a condition which usually occurred on young trees where apple foliage was near the ground. Most observations of the occurrence of this species in Washington orchards have been in situations where the foliage of fruit trees has been used for pupation sites (Brunner personal communication). There have been no studies to determine if foliage of fruit trees is suitable for larval development. While the literature suggests that fruit crops do not normally serve as oviposition sites, there have been no definitive studies to prove this contention.

MATERIALS AND METHODS

Development Experiments

A laboratory colony of *X. pallorana* was initiated from individuals collected from alfalfa in the Columbia Basin in June, 1996, near Quincy Washington. The source of newly eclosed *X. pallorana* neonates was a laboratory colony reared on a modified pinto bean-based diet for noctuids (Shorey and Hale 1965) (24 ± 2°C, 16:8 (L:D) photoperiod). In the first experiment, 40 newly eclosed *X. pallorana* larvae were placed individually into a petri dishes (Falcon 5009, 50 x 9 mm) containing a disk of apple, pear or cherry foliage or a sprig of alfalfa. Fruit foliage was obtained from unsprayed orchards at the Washington State University Tree Fruit Research and Extension Center (WSU-TFREC), Wenatchee. Petri dishes were placed in a controlled

environment growth room at 24 ± 2°C, 50% RH, and 16:8 (L:D) photoperiod. Larval development was examined daily and stage changes and mortality were recorded. Food was changed as needed, when it had been consumed, or if mold was observed.

In a second experiment, *X. pallorana* was reared on excised plant material in order to reflect more natural conditions of the plant. Sections of growing shoots or stems were collected in the field from unsprayed apple, pear, or cherry orchards and from alfalfa. These shoots/stems were inserted immediately into common water pix ("stickpic" model 55-37) used by florists to keep flowers fresh. The water pix was inserted into a 946 ml Styrofoam container and covered with a clear 473 ml plastic cup (Fig. 1.1). Foliage in each container was checked daily and replaced when necessitated by feeding or deterioration of quality. Forty neonate *X. pallorana* larvae were introduced individually onto the foliage in each container then followed for development. The containers were kept in a controlled environment growth room 24 ± 2°C with a relative humidity of 70% and a 16:8 (L:D) photoperiod. Larvae were observed daily and the approximate stage (size) and mortality were recorded. Developmental times from hatch to pupation and pupation to adult eclosions were recorded. Pupating larvae were set aside and weighed within 24 hrs of pupal formation but after they had completed melanization. Sex of newly emerged adults was recorded. Sex of newly emerged adults was recorded. Developmental time and pupal weight for larvae reared on each host plant was analyzed using standard analysis of variance (ANOVA) (SuperANOVA accessible general linear model program; (Abacus Concepts, Berkeley, CA). Mean separation was done with the Fisher protected least significant difference (LSD) (P = 0.05).

Oviposition Experiments: No-Choice Test

This experiment was conducted to determine whether *X. pallorana* would oviposit on apple foliage in a no-choice situation. Twelve sleeve cages made of cloth and wire screen, approximately 98 cm long and 56 cm in diameter were placed over developing apple foliage in the field. Two newly emerged male and female *X. pallorana* adults obtained from a laboratory colony reared on a modified pinto bean diet for noctuids (Shorey and Hale 1965) were released into each cage. After a period of seven days, each cage was removed and all egg masses were collected and their location recorded.

Oviposition Experiments: Choice Test

To determine if *X. pallorana* females would discriminate between a fruit tree host and their "normal" host, experiments were conducted that provided moths with a choice of oviposition site. Nylon organdy mesh cages with approximate dimensions of 1.22 m x 1.22 m x 1.22 m were suspended from a frame of plastic irrigation pipe (PVC, 2 cm) and placed over small potted apple trees in an area of orchard where an already established alfalfa plant was located. Five newly emerged male and female *X. pallorana*, from a laboratory colony were released into each cage and allowed to mate and oviposit for seven days. Ten cages were set-up. Seven days after the moths were released, cages were removed and egg masses were collected from foliage within each cage. The number of egg masses and type of foliage each egg mass was deposited on was recorded. The average number of egg masses deposited on each host was analyzed using a t-test for means.

RESULTS

Developmental Experiments

In the two experiments examining developmental rates on different host plants, males and females were analyzed separately due to differences in size and developmental rates. In the first experiment, where leaf disks and petri dishes were used, male larval developmental times were significantly longer ($P < 0.05$) for larvae reared on apple, pear, and cherry compared to those reared on alfalfa (Table 1.1). Male pupal developmental times were not significantly different ($P > 0.05$) for larvae reared on apple, pear, and cherry than those reared on alfalfa. Male pupae reared on cherry and apple weighed significantly more, while those reared on pear weighed significantly less than those reared on alfalfa (Table 1.2).

Female developmental rates and pupal weights followed the same general trend as males. Female larval developmental times tended to be longer in those reared on apple, pear, and cherry compared to those reared on alfalfa; however, only for larvae reared on cherry were the differences significant at $P < 0.05$ (Table 1.1). Pupal developmental time for females tended to be longer for larvae reared on apple, pear, and cherry than those reared on alfalfa, but the differences were not statistically significant. All female pupal weights were significantly higher for those reared on apple, pear, and cherry than those reared on alfalfa (Table 1.2).

In experiment two (Table 1.3) where excised shoots of host plants were used, data were analyzed the same as in experiment one. Male larval developmental time was not significantly different for those reared on apple, cherry, and pear when compared to those reared on alfalfa.

Pupal weight for males was significantly higher for those reared on apple, pear, and cherry than those reared on alfalfa (Table 1.2).

Female developmental time and pupal weight data followed the same general trend as male data. There were no significant differences in female larval developmental period for individuals feeding on any of the foliage types (Table 1.3). Pupal developmental time was similar for those reared on apple, cherry, pear, and alfalfa. Female pupal weight was found to be significantly higher for those reared on apple, cherry, and pear compared to those reared on alfalfa (Table 1.2).

Oviposition Experiments: No-Choice Test

Xenotemna pallorana was found to oviposit on apple foliage when given no choice. Egg masses were found on apple foliage in eight out of 12 sleeve cages, with an average of 2.08 egg masses per cage. Egg masses were only found on the upper surface of apple leaves.

Oviposition Experiments: Choice Test

A total of 51 egg masses were collected from the 12 cages, with an average of 4.25 egg masses per cage (Table 1.4). Thirty-eight, or 74.5%, of the egg masses were found on alfalfa, while 12, or 23.5%, were found on other ground cover plants, and only one egg mass was collected from apple foliage, or 1.96% of the total egg masses collected. The number of egg masses collected on both apple and other ground cover plants was significantly lower ($P < 0.05$) than the number collected on alfalfa (Table 1.4). The number of egg masses collected on other

ground cover plants was found to be significantly higher than those found on apple ($P < 0.05$) (Table 1.4).

DISCUSSION

Xenotemna pallorana is a polyphagous tortricid moth that has been found to use apple foliage for both pupation sites and feeding when ground cover is depleted (Chapman and Lienk 1971). This moth prefers to oviposit on its primary hosts of either alfalfa or white sweet clover (Chapman and Lienk 1971). My studies confirm these two statements and offer data to substantiate them.

Regardless how host plant material was offered to *X. pallorana,* either leaf disks or whole shoots/stems, it was able to complete its life cycle on apple, cherry, and pear. When host plant material was offered in petri dishes as leaf disks, data on development and pupal weight tended to be less consistent than when host plant material was offered as excised shoots/stems. This was most likely due to the quality of plant material. Foliage broke down much faster in the petri dish environment. The second experiment exhibited a more natural scenario.

Larvae development was the same as, or tended to take slightly longer, on apple, cherry, and pear compared to alfalfa. The single exception was noted on pear where *X. pallorana* larval development was shorter than on alfalfa and the other fruit tree hosts. Development of pupae was very consistent, ranging between 9.9 and 11.9 days, and while there were some significant differences in pupal duration noted there did not appear to be any consistent relationship between pupal duration, pupal weight, larval duration, or plant diet.

Pupal weights were consistently higher for larvae reared on apple, cherry, and pear compared to those reared on alfalfa. Using both developmental time and pupal weight as measures of host plant suitability, it must be concluded that apple, pear, and cherry are suitable hosts for *X. pallorana.* It was able to complete its life cycle on the foliage of all plants, although development generally took longer and pupal weight was greater than when alfalfa was the host plant. Higher mortality was recorded for larvae reared on alfalfa than the other host plants. This was most likely due to the physiology of alfalfa leaves, which are more susceptible to mold and breakdown than apple, cherry, or pear in the arenas used in these studies.

Offered no choice, *X. pallorana* was shown to oviposit on apple foliage. This was not unexpected since in our colony these moths oviposit on wax paper and have been observed to lay on other smooth material such as glass or plastic. What this experiment did show was that *X. pallorana* mimics similar behavior on apple as on its natural hosts by laying egg masses on the upper surface of leaves.

When provided a choice, *X. pallorana* strongly preferred to lay eggs on alfalfa rather than other groundcover plants and almost totally ignored apple. Ground cover plants on which egg masses were found included blades of grass, family Poaceae; dandelion, *Taraxacum officinale* Weber in Wiggers; and field bindweed, *Convolvulus arvensis* L. The single egg mass found on apple may have been the result of the location of foliage. The small potted apple trees used in the experiment had a few branches near to or even touching the cover crop. It was in this situation that the egg mass of *X. pallorana* was found on apple. It can therefore be assumed that it is not the physical properties of the plant that determines oviposition

preference but a behavioral response by female *X. pallorana* to habitat. This is the most likely explanation of why *X. pallorana*, while occurring in fruit growing areas of Washington, is not found feeding on apple or other fruit trees and why it is rarely collected in orchards.

In conclusion, *X. pallorana* can complete development on apple, cherry, and pear foliage as well as it can on alfalfa, although developmental time is longer in most cases. There is no aversion of females to oviposition on apple foliage, but under natural conditions it seems clear that they preferentially select ground cover habitats as sites to search for host plants. It may well be that *X. pallorana* does not need to deposit eggs exclusively on hosts such as alfalfa as long as suitable hosts are nearby. Larvae are likely capable of searching out hosts to some extent following hatch. It is also likely that *X. pallorana* has a relatively broad host range.

The potential to use *X. pallorana* as an alternate host for leafroller parasites by culturing it and its host plants, specifically alfalfa, in the orchard cover crop seems plausible given the results of these experiments. Certainly it would be preferred that an alternate host for leafroller parasites would have no attraction to fruit trees as host plants. However, the *X. pallorana*-alfalfa combination offers a low risk model for the orchard environment to test the hypothesis that cover crop management could be used to enhance leafroller biological control. The question remained if *X. pallorana* was a suitable host for leafroller parasites. This was addressed in a second set of experiments reported in Chapter 2.

Figure 1.1. Chamber used in second development experiment.

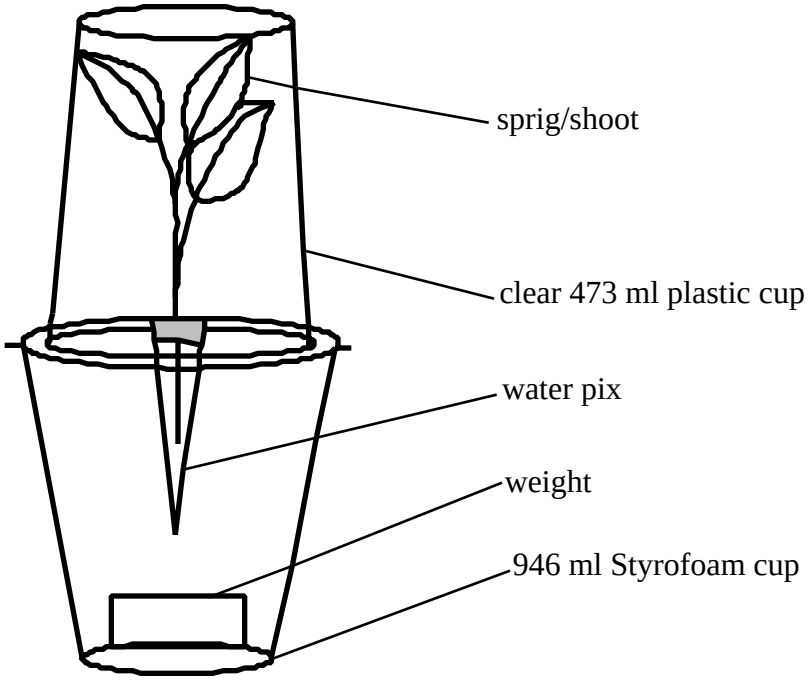

Table 1.1. Development of *X. pallorana* reared on different hosts in Petri dishes.

Host	Sex	n	Larval developmental time (days)	Pupal developmental time (days)
Cherry	Male	13	35.6a	11.9a
Pear	Male	21	32.0b	11.8a
Apple	Male	11	34.4ab	11.9a
Alfalfa	Male	14	25.8c	11.9a
Cherry	Female	13	40.8a	11.1a
Pear	Female	8	36.5b	10.9a
Apple	Female	19	35.8bc	10.8a
Alfalfa	Female	9	33.1bc	10.2a

Means within a column followed by the same letter are not significantly different ($P > 0.05$) Fisher's protected LSD test.

Table 1.2. Pupal weight of *X. pallorana* reared on different hosts.

Host	Sex	n	Pupal weight (g) in petri dish	n	Pupal Weight (g) on Excised Shoot
Cherry	Male	13	0.055b	19	0.062b
Pear	Male	22	0.038d	12	0.054c
Apple	Male	11	0.073a	12	0.074a
Alfalfa	Male	14	0.047c	4	0.038d
Cherry	Female	13	0.058b	11	0.083b
Pear	Female	7	0.044c	16	0.062c
Apple	Female	19	0.094a	11	0.102a
Alfalfa	Female	9	0.042c	9	0.045d

Means within a column followed by the same letter are not significantly different ($P > 0.05$) Fisher's protected LSD test.

Table 1.3. Development of *X. pallorana* reared on different hosts using excised shoots.

Host	Sex	n	Larval developmental time (days)	Pupal developmental time (days)
Cherry	Male	19	29.9a	11.8a
Pear	Male	12	24.5c	10.5b
Apple	Male	12	27.3b	11.8a
Alfalfa	Male	4	27.8abc	11.2ab
Cherry	Female	11	32.8a	10.6a
Pear	Female	16	28.9a	9.9a
Apple	Female	31	31.0a	10.5a
Alfalfa	Female	9	32.6a	10.7a

Means within a column followed by the same letter are not significantly different ($P > 0.05$) Fisher's protected LSD test.

Table 1.4. Oviposition choice test.

Foliage type	Total # collected	Percent of total	Average # per cage
Apple	1	2.0	0.08c
Other	12	23.5	1.00b
Alfalfa	38	74.5	3.17a

Means within a column followed by the same letter are not significantly different (P > 0.05) chi square test of independence.

CHAPTER TWO

THE POTENTIAL OF *XENOTEMNA PALLORANA* (LEPIDOPTERA: TORTRICIDAE), A LEAFROLLER FOUND IN ALFALFA, TO ACT AS AN ALTERNATIVE HOST OF *COLPOCLYPEUS FLORUS* (HYMENOPTERA: EULOPHIDAE) IN ORCHARDS

INTRODUCTION

Colpoclypeus florus is a gregarious eulophid ectoparasitoid that in Europe has been known to attack over 30 species of leafrollers in the family Tortricidae (Gruys and Vaal 1984). It was first reported in Washington State in 1992 when it parasitized over 80% of leafroller larvae in an unsprayed orchard (Brunner 1996a). In The Netherlands, *C. florus* has been found to parasitize up to 90% of the leafrollers in orchards during July and August (van Veen and Wijk 1987). Various methods have been employed to establish and sustain *C. florus* in orchard situations as a means for biological control of leafrollers.

This wasp locates a host by olfactory cues emitted from the silk of the host's retreat. Once a suitable host has been located, the female stings the leafroller in the head capsule. The number of stings *C. florus* inflicts is relative to the weight of the host (van Veen and Wijk 1987). The main effect of the venom is not to paralyze the host but to arrest its development and elicit a behavioral response, which causes the host to enclose itself tightly with silk in its retreat. Female wasps generally stay with the host for 2 to 26 hours (van Veen and Wijk 1987). This is also dependent upon the size of the host. Eggs are deposited on the silk near the host. *Colpoclypeus florus* females can control clutch size, but the number of males within each tends to be fairly consistent at two to three per clutch (van Veen and Wijk 1987). They are arrhenotokous, which means that fertilized eggs produce females, while unfertilized eggs produce males. Females can parasitize more than one host (Gruys and Vaal 1984) but generally do not parasitize many due to the length of time spent with each.

Shortly after emergence from the egg, *C. florus* larvae locate the host and begin feeding on the outside of the host's body. Up to 50 or more wasps can be produced from a single host

(Brunner 1996a, Dijkstra 1986). Adult males emerge first within the host's retreat and mate with the females as soon as they emerge. *Colpoclypeus florus* is very susceptible to both temperature extremes and pesticides (Brunner personal communication). It has been shown that *C. florus* fail to pupate after being directly sprayed with fenoxycarb (de Reede et al. 1984). Insect growth regulators, however, seem to have little or no effect on *C. florus* populations (de Reede et al. 1984).

In Washington State there are two leafroller species that cause significant damage to tree fruits. The obliquebanded leafroller (OBLR), *C. rosaceana* is named for the obliquely directed median band on the forewing of the adult. The larvae of this species are readily identified in the field with their characteristic black head capsules and green bodies. The pandemis leafroller (PLR), *P. pyrusana,* looks much like the obliquebanded leafroller in the adult stage but, in contrast, the larva has a green head capsule. The seasonal life histories of both species are similar and fairly synchronous. Within an orchard only one species or the other is usually found.

Leafroller larvae tie leaves together, either singularly or in a group, using silk produced by glands near their mouthparts. These leaf refugia serve as retreats from which larvae feed and develop. Larvae sometimes build retreats by tying leaves to adjacent developing fruit. When a site becomes unsuitable for feeding, they move to another site within the canopy and build a new retreat. This movement can occur several times before the leafroller completes its larval development. Initial feeding sites by larvae up to the third instar are usually along the midrib of the leaf under a protective layer of silk (Chapman and Lienk 1971). Fourth and fifth instars

exhibit the typical “rolled” damage we see most often with this group. As with other Lepidoptera, the penultimate immature stage causes the most serious defoliation.

OBLR and PLR overwinter in stages that evidently do not support *C. florus*, which overwinters as a mature larva within the silken retreat of the host (Gruys and Vaal 1984). This separation in generational sequence does, however, suggest that *C. florus* leaves the orchard in the fall and overwinters on some unknown hosts (van Veen et al. 1986). This has led to an investigation for alternative hosts of *C. florus* that could provide both a stable supply of hosts for spring and summer generations and hosts in the appropriate stages for overwintering.

One well-known success story in alternative host implementation is that of the mymarid egg parasite, *Anagrus epos* Girault. The grape leafhopper, *Erythroneura elegantula* Osborn, was a serious pest to vineyards of the Okanagan Valley of British Columbia (McKenzie and Beirne 1972). This pest was found to overwinter at an unsuitable stage for *A. epos* to successfully establish itself in vineyards. Like *C. florus* in the orchard, *A. epos* was assumed to overwinter outside of the crop system. It was found that *A. epos* in particular areas overwintered on the eggs of *Edwardsiana rosae* (Linnaeus), the rose leafhopper. The proximity of rose plants to the vineyard was directly proportional to the success rate of parasitism of *A. epos* on the grape leafhopper in the spring and summer (McKenzie and Beirne 1972). The same scenario was observed with *A. epos* in California using the eggs of *Dikrella cruentata* Gillette on wild blackberry (McKenzie and Beirne 1972) and the prune leafhopper, *Edwardsiana prunicola* Edwards on French prune trees (Wilson et al. 1989).

Altieri and Schmidt (1985) reported that manipulation of cover crops could result in enhanced biological control of specific pests in orchards and vineyards. Legumes have

specifically been evaluated, because they can offer advantages by increasing beneficial arthropod densities and also aid in supplying nitrogen to the soil (Smith et al. 1995). *Xenotemna pallorana* Robinson is a leafroller that is commonly found in alfalfa fields of the Columbia Basin and Yakima Valley in Washington State (Newcomer and Carlson 1952). This leafroller is native to North America and its preferred hosts are alfalfa, *Medicago sative* L., and white sweet clover, *Melilotus alba* Desr. *Xenotemna pallorana* has been found in orchards but has never been a serious pest of fruit, except where they partially defoliated young trees when ground cover was depleted (Newcomer and Carlson 1952).

This leafroller is nondescript and straw colored as an adult. The larvae have green bodies and head capsules. They are easily found in the field due to the characteristic rolling of racemes and leaves on the terminal end of host plants. Its original host plant is most likely a native legume because neither alfalfa nor white sweet clover is endemic to North America. *Xenotemna pallorana* is recorded as a pest of rose (Schott 1925), lucerne (Anon. 1928), strawberries (Smith 1941), birdsfoot trefoil (Neunzig and Gyrisco 1955), seed alfalfa (Snow and McClellan 1951), and white pine (McDaniel 1936). In strawberries it did not cause significant damage, but it did mislead growers in timing of spraying for the strawberry leafroller, *Ancylis comptana* Frolich, due to misidentification (Smith 1941). In some white pine nurseries in Michigan, however, it has been known to damage up to 95% of all new growth (McDaniel 1936).

The life cycle of *X. pallorana* is similar to that of the obliquebanded and pandemis leafrollers, but Chapman and Lienk (1971) suggest that *X. pallorana* overwinters at a more advanced stage. The pupal stage generally lasts much longer, which suggests less likelihood of

generation overlap with orchard leafrollers. Lastly, oviposition is generally low to the ground and essentially limited to primary hosts. These factors may make *X. pallorana* a suitable alternative host for *C. florus* in orchards using alfalfa as ground cover. The following addresses the question of whether there is a preference by *C. florus* for either the obliquebanded leafroller or *X. pallorana* and the preference for either ground cover or crop canopy habitats.

MATERIALS AND METHODS

HOST SUITABILITY

General Parasitism in the Lab

The source of OBLR larvae was a laboratory colony reared on a modified pinto bean-based diet for noctuids (Shorey and Hale 1965) (24 ± 2°C, 16:8 (L:D) photoperiod). *Xenotemna pallorana* larvae were field collected in June from alfalfa in the Columbia Basin, near Quincy Washington. Fifty OBLR fourth instar larvae and 50 *X. pallorana* fourth instar larvae were placed individually into petri dishes (Falcon 5009, 50 x 9 mm) containing a small piece, approximately 1 cm square, of artificial diet. The lid of each was replaced, and each dish was set aside for approximately one hour to allow the leafroller to build a silken retreat. After this period of time, one mated *C. florus* female that had emerged no more than two days earlier, was placed into each petri dish using a camel's hair paint brush. Each lid was replaced and containers were placed in a room at 23 ± 2 °C and a photoperiod of 15:9 (L:D). Petri dishes were individually marked and observed daily for the presence of *C. florus* eggs, larvae, pupae, and adults. Adults were recorded only after they had emerged from within the host's retreat. The date was noted for each stage and the number of surviving progeny were recorded and

sexed. Number of progeny, sex ratio, and development time for *C. florus* was analyzed using a t-test assuming unequal variances. Percent parasitism and percent parasitoid induced mortality were analyzed using the binomial test of proportions.

Host Preference in the Lab

Arenas were assembled (Figure 2.1) using 14 x 3 cm petri dish bottoms, two 1/2dram shell vials placed about 8 cm from one another, and covered by a 473 ml clear plastic cup. A small portion of artificial diet was placed into each shell vial. The source of both OBLR and *X. pallorana* larvae was from laboratory colonies (24 ± 2°C, 16:8 (L:D) photoperiod). One fourth instar OBLR was placed into one of the shell vials, and one *X. pallorana* fourth instar was placed in the other. Each shell vial was put aside for one hour to allow the leafroller inside to build a silken retreat. After that period of time, one mated *C. florus* female was transferred to each arena using a small camel's hair brush. Each arena was checked at 12 hr, 24 hr, and 36 hr to determine which leafroller host *C. florus* chose. At each interval, the shell vial containing the particular leafroller species that *C. florus* was inside of was noted. If *C. florus* was not in one of the shell vials, but somewhere else in the arena, the sample was marked as "no-choice." Host preference was determined for each interval, and *C. florus* female's first choice of leafroller species was also determined. Data were analyzed using chi-square goodness-of-fit tests.

Host Preference in the Field

In an unsprayed apple orchard with a known background population of *C. florus*, 15 trees were selected at random. Half of each tree was infested with 15 OBLR late third to early

fourth instars, while the other half was infested with 15 *X. pallorana* late third to early fourth instars. The source of both OBLR and *X. pallorana* larvae was from laboratory colonies (24 ± 2°C, 16:8 (L:D) photoperiod). Larvae were transferred from each rearing cup using soft forceps to random leaves within the canopy at approximately 2 meters above the ground. Larvae of both species were collected 17 days later, marked, and transferred to individual petri dishes (Falcon 5009, 50 x 9 mm). From these, percent parasitism, number of progeny, and sex ratio of *C. florus* were determined. The number of *C. florus* progeny and sex ratios were analyzed using a t-test assuming unequal variances. Percent parasitism was analyzed using the chi-square test of independence.

HABITAT PREFERENCE

Caged Experiment 1

To determine if there is a habitat preference between ground cover and tree canopy by *C. florus*, experiments were conducted that provided wasps with leafrollers in differing habitat situations. Nine nylon organdy mesh cages with approximate dimensions of 1.22 m x 1.22 m x 1.22 m, were suspended from a frame of plastic irrigation pipe (PVC, 2 cm) and placed over small potted apple trees in an area of orchard where an already established alfalfa plant was located. In each cage, 15 OBLR fourth instar larvae were placed randomly on the leaves of the tree and 15 *X. pallorana* fourth instar larvae were placed randomly on the apical ends of alfalfa shoots. Cages were then undisturbed for one hour to allow the leafrollers to build retreats in the foliage. After this period, 30 mated *C. florus* females were released into each cage. The source of both OBLR and *X. pallorana* larvae was laboratory colonies (24 ± 2°C, 16:8 (L:D)

photoperiod). Ten days following the release of *C. florus* females, leafrollers were collected from both apple and alfalfa foliage, marked accordingly, and placed into petri dishes (Falcon 5009, 50 x 9 mm) to determine percent parasitism in each habitat. Data were analyzed using the chi-square test of independence.

Open Experiment 1

This experiment was conducted much like caged experiment 1; however, trees and alfalfa were in an open field near an orchard with a background population of *C. florus*. Ten small potted apple trees were placed in an area of orchard where an already established alfalfa plant was located. In each situation, 15 OBLR fourth instar larvae were placed randomly on the leaves of the tree and 15 *X. pallorana* fourth instar larvae were placed randomly on the apical ends of alfalfa shoots near the base of each tree. The source of both OBLR and *X. pallorana* larvae was laboratory colonies (24 ± 2°C, 16:8 (L:D) photoperiod). Ten days later, leafrollers were collected from both apple and alfalfa foliage, marked accordingly, and placed into petri dishes (Falcon 5009, 50 x 9 mm) to determine percent parasitism in each habitat.

Caged Experiment 2

To further determine if there is a habitat preference between ground cover and tree canopy by *C. florus*, experiments were conducted that provided wasps with a single leafroller species in differing habitat situations. Nine nylon organdy mesh cages with approximate dimensions of 1.22 m x 1.22 m x 1.22 m, were suspended from a frame of plastic irrigation pipe (PVC, 2 cm) and placed over small potted apple trees in an area of orchard where an already

established alfalfa plant was located. In each cage, 15 *X. pallorana* fourth instar larvae were placed on the leaves of the tree, and 15 *X. pallorana* fourth instar larvae were placed on the apical ends of alfalfa shoots. Cages were then undisturbed for one hour to allow the leafrollers to build retreats in the foliage. After this period, 30 mated *C. florus* females were released into each cage. The source of both OBLR and *X. pallorana* larvae was laboratory colonies (24 ± 2°C, 16:8 (L:D) photoperiod). Ten days following the release of *C. florus* females, leafrollers were collected from both apple and alfalfa foliage, marked accordingly, and placed into petri dishes (Falcon 5009, 50 x 9 mm) to determine percent parasitism in each habitat.

Open Experiment 2

This experiment was conducted much like caged experiment 2; however, trees and alfalfa were in an open field near an orchard with a known background population of *C. florus.* Ten small potted apple trees were placed in an area of orchard where an already established alfalfa plant was located. In each situation, 15 *X. pallorana* fourth instar larvae were placed on the leaves of the tree, and 15 *X. pallorana* fourth instar larvae were placed on the apical ends of alfalfa shoots near the base of each tree. The source of both OBLR and *X. pallorana* larvae was laboratory colonies (24 ± 2°C, 16:8 (L:D) photoperiod). Ten days following the release of *C. florus* females, leafrollers were collected from both apple and alfalfa foliage, marked accordingly, and placed into petri dishes (Falcon 5009, 50 x 9 mm), to determine percent parasitism in each habitat.

RESULTS

HOST SUITABILITY

General Parasitism in the Lab

There were no significant differences ($P > 0.05$) in larval, pupal, or total developmental time for *C. florus* reared on either OBLR or *X. pallorana* (Table 2.1). The average number of progeny per host for *C. florus* reared on OBLR was 11.2 and on *X. pallorana* was 14.2. These were not significantly different. Sex ratios were not significantly different for *C. florus* reared on OBLR (0.7:10.6 (M:F)) and *X. pallorana* (1.1:13.1 (M:F)). Percent parasitism, that is, larvae attacked that produced progeny, of OBLR larvae was 58.0% and was 76.9% for *X. pallorana.* There was no difference in the percent of parasitoid induced mortality, which for OBLR was 30.0% and 15.4% for *X. pallorana.*

Host Preference in the Lab

This experiment was performed to see whether *C. florus* preferred OBLR or *X. pallorana* as a host. After 12 hours of exposure *C. florus* was observed to be associated with OBLR in 10 of the 50 arenas (Table 2.2). And in the same period, *C. florus* was observed to be associated with *X. pallorana* in 9 of the 50 arenas. After 24 hours, *C. florus C. florus* was observed to be associated with OBLR in 16 of the arenas and associated with *X. pallorana* in 23 of the 50 arenas. After 36 hours, *C. florus* was observed to be associated with OBLR in 11 of the arenas, while it was associated with *X. pallorana* in 13 of the arenas. At all of these time intervals the number of *C. florus* observed to be associated with either leafroller species was not significantly different ($P = 0.05$). OBLR was chosen by *C. florus* first in 18, arenas and *X.*

pallorana was chosen first in 22. There was no observed preference for either host species as measured by the first choice of *C. florus* females.

Host Preference in the Field

This experiment was performed to determine if there was a preference for either OBLR or *X. pallorana* as hosts in the field given the same habitat (apples). The observed rate of parasitism of OBLR and *X. pallorana* by *C. florus* was 36.8% and 58.5%, respectively (Table 2.3). Parasitism of *X. pallorana* was significantly higher ($P < 0.05$). The average number of *C. florus* progeny reared from OBLR and *X. pallorana* was 26.6 and 24.8, respectively, and these were not significantly different ($P > 0.05$). Sex ratio for progeny reared from either leafroller species was not significantly different.

HABITAT PREFERENCE EXPERIMENTS

In the first caged experiment, the rate of parasitism by *C. florus* of OBLR in the tree was 49.43% and of *X. pallorana* in the ground cover was 3.77% (Table 2.4). In the second caged experiment the rate of parasitism by *C. florus* of *X. pallorana* in the tree was 79.63% and in the ground cover was 31.17% (Table 2.4). In both experiments, the differences in parasitism rates were significantly different ($P < 0.05$).

In the first open environment experiment, parasitism by *C. florus* of OBLR in the tree was 95.74%, and parasitism of *X. pallorana* in the ground cover was 13.64% (Table 2.4). In the second open environment experiment, parasitism by *C. florus* of *X. pallorana* in the tree was

100% and in the ground cover was 28.26% (Table 2.4). In both experiments the differences in parasitism rates were significantly different ($P < 0.05$).

DISCUSSION

The first questions posed in determining if *X. pallorana* would be a suitable alternative host for *C. florus* were if it would reproduce and how its population growth compared to other leafroller species. Results from the no choice laboratory experiment demonstrated clearly that *X. pallorana* is a suitable host for *C. florus* as OBLR. For both leafroller species the number of progeny and sex ratio was similar and, while the percent of parasitoid induced mortality was slightly higher for OBLR than that of *X. pallorana,* the differences were not statistically significant. In similar comparative laboratory studies, OBLR and PLR were shown to be equally acceptable as hosts by *C. florus* (Brunner, personal communication). Since *C. florus* is known to attack over 30 species of leafrollers in Europe (Gruys and Vaal 1984) and is reported attacking at least three species in the U.S. (Brunner 1996a, Hagley and Barber 1991), it was not surprising that parasitism rates for both OBLR and *X. pallorana* larvae were similar in laboratory experiments. These results were quite similar to those reported by Gruys and Vaal (1984) and van Veen and Wijk (1987) using *A. orana* as a host for *C. florus.* There was some background parasitism by an internal ichneumonid parasitoid to the field collected *X. pallorana* larvae. These individuals were not included in the results.

The next question posed was whether *C. florus* would show a preference for OBLR or *X. pallorana* where choices were allowed. Laboratory results showed that *C. florus* had had no overriding preference for one leafroller over the other. While *C. florus* tended to choose *X.*

pallorana over OBLR as their first choice, the numbers were not significantly higher. In field studies where there was a naturally occurring population of *C. florus*, the number of progeny per host was very high, probably being exaggerated due to superparasitism caused by the level of parasites in the orchard. Superparasitism is only assumed but is based on the observation that many of the leafrollers collected from the field had one or more *C. florus* female within the host's retreat. While the number of progeny and sex ratio of *C. florus* were exceptional, data were consistent for both leafrollers and showed no significant differences except in the rate of parasitism of *X. pallorana,* which was significantly higher than that of OBLR. Some leafrollers of both species were also parasitized by a tachinid parasitoid, but previous investigations have shown that this parasitoid's internal feeding does not affect the external feeding and development of *C. florus* (R. S. Pfannenstiel, personal communication).

One important aspect of whether *X. pallorana* could potentially serve as an alternative host for *C. florus* was whether habitat could affect the wasp's ability to find and parasitize its host. To assess this, the first two experiments looked at a normal scenario that would be found in the field, i.e. with OBLR in the tree and *X. pallorana* in the ground cover. One experiment was conducted in a caged situation, while the other was in a more natural, "open" environment. Both experiments showed that there was a marked difference in level of parasitism in the different components of the orchard habitat. In both experiments, parasitism was significantly lower on hosts in the ground cover compared to apple. Since there was no difference in the preference by *C. florus* for either leafroller species, the differences observed in the field must be associated with the parasite's tendency to search more in trees or that it is a more efficient searcher in apple trees compared to the ground cover.

To again make sure that there was no secondary effect of host preference by *C. florus*, a second set of experiments was conducted looking at parasitism rates given different habitats, but this time using the same leafroller host in both habitats. When *X. pallorana* was placed in both the tree and ground cover, *C. florus* attacked those in the tree more than the cover crop. This result was consistent for both caged and open environment experiments. Dijkstra (1986) looked at habitat response within the canopy of the tree and found that larvae feeding at the tops of long shoots had a higher rate of parasitism This is a behavior exhibited by *C. florus* to help minimize search time (Dijkstra 1986). This could be one explanation for the lower degree of parasitism found in ground cover than in the apple tree canopy. In these final experiments looking at habitat preference, we once again had multiple parasitism on some of the leafrollers collected by *C. florus* and an internal tachinid parasitoid. As stated previously, they had little or no effect on the parasitism by *C. florus* in these experiments.

In conclusion, *X. pallorana* was shown to be as suitable a host physiologically for *C. florus* as OBLR in both laboratory and field studies. *Colpoclypeus florus* showed no preference between OBLR and *X. pallorana* in choice situations. There was a preference by *C. florus* for tree canopy habitats rather than cover crop habitats; however, there was still substantial parasitism of *X. pallorana* in cover crop. Therefore, in orchards using alfalfa as ground cover, *X. pallorana* could serve as a reservoir for parasites like *C. florus* during the spring and summer and possibly also serve as an alternative overwintering host for *C. florus* within orchards.

Figure 2.1. Chamber used in laboratory host preference experiment.

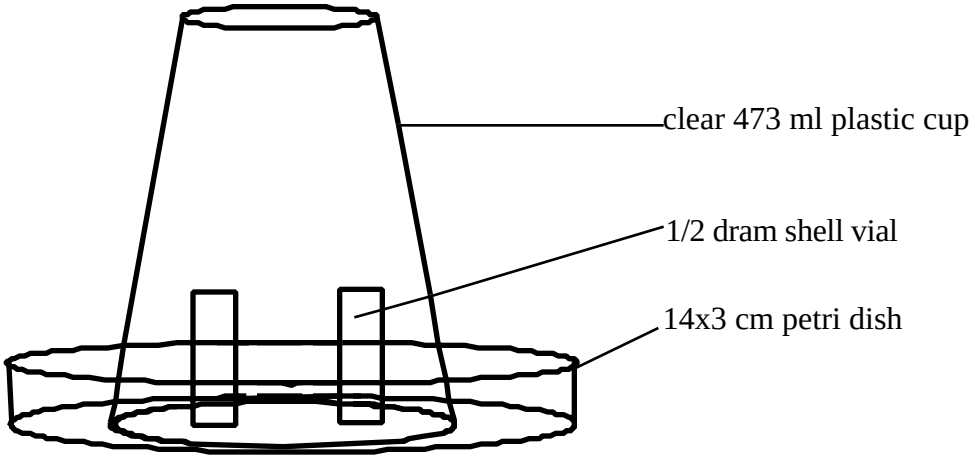

Table 2.1. Laboratory parasitism by *Colpoclypeus florus.*

	Host	
Variable	***X. pallorana***	***C. rosaceana***
n	39	50
Larval developmental time (d)	4.9a	5.2a
Pupal developmental time (d)	7.0a	7.0a
Total developmental time (d)	16.6a	16.7a
Avg. # adult C. florus	14.2a	11.2a
Avg. # females	13.1a	10.6a
Avg. # males	1.1a	0.7a
% parasitism	76.9a	58.0a
% parasitoid induced mortality	15.4a	30.0a

Values within a row followed by the same letter are not significantly different ($P > 0.05$) t-test assuming unequal variance and binomial test of proportions.

Table 2.2. Host preference of *Colpoclypeus florus* in the laboratory.

Preference	12 hr	24 hr	36 hr	First choice
C. rosaceana	10a	16a	11a	18a
X. Pallorana	9a	23a	13a	22a

Values within the same column followed by the same letter are not significantly different (P > 0.05) Pearson's chi-square goodness-of-fit test.

Table 2.3. Host preference of *Colpoclypeus florus* in the field.

Host	% parasitism	Avg. # progeny	Avg. # females	Avg. # males
X. pallorana	58.5a	24.8a	17.9a	6.9a
C. rosaceana	36.8b	26.6a	19.0a	7.6a

Values within a column followed by the same letter are not significantly different ($P > 0.05$) t-test assuming unequal variances and chi-square test of independence.

Table 2.4. Rate of parasitism by *Colpoclypeus florus* in different habitats.

Habitat	Exp. 1 Caged	Exp. 1 Open	Exp. 2 Caged	Exp. 2 Open
Apple	49.43%a	95.74%a	79.63%a	100.00%a
Alfalfa	3.77%b	13.64%b	31.17%b	28.26%b

Values within a column followed by the same letter are not significantly different ($P > 0.05$) chi-square test of independence.

SUMMARY

The Food Quality Protection Act of 1996 promises to eliminate or severely restrict the use of organophosphate insecticides which are relied upon heavily for leafroller control in Washington orchards. With the implementation of mating disruption as a primary control tactic for the key pest, codling moth, and use of softer pesticide programs for other pests, leafrollers have risen to major pest status in pome fruit orchards in Washington. These two factors have increased the urgency to find alternative means for controlling leafrollers. With an uncertain future for broad-spectrum pesticides, the development of new insecticide chemistries that are highly selective, and the increasing adoption of mating disruption as a control for codling moth, the window of opportunity for making better use of biological control in orchards has never been greater. *Colpoclypeus florus*, a parasitic wasp in the family Eulophidae, has shown promise as a biological control agent for leafrollers in Europe and Washington. However, although *C. florus* parasitism of *P. pyrusana* reaches very high levels (>80%) in the summer, it has not been completely effective at controlling leafroller populations. The lack of suitable overwintering hosts may result in local the extinction of *C. florus* populations, necessitating reestablishment in the orchards the following year from non-orchard habitats. The two main leafrollers found in orchards, *C. rosaceana* and *P. pyrusana,* do not overwinter in stages suitable for *C. florus*.

Xenotemna pallorana is a leafroller whose hosts are primarily alfalfa and white sweet clover. In orchards that use alfalfa for ground cover, populations of *X. pallorana*

could be propagated and serve as an alternative host for *C. florus*. Not only might this provide for a more suitable overwintering host, but it might also enhance biological control of pest species of leafroller in summer by increasing the number of *C. florus* produced in orchards.

The first chapter of this thesis looked at the development of *X. pallorana* on the foliage of fruit crops, apple, cherry, and pear in comparison to alfalfa. It was somewhat troubling to find that *X. pallorana* was able to develop adequately on all three orchard plants. If *X. pallorana* could develop on all three fruit plants it would seem a risky suggestion to propose to introduce them into an orchard environment, even on the cover crop. However, the lack of *X. pallorana* presence in orchards even though they were evidently common in environments around many orchards suggested that other factors might be important in this leafroller choosing its host plant. When oviposition preference was tested using apple and alfalfa, *X. pallorana* females laid on apple foliage when given no other choice. However, when provided a choice in a natural setting *X. pallorana* showed strong, almost exclusive, preference for ground cover foliage, the most preferred being alfalfa.

In the second chapter the focus was on the activity of *C. florus* by examining host and habitat preferences. *Colpoclypeus florus* showed no preference between OBLR and *X. pallorana* larvae in laboratory and field studies. Habitat preference studies showed that *C. florus* had a fairly strong preference for apple, compared to ground cover habitats when given the choice of finding host larvae in both locations. From these studies it seems that *X. pallorana* could serve as an alternative host for *C. florus* in orchards without increasing the risk

of crop loss. At the very least, *X. pallorana* and an alfalfa cover crop could be used as a model to study the potential of enhancing leafroller biological control in orchards by augmenting populations of an alternative host for a parasite instead of the parasite population. It would seem easier to rear and augment leafroller populations in a cover crop than to rear parasites in an artificial environment, i.e. mass rearing, where concerns over fitness always abound.

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