

Development and oviposition preference of *Xenotemna pallorana* Robinson (Lepidoptera: Tortricidae) on alfalfa and fruit tree foliage

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ABSTRACT

Xenotemna pallorana Robinson (Lepidoptera: Tortricidae) is an alfalfa (*Medicago sativa* L., Fabaceae) feeding leafroller that has been considered for incorporation into apple (*Malus domestica* Borkh., Rosaceae) orchard ecosystems in Washington State, U.S.A., as an alternative host for the leafroller parasitoid *Colpoclypeus florus* Walker (Hymenoptera: Eulophidae). *Xenotemna pallorana* has been observed to feed on apple foliage when populations deplete the foliage of alfalfa in a groundcover, but there have been no studies to determine if foliage of fruit trees is suitable for full larval development or would be attractive as oviposition sites. Leafroller larvae were fed apple, cherry (*Prunus avium* L., Rosaceae), pear (*Pyrus communis* L., Rosaceae), and alfalfa foliage, all of which proved suitable for development; although development time and pupal weights varied among foliage types. Adult female *X. pallorana* exposed to apple foliage under no-choice conditions oviposited on the upper side of apple leaves. In a choice test between apple foliage and ground cover including alfalfa, *X. pallorana* females preferentially selected alfalfa and other components of the ground cover (98.04%) over the apple foliage (1.94%) for oviposition. Despite the ability of *X. pallorana* to develop on fruit tree foliage, its distinct preference for ovipositing on alfalfa suggests that it is unlikely to damage fruit. Therefore, *X. pallorana* presents a low-risk opportunity to study enhancement of biological control of leafrollers in orchards through ground cover management and host augmentation.

Key Words: *Colpoclypeus florus*, *Xenotemna pallorana*, ground cover, alternative host

INTRODUCTION

Once considered secondary or minor pests, the leafrollers *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) and *Pandemis pyrusana* Kearfott (Lepidoptera: Tortricidae) are now two of the most important pests in Washington pome fruit orchards (Brunner and Beers 1990; Brunner 1994; Brunner 1996b; Brunner 1999). This is especially true in orchards that use pheromone-based mating disruption for the control of codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) (Gut and Brunner 1998). The seasonal life histories of *C. rosaceana* and *P. pyrusana* are similar and fairly synchronous, with both species having two generations per year in Washington State and overwintering as small larvae (Beers *et al.* 1993). Control of leafrollers in orchards has historically relied on broad-spectrum insecticides (Beers *et al.* 1993; Brunner 1999); however, the development of insecticide resistance has made control difficult (Sial and Brunner 2010a). Additionally, even the integration of insecticides with newer chemistries might lead to resistance onset within 5 to 10 generations (Sial and Brunner 2010b) if not carefully managed. The loss of conventional control products and continued evolution of resistance to pesticides greatly increase the need for non-chemical alternatives to suppress leafroller populations in orchards.

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Biological control may play a significant role in an integrated approach for leafroller control. One natural enemy that has shown promise against leafrollers in Washington State is the gregarious ectoparasitoid *Colpoclypeus florus* Walker (Hymenoptera: Eulophidae). This species was first discovered in Washington State in 1992, where it parasitized about 80 percent of the summer generation *P. pyrusana* in an unsprayed apple orchard (Brunner 1996a). In Europe, *C. florus* parasitizes more than 30 species of tortricid larvae (Dijkstra 1986) and has been reported to parasitize large numbers of leafrollers in Europe that feed on both apple and strawberries (Gruys and Vaal 1984). However, it is absent or rare in spring, although it can be common in summer and early fall, causing significant mortality of leafroller larvae (Gruys 1982). The absence of *C. florus* in spring limits its impact as a biological control agent in Europe. The disparity in the seasonality of parasitism is presumably caused by a lack of synchrony between the phenology of *C. florus* and leafroller species present in European orchards (Gruys 1982). *Colpoclypeus florus* attacks relatively large leafroller larvae (Gruys and Vaal 1984; Dijkstra 1986); however, most tortricids found in orchard systems overwinter as early larval instars that are not suitable hosts for *C. florus* in Europe (Gruys and Vaal 1984). Female *C. florus* searching for suitable hosts in the fall within orchards cannot find them and evidently leave the orchard environment in search of suitable overwintering hosts (van Veen and Wijk 1987). In Europe, no leafrollers have been found that provide overwintering opportunities in orchards for *C. florus* (Evenhuis and Vlug 1983). However, if suitable hosts are placed within orchards, parasitism is high (Pfaffenstiel *et al.* 2012).

Parasitism of the summer leafroller generation in Washington by *C. florus* can be high (>60%) (Brunner 1996a), but spring parasitism is generally low, and parasitism of the summer generation is often insufficient to prevent fruit damage (Unruh *et al.* 2001). As in Europe, the leafroller pests in Washington orchards, *C. rosaceana* and *P. pyrusana*, overwinter as instars that are typically too small to be suitable hosts for *C. florus* (Pfaffenstiel and Unruh 2003; Pfaffenstiel *et al.* 2010). One possible tactic to augment biological control of leafrollers would be to provide alternate overwintering hosts for *C. florus*.

A leafroller being considered for this is *Xenotemna pallorana* Robinson (Lepidoptera: Tortricidae). This leafroller is an alfalfa-feeding species that is in the same tribe (Archipini) as both *C. rosaceana* and *P. pyrusana*, and may overwinter in an appropriate stage for *C. florus* to attack in the fall. *Xenotemna pallorana* is common in alfalfa crops in some parts of the Columbia Basin (CAN and RSP personal observation). Alfalfa occurs in orchard groundcovers in Central Washington, but currently *X. pallorana* is not commonly observed in orchards (RSP personal observation). Establishment of *X. pallorana* in orchard groundcovers in the fall would allow *C. florus* to locate hosts for overwintering within the orchard and, thus, be more abundant in orchards the following spring. *Xenotemna pallorana* is bivoltine and highly polyphagous (Chapman and Lienk 1971), having been reported feeding on 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).

Preliminary evaluations demonstrated that *X. pallorana* is a suitable host for *C. florus* (CAN and RSP, unpublished data). When host foliage is depleted, *X. pallorana* will feed on apple leaves on young trees where foliage is near the ground (Chapman and Lienk 1971). The occurrence of *X. pallorana* in Washington apple orchards is associated with mature larvae using foliage for pupation sites (JFB personal observation).

Before *X. pallorana* can be recommended as a potential alternative host for *C. florus* in orchard ground covers, it is necessary to demonstrate that it would not consistently feed on and damage fruit trees. Our goal in this study is to determine whether *X. pallorana* feed and develop normally on foliage of three tree fruit species, whether adults oviposit on apple foliage under no-choice conditions, and finally whether *X. pallorana*

prefers to oviposit on apple tree foliage when an alfalfa-dominated ground cover is available.

MATERIALS AND METHODS

A laboratory colony of *X. pallorana* was initiated from individuals collected from alfalfa in the Columbia Basin near Quincy, WA, in June 1996. *Xenotemna pallorana* larvae were reared in groups (8) within 118-ml plastic portion cups (Solo®), on a modified pinto bean-based diet (Shorey and Hale 1965) at 24 ± 2 °C with a 16:8 (L:D) photoperiod. Pupae were collected and placed into a cylindrical wire mesh oviposition cage 20 cm tall and 10 cm in diameter. Waxed paper was placed into oviposition cages, and *X. pallorana* was allowed to eclose, mate and oviposit on the waxed paper. Exposure to natural light greatly increased oviposition, so oviposition cages were positioned near windows in the rearing rooms. Egg masses were cut from the waxed paper, washed in a 5% sodium hypochlorite solution, and then rinsed with distilled water. Eggs were carefully peeled from the waxed paper and placed into petri dishes (Falcon 5009, 50 x 9 mm), with a 1-cm cube of artificial diet. Neonates were transferred in groups of eight to 104-ml portion cups, with the bottoms filled approximately 1 cm deep with artificial diet for larval development.

Development on fruit tree hosts. Forty newly hatched *X. pallorana* larvae were placed individually into Petri dishes (Falcon 5009, 50 x 9 mm) containing an approximate 4-cm² portion of a mature leaf from a growing shoot (4 to 10 leaves from shoot apex) of apple (*Malus domestica* Borkhausen var. Red Spur Delicious), pear (*Pyrus communis* L. var. Bartlet), or cherry (*Prunus avium* L. var. Bing). For alfalfa, the apical 4 to 5 leaves of a non-blooming shoot were collected and placed in the same arena type as fruit tree foliage. Our goal here was simple; to see if the larvae would complete development to adult on the different foliage types. The use of cut foliage, which can be lower in quality, provides a conservative estimate of suitability and allowed the study to be conducted under controlled conditions. Conducting this study on growing trees in the orchards would have exposed larvae to significant environmental variation and mortality that might have masked the effects of foliage type and prevented estimation of plant-based survival rates.

Fruit tree foliage was obtained from multiple trees for each type within unsprayed orchards at the Washington State University Tree Fruit Research and Extension Center (WSU-TFREC) in Wenatchee, WA. Alfalfa foliage was obtained from plants transplanted from the field to 3.8-l pots in the greenhouse containing 3-l of Sunshine potting mix #1 (Sungro Horticulture, Agawam, MA, USA) with 30 ml of long-release fertilizer granules spread over the surface (Osmocote, Everris NA). *Xenotemna pallorana* larvae were placed in a controlled environment at 24 ± 2 °C, 70 ± 10 % RH, and 16:8 (L:D) photoperiod. Larvae were examined daily, and mortality was recorded. Food was changed as needed, when it had been consumed, or if a decline in apparent quality was observed, but no less than twice per week. Pupating larvae were set aside and weighed within 48 h of pupal formation but after they had completed melanization. Chi-square analysis was used to determine if food source affected survivorship of *X. pallorana*. The sex of newly emerged adults was recorded. Larval and pupal development time and pupal weights for each sex reared on each foliage type were analyzed using one-way analysis of variance (ANOVA; Super ANOVA general linear model program; Abacus Concepts, Berkeley, CA). Mean separations were done with the Fisher protected least significant difference test (LSD $\alpha = 0.05$).

Oviposition Experiments. Experiments were conducted to determine whether *X. pallorana* would oviposit on apple foliage in choice and no-choice situations. For the no-choice studies, 12 sleeve cages made of cloth and wire screen (window screen tubes, approximately 50 cm long and 25 cm in diameter) were placed over unsprayed apple (c.v. Red Spur) foliage in the field. Newly emerged male and female *X. pallorana* adults

(<12h old, two of each) obtained from the laboratory colony were released into each cage. After a period of seven days, each cage was removed, all egg masses were collected, and their locations were recorded.

A second experiment was conducted to determine if *X. pallorana* females prefer to oviposit on foliage of a fruit tree host, apple, or their typical field host, alfalfa, as part of a groundcover. Four nylon organdy mesh cages (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 patches of alfalfa plants in a fescue (*Festuca* spp; Poaceae) dominated groundcover. A single potted apple tree (c.v. "Oregon Spur", ~1 m tall, with at least 30 fully developed leaves) was placed in each cage. Alfalfa typically comprised <50% of the ground cover foliage. Although the leaf area was not directly measured for each foliage type, alfalfa foliage made up a smaller proportion of the potential oviposition substrates than the apple and fescue foliage (CAN and RSP personal observation). Ten newly emerged colony-reared *X. pallorana*, (1:1 sex ratio) were released into each cage and allowed to mate and oviposit for seven days. Seven days after the moths were released, cages were removed, and egg masses were collected from foliage within each cage ($n=12$). The proportion of egg masses deposited on apple foliage vs. alfalfa and other groundcover plants was compared to the null hypothesis of no preference (50:50) using Chi-square analysis.

RESULTS AND DISCUSSION

A similar number of *X. pallorana* survived to adulthood when reared on the various foliage types ($\chi^2 = 3.42$, df = 3, $P = 0.3313$). Survival of *X. pallorana* on the factitious hosts was 65% on cherry, 72.5% on pear, and 75% on apple foliage. Survival on alfalfa was the lowest overall, at 56%; most likely, this is an artifact related to the quality of the alfalfa foliage declining more quickly than the apple foliage in the bioassay arena. The host material that larvae were reared on significantly affected larval developmental time for both males and females ($F = 16.155$; df = 3; $P < 0.0001$ and $F = 7.647$; df = 3; $P = 0.0003$, respectively), whereas pupation time was not different (Table 1). There was also a significant effect of host material on pupal weight for both males and females ($F = 57.477$; df = 3; $P < 0.0001$ and $F = 43.864$; df = 3; $P < 0.0001$, respectively; Table 2). *Xenotemna pallorana* was able to complete its life cycle on the foliage of apple, cherry, and pear, in addition to alfalfa. Larval development was fastest on alfalfa. Pupal weights were consistently higher for larvae reared on apple and cherry foliage than on alfalfa and pear. Adults from the heaviest pupae (reared on apple and cherry foliage) had unusually large abdomens in proportion to their wing size and had difficulty flying (CAN and RSP, personal observation). It would have been difficult for these adults to disperse and either mate or deposit egg masses. It may be that the larvae developing on apple and cherry develop to such a large size through a supernumerary instar, which would account for both the longer development time and the larger pupal size. Adults that developed on alfalfa and pear foliage had physical proportions similar to those of individuals collected from the field or reared on artificial diet.

In no-choice trials, *X. pallorana* readily oviposited on apple foliage. Egg masses were found on apple foliage in 8 of 12 sleeve cages, with an average of 2.08 egg masses per cage. These egg masses successfully hatched, and the larvae were observed to begin feeding on the leaves; however, further development was not monitored. Egg masses were found only on the upper surface of apple leaves. Therefore, without a choice, *X. pallorana* will accept apple foliage for oviposition. Because *X. pallorana* oviposits on wax paper in the laboratory and has been observed to lay egg masses on other smooth material such as glass or plastic, oviposition on apple foliage when confined was not surprising.

Table 1
Development time of *X. pallorana* reared on different hosts.

Host	Sex	n	Larval developmental time (mean days \pm SEM)	Pupation time (mean days \pm SEM)
<i>Cherry</i>	Male	13	35.6 \pm 1.1a	11.9 \pm 0.2a
<i>Pear</i>		21	32.0 \pm 0.9b	11.8 \pm 0.2a
<i>Apple</i>		11	34.4 \pm 1.2ab	11.9 \pm 0.3a
<i>Alfalfa</i>		14	25.8 \pm 1.1c	11.9 \pm 0.2a
<i>Cherry</i>	Female	13	40.8 \pm 1.1a	11.1 \pm 0.2a
<i>Pear</i>		8	36.5 \pm 1.4b	10.9 \pm 0.3a
<i>Apple</i>		19	35.8 \pm 0.9b	10.8 \pm 0.2a
<i>Alfalfa</i>		9	33.1 \pm 1.3b	10.2 \pm 0.3a

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

Table 2
Pupal weight of *X. pallorana* reared on different hosts.

Host	Sex	n	Pupal weight (g)
			(mean \pm SEM)
<i>Cherry</i>	Male	13	0.055 \pm 0.002b
<i>Pear</i>		22	0.038 \pm 0.002d
<i>Apple</i>		11	0.073 \pm 0.002a
<i>Alfalfa</i>		14	0.047 \pm 0.002c
<i>Cherry</i>	Female	13	0.058 \pm 0.004b
<i>Pear</i>		7	0.044 \pm 0.005c
<i>Apple</i>		19	0.094 \pm 0.003a
<i>Alfalfa</i>		9	0.042 \pm 0.005c

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

In choice trials, *X. pallorana* were presented with apple trees and alfalfa in a fescue-dominated ground cover. A total of 51 egg masses were collected (Table 3), with an average of 4.25 egg masses per cage ($n = 12$). The number of egg masses found on alfalfa and other ground cover plants was significantly higher than on apple, with slightly over 98% of the egg masses (50) found on alfalfa and other ground cover plants (74.5% on alfalfa and 23.5% on other ground cover plants; $\chi^2 = 31.5$, df = 1, $P << 0.0001$) when compared to the null hypothesis of no preference. Only one egg mass was found on apple foliage—less than 2% of the total collected.

Table 3

Oviposition choice of *X. pallorana* females between apple foliage and an alfalfa-dominated cover crop.

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

Therefore, when provided a choice, *X. pallorana* females predominantly deposited their eggs on alfalfa and other groundcover plants over apple foliage. Other than alfalfa, ground cover plants on which egg masses were found included blades of fescue (Poaceae); dandelion (*Taraxacum officinale* Weber, Asteraceae); and field bindweed (*Convolvulus arvensis* L., Convolvulaceae). The single egg mass found on apple, 2% of the total egg masses, may have been the result of the location of foliage in close proximity to the ground cover. *Xenotemna pallorana* will lay eggs on apple foliage under no-choice situations. The species can be locally abundant in alfalfa fields in the Columbia Basin, but are not observed frequently in orchards (CAN and RSP, personal observation). Oviposition preference for alfalfa most likely explains why *X. pallorana*, although occurring in fruit growing areas of Washington and commonly observed in alfalfa fields, is not commonly observed feeding on apple or other fruit trees. Additionally, the density of alfalfa in eastern Washington apple orchards varies considerably, and most orchards use pesticides for insect control, making observations of *X. pallorana* less likely.

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. Although females may oviposit almost exclusively on a limited range of hosts, they may develop as fast and as well on non-preferred hosts (Thompson 1988). There is no aversion of females to oviposition on apple foliage when not given a choice, but under natural conditions it is clear that they preferentially select ground cover habitats for oviposition sites. It may be that *X. pallorana* doesn't need to deposit eggs exclusively on hosts such as alfalfa as long as suitable hosts are nearby, which may explain the frequency of oviposition on non-alfalfa groundcover plants. Larvae may search for and disperse to alfalfa in groundcover to some extent following hatch. Although this research shows that at least three orchard crops are suitable hosts for *X. pallorana*, we believe the strong oviposition preference for alfalfa would preclude any pest potential by this species. The *X. pallorana*-alfalfa combination may offer a low-risk model for the orchard environment to test the hypothesis that cover-crop management could be used to enhance leafroller biological control.

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