

Apple Leafroller (Lepidoptera: Tortricidae) Populations and Parasitism in an Orchard Managed With Either a Grass or Alfalfa Cover and Without Insecticides Over Four Growing Seasons

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Northwest U.S. apple growers are concerned that with reduced insecticide use leafrollers will become serious economic pests. Concomitantly, interest in integrating legume covers in the agro-ecosystem, to augment orchard nitrogen nutrition, increases. We evaluated leafroller populations and parasitism in apples with grass or alfalfa covers and curtailed insecticide use. For both covers, the leafroller populations rose to high levels then dramatically declined the fourth year of the study. Parasitoids modestly contributed to leafroller biological control. Only in a few instances, parasitism rates were higher in alfalfa cover plots compared with grass cover plots. Six parasitoid species contributed to leafroller biocontrol. Yield was not substantially adversely affected by leafrollers.

KEYWORDS *apple leafroller, Malus domestica, legume cover, alfalfa cover, leafroller parasitoids, biological control*

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INTRODUCTION

Washington State, located in the USA, is a leading national producer of apples [*Malus x sylvestris* (L.) Mill. var. *domestica* (Borkh.)]. Leafrollers (Lepidoptera: Tortricidae) have become pests inhabiting commercial apple orchards in North America (Mayer and Beirne, 1974; Weires and Riedl, 1991) and in Washington State the pandemis leafroller, *Pandemis pyrusana* (Kearfott) and the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) are the most prevalent and destructive species (Beers et al., 1993; Brunner et al., 2001). As growers adopt pheromone-based mating disruption technology for management of the codling moth (*Cydia pomonella*) (Beers, 1998; Brunner et al., 1994), many are concerned leafrollers will assume premiere pest status (de Reede et al., 1985; Gut et al., 1995; Knight and Turner, 1999; Knight et al., 2000; Phannensteil et al., 1997).

Concomitantly, orchardists face increasing costs for nitrogenous fertilizers. Synthetic nitrogen fertilizer in urea form costs the grower in central Washington State about \$1.39 per kg N on average over the last five years (N. Squires, Northwest Wholesale, Wenatchee, WA, USA, personal communication) while calcium nitrate costs about \$4.05 per kg N (K. Lorentz, Quincy Farm Supply, Quincy, WA, USA, personal communication). Organic nitrogen sources typically range in price from \$3–13 per kg N (dry basis) (D. Granatstein, unpublished data). As costs for N fertilizers increase, Northwest apple growers are interested in the potential of legume covers to supply a portion of tree N need (Granatstein and Sanchez, 2009) and possibly contribute to other agro-ecological functions (Altieri and Nickolls, 1999; Brown and Schmitt, 1996; Prokopy, 1994; Stary and Pike, 1999). Currently, grass covers are standard for both conventional and organic apple orchards in Washington State.

Maxwell (1999) suggested that the study of general patterns of variations and behavior in pest populations and communities in response to agro-ecosystem manipulations (as opposed to reductionist investigation leading to prescriptive management tactics) is an appropriate mode of experimentation leading to ecologically based pest management (Lewis et al., 1997; National Research Council, 1996). As such, our study addressed three hypotheses: (1) leafroller populations will not exceed threshold levels in the absence of broad spectrum insecticide use; (2) parasitism will contribute to leafroller biological control; and (3) an alfalfa cover will not exacerbate leafroller populations or economic injury.

MATERIALS AND METHODS

Our study was conducted in north central Washington State over four growing seasons. Plots for this experiment were established in a 3.12-hectare block of 5th leaf, mature-bearing, Fuji (BC 2)/M9 apple planted

1.2 m × 4.0 m (1,977 trees/ha), trellised and trained to a central axis. The block was divided into six contiguous half hectare plots, configured to maximize distance between a designated 0.12 ha core-sampling area situated in the center of each plot. At least 31.6 m separated each core-sampling area from adjacent ones. A randomized block design with three replicates of each of two treatments was utilized for this experiment (Davis, 2000).

Experimental treatments were (1) reduced insecticides/alfalfa cover and (2) reduced insecticides/grass cover (= control). A standard orchard cover crop mix of 40% 'Penguin' perennial ryegrass (*Lolium perenne* [L.]), 40% 'Omni' perennial ryegrass, and 20% chewing's fescue (*Festuca rubra* [L.] subsp. *commutata* Gaudin) (Cenex Ag Supply Co., Wenatchee, WA, USA) was utilized for the grass cover, while 'Vernal' alfalfa (Cenex Ag Supply Co., Wenatchee, WA, USA) was chosen for its drought tolerance and lower vigor. Plots were sown with their respective covers in mid-May of the initial year of the experiment.

The orchard block was farmed utilizing standard practices with the exception of arthropod pest and cover crop management. Both covers were mowed with a flail-type mower three to four times per year: the alfalfa before flowering and to a height of approximately 15 cm to avoid crown damage, the grass to about 9 cm and before seed heads formed. Pheromone-based mating disruption technique alone was used for codling moth management. Isomate-C (Pacific Biocontrol, Vancouver, WA, USA) pheromone loaded emitters were dispensed in all treatment plots, within recommended rates, at 500 emitters/ha. Other than a single, delayed dormant superior oil application (rate = 11.3 l/378 l of water, 9,351 l/ha), no insecticide treatments were utilized in the plots for arthropod management during the course of the experiment. However, carbaryl (Sevin XLR) (Bayer CropScience, Research Triangle Park, NC, USA) was used, per standard practice, as a fruitlet thinner (rate = 0.23 or 0.5 l/379 l water depending on the season, 9,351 l/ha) tank mixed with other non-insecticidal growth regulating substances and a surfactant, applied once per season, in the first two experimental seasons but not thereafter.

P. pyrusana populations and rates of parasitism were monitored using the following methods (Beers et al., 1993; Edwards, 1998; Swezey et al., 2000).

Leafroller Infestation

1. Tight cluster (pre-bloom, approximately mid-April) larva infestation rate was assessed by randomly collecting and examining under a microscope ten upper canopy fruit buds from each of 30 trees in each replicate.
2. Petal fall (approximately the first week of May) and summer generation (the first and last year only) larva infestation rates were assessed by inspecting 10 randomly selected upper canopy shoots on 30 trees per

plot for active leafroller feeding sites. Counts were conducted weekly for three consecutive weeks.

3. Overwintering larva were trapped in 2.5 cm-wide corrugated cardboard strips placed around the base of 30 sample trees in each replicate in mid-August. The bands were retrieved in mid-October and stored in sealed plastic bags at 5.7°C until January. The bands, still in plastic bags, were then removed from refrigeration, left at room temperature for a period of approximately one month, and leafrollers counted.

Parasitism of Larva

1. Larva collected at tight cluster (see protocol 1 above) were placed in a clear plastic cup that contained an artificial diet (Nobbs, 1997) and were reared in the laboratory at room temperature until death or until an adult leafroller or parasitoid emerged.
2. Petal fall (mid-May) when overwintering generation larvae were nearly mature we searched (30 min maximum) for and collected up to 100 larvae within their retreats and reared them in the laboratory.
3. Summer generation parasitism was assessed by collecting 100–300 larvae, within their retreats, per plot, and rearing them out. Collection times varied from mid to late summer.
4. Sentinel obliquebanded leafroller larva were set out every ten days beginning in late June and continuing through mid-October on ten shoots in each plot and retrieved ten days later. Twenty 3rd-instar larva/plot were placed on shoots selected for consistent location in the canopy (approximately chest to shoulder high), moderate vigor, and evidence of new growth. Retrieved larvae, within their retreats, were brought to the lab for rearing out.

Parasitoids reared from *P. pyrusana* and obliquebanded leafrollers were submitted to the M.T. James Entomological Collection at Washington State University for identification (by Dr. Richard S. Zack).

Yield per plot was calculated based on the number of bins harvested plus the estimated quantity of fruit culled during harvest. Insect pest damage cause and rate to harvested fruit was evaluated by randomly selecting and assessing 100 cull apples from bins in each plot. Cullage of harvested fruit due solely to leafroller damage was estimated for year 4 only.

Data were analyzed using ANOVA (Statistix 7, Analytical Software, Tallahassee, FL, USA) to compare between treatments in single years only and are thus reported as trends over time.

RESULTS AND DISCUSSION

P. pyrusana Infestation

Infestation rates at tight cluster and petal fall demonstrated similar trends for both cover treatments during year 1 (Table 1). Some statistical differences

TABLE 1 Leafroller Larvae Infestation Rates for Three Sampling Times

Year	Tight cluster, % buds		Petal fall, % shoot tips		Summer, % shoot tips	
	Alfalfa	Grass	Alfalfa	Grass	Alfalfa	Grass
1	0.4a	0.9a	0.8a	0.3a	56.7a	43.3a
2	2.4a	0.2b	19.8a	18.8a	No data	No data
3	40.7a	40.0a	38.9a	57.8b	No data	No data
4	5.4a	5.0a	12.9a	11.6b	21.5a	23.0a

Each stage and each year were analyzed separately. Means within rows followed by the same letter are not significantly different ($p = 0.05$).

were recorded between cover crop treatments during years 2, 3, and 4. Tight cluster populations peaked in year 3, for both cover treatments, with nearly half of the flower clusters being infested, followed by a precipitous population decline in year 4. Infestation rates only differed between cover treatments in year 2 when alfalfa treatment infestation levels were significantly greater ($p = 0.014$) than grass, though both were relatively low. In year 3, the grass treatment petal fall infestation rate was significantly greater than for the alfalfa treatments ($p = 0.026$). Conversely, year 4 petal fall infestation levels were significantly greater for alfalfa plots than for grass ($p = 0.013$). However, these differences seemed of little practical significance.

Summer generation shoot infestation levels were higher for both cover treatments compared with tight cluster and petal fall in years 1 and 4 (no data for years 2 and 3). There was no difference between alfalfa and grass treatments for either year.

There were no differences in overwintering leafroller trap catch between cover treatments during any year of this study (Table 2). The greatest numbers, however, were detected in year 2 and preceded the growing season (year 3) having the greatest leafroller infestation levels any year of the study. Overwintering trap catch decreased in year 3, to nearly nil in year 4. Decline in overwintering trap catch preceded observed growing season population declines. Trapping overwintering larvae may be a valuable predictive tool under such a management regime.

TABLE 2 Mean Number of Overwintering Stage Leafrollers Caught in Cardboard Band Traps

Year	Alfalfa	Grass
1	0.0a	0.0a
2	29.9a	39.3a
3	11.7a	13.0a
4	2.0a	1.3a

Means within rows followed by the same letter are not significantly different ($p = 0.05$). Treatments were compared for each year separately.

P. pyrusana Parasitism

Throughout the duration of this experiment, early spring (tight-cluster) parasitism was low and in no year was the rate different between treatments, though year 4 parasitism in the alfalfa plots was 8.2%, while for the grass treatment, none was detected (Table 3). While not statistically significant in this experiment, this may be an indication that alfalfa cover may encourage leafroller parasitism. In alfalfa plots, parasitism increased each year and achieved the highest level during year 4 of the experiment, while grass treatment parasitism rates remained fairly constant and lower overall.

Later spring (at petal fall) parasitism rates were higher overall than earlier in the growing season (Table 3). At this time, in years 3 and 4, alfalfa plot parasitism was significantly greater ($p < 0.001$) but in year 2 grass plot parasitism was greater ($p < 0.001$). The single highest rate (18.7%) for all years regardless of treatment was in alfalfa for year 4.

Parasitism rates of the summer generation were often relatively high, for the most part greater in the first years and lowest the fourth year of the experiment (Table 3). Collection timing for summer generation *P. pyrusana* parasitism assessment was somewhat inconsistent between years. The extremely high summer generation parasitism rates, especially in year 2 of the study, may be partly attributable to sample timing, which may have been when few summer generation *P. pyrusana* larvae were present and subjected to intense parasitoid pressure.

Seemingly, the endemic leafroller population outpaced the parasitoid population increases during the summer of year 2 so that parasitoid populations lagged substantially behind during years 3 and 4 resulting in overall low rates of parasitism in these years.

Parasitism of Sentinel Obliquebanded Leafroller

Parasitism rates of sentinel larva did not increase over the duration of the study. For alfalfa plots, parasitism was lowest in the final year and for grass

TABLE 3 Leafroller Larvae Parasitism Percentage Rates for Three Sampling Times

Year	Tight cluster		Petal fall		Summer	
	Alfalfa	Grass	Alfalfa	Grass	Alfalfa	Grass
1	0.0a	0.0a	No data	No data	41.7a	85.0a
2	0.0a	12.5a	3.26a	4.9a	93.3a	97.3a
3	1.9a	2.31a	12.9a	7.66b	51.4a	52.8a
4	8.2a	0.0a	18.8a	5.6b	42.6a	36.9a

Each stage and each year were analyzed separately. Means within rows followed by the same letter are not significantly different ($p = 0.05$).

TABLE 4 Overall Annual Retrieval and Parasitism Rates (%) of Sentinel Obliquebanded Leafroller Larvae

Year	Alfalfa	No. parasitized/total collected	Grass	No. parasitized/total collected
1	38.5a	34/88	26.4a	41/155
2	47.0a	108/229	43.2a	73/168
3	32.4a	86/265	33.5a	85/253
4	27.1b	61/225	29.2a	71/243

Means within rows followed by the same letter are not significantly different ($p = 0.05$). Treatments were compared for each year separately.

the final year nearly equaled year 1. Grass plot parasitism was greater than alfalfa plots ($p = 0.033$) in year 4 only (Table 4). Parasitism rates between 10-day sampling periods, for both covers, varied substantially, as did parasitism rates between treatment replicates for any given 10-day set. Similar to *P. pyrusana* parasitism, the highest parasitism rates seemed to correspond to lower larvae retrieval rates. In year 4, several times as few as 5 larvae were retrieved from a set of 20 in a plot. We noted many spiders and earwigs in empty retreats when searching for sentinel larvae. It may be that increased predation caused some leafroller mortality.

Parasitoid Species

In addition to the eulophid *Colpoclypeus florus*, four different braconids were reared from collected leafroller larva; two *Apanteles* spp. and two *Bracon* spp.; identification at the species level was not made and are referred to herein as Braconidae. The tachinid reared from leafrollers was identified as *Nemorillia pyste* (Walker).

At tight cluster for both treatments in all years, only Braconidae were reared from *P. pyrusana* and in year 3, when *P. pyrusana* densities peaked, they were found from all plots. In the final year of the study, parasitoids were reared only from leafroller larvae collected in the alfalfa treatments at this sampling time.

In year 1, only tachinids were reared from sentinel obliquebanded leafroller larvae, Braconidae and *C. florus* were found in subsequent years. Generally, tachinids accounted for most early season parasitism of sentinel obliquebanded leafroller larvae while Braconidae and *C. florus* dominated later season.

Fruit Cullage From Leafrollers

Yield for both covers during all years of the study were acceptable and reflective of commercial orchards in the region (Fiji has a proclivity for alternate bearing). Excessive cullage was largely due to codling moth injury (data not reported). Damage to fruit from leafrollers increased annually for

TABLE 5 Yield and Cullage Rates From All Causes and From Insect and Leafroller Damage Specifically for Each Experimental Year

	Total yield bins/plot	Total cullage bins/plot	Percent cullage all causes	Percent of culls w/ insect damage	Percent of culls w/ leafroller damage	Cullage- bins/acre w/ leafroller damage
Year 1						
Alfalfa	40.3	12.9	31.3	36.0	11.3	1.5
Grass	31.3	10.5	33.5	56.0	33.0	3.4
Year 2						
Alfalfa	29.3	6.9	24.0	56.0	33.3	2.3
Grass	34.7	10.1	29.1	58.6	37.0	3.7
Year 3						
Alfalfa	38.8	13.4	34.5	73.0	54.3	7.3
Grass	37.3	10.3	27.6	60.0	49.3	5.1
Year 4						
Alfalfa	40.2	19.1	47.5	30.0	17.0	3.2
Grass	25.7	13.4	52.1	31.0	18.0	2.4

the first three years and then decreased in year 4 (Table 5). In year 4, alfalfa and grass treatment damage were one-third that of the previous year. In year 4, cullage exclusively from leafroller damage for the alfalfa treatment was 8.6% and for grass 9.0% of total cullage of harvested fruit. It is generally accepted that cullage rates for Fuji apples are typically greater than for most other commercial cultivars grown in North Central Washington, and in any given year leafroller damage, under conventional management regimes, will account for approximately 6 to 10% of fruit cullage. Thus, it would appear that, without insecticides, leafroller damage for both covers was not substantially greater in year 4 than what might be expected.

In year 4, based on our evaluation of cullage, alfalfa plots lost 1.64 bins of fruit per acre and grass plots 1.2 bins attributable to leafroller damage exclusively. Cost of production for Fuji apple is about \$150 per bin (Hinman et al., 1998). The cost of conventional leafroller control in Washington apple orchards (a delayed dormant chlorpyrifos application and two *Bt* covers) this same year was approximately \$155.00 per acre (H. Teas, Northwest Wholesale, Wenatchee, WA, USA, personal communication). Thus, the cost of management would exceed the value of damaged fruit. Net loss exclusively from leafroller damage the previous year (year 3), when leafroller populations peaked, was likely much greater and over the course of four years it may have been that total fruit loss to leafroller damage would have exceeded cumulative costs of control.

CONCLUSIONS

Our findings do suggest that in the absence of broad-spectrum insecticide treatment *P. pyrusana* populations can increase substantially over a

three-year period before some reduction is noted. In the final year of this study, leafroller populations for both cover treatments were somewhat higher than would typically be tolerated in a commercial orchard under a pesticide-based management system. That population, however, may not necessarily be an indication of an economically consequential infestation level as elevated leafroller populations and the fruit damage they caused did not appreciably affect overall cullage rates. This is a point for further investigation.

We detected no adverse effect from the alfalfa cover relative to leafroller population or biological control. For both of the experimental management regimes there was, at first, a substantial increase and then an equally substantial decrease in population levels of *P. pyrusana* but only a modest to negligible gain in parasitoid activity (Bostanian et al., 2001; Brown and Adler, 1989; Brown and Welker, 1992). However, slightly increased parasitism rates in alfalfa treatment plots may indicate the possibility of enhanced parasitism with an alfalfa cover, particularly in the later spring. Of the six parasitoids detected, tachinids were more prevalent in the early season and may have potential for further augmentation to provide overwintering generation biocontrol. Braconidae dominated the mid-season and *C. florus* was not prevalent until fall, supporting the understanding that it does not overwinter in the orchard or in association with orchard leafrollers (Brunner, 1996; Evenhuis, 1974; Nobbs, 1997).

A differential host preference may have been exhibited by the various parasitoids. Braconidae proved to be a prevalent early season *P. pyrusana* parasitoid, while tachinids dominated early season obliquebanded leafroller parasitism. Altogether Braconidae and *C. florus* were the dominant parasitoids of *P. pyrusana* with tachinids only occasionally detected. Conversely, tachinids were a more substantial component of the obliquebanded leafroller parasitoid complex each year of the experiment.

Brunner et al. (2001) demonstrated a rather profound sensitivity of *C. florus* to carbaryl. It may be that the carbaryl used as a fruit-thinning agent the first two experimental seasons adversely affected *C. florus* and other parasitoids. The elimination of carbaryl as a thinning agent in late spring and early summer could be an important step in conserving parasitoids and increasing early season parasitism.

The sharp decline in *P. pyrusana* populations, in both treatments, cannot be attributed to parasitism. A granulovirus, as reported by Phannensteil et al. (2004), may have contributed. Confirmation that *P. pyrusana* larvae were infected with this virus was made via evaluation of symptoms in consultation with a USDA insect pathologist (L. Lacey, personal communication). Lacey et al. (2001) discuss the potential of granuloviruses for the biological control of lepidopteran pests in apples. Additionally, generalist predators may have contributed to *P. pyrusana* biological control in test plots (Bostanian et al., 1984). Though it was not within the scope of this experiment to quantify the presence or predatory activity of spiders (Araneae)

and earwigs *Forficula auricularia* (Linnaeus), they became increasingly abundant and were readily found on trees and in leafroller retreats in all plots in year 4. Generalist predator feeding on leafroller eggs and larvae in apple orchards might contribute substantially to natural control of leafrollers (Miliczky and Calkins, 2002).

This experiment does not allow us to posit that endemic leafroller populations stabilized in this agro-ecosystem or whether the legume cover was advantageous from an orchard agro-ecosystem design and management perspective (Hill et al., 1999). However, the legume cover did not result in elevated leafroller populations over the standard grass cover or exacerbate leafroller injury to fruits.

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