Biological Control of the Ambermarked Birch Leafminer (Profenusa thomsoni) in Alaska

BIOLOGICAL CONTROL OF THE AMBERMARKED BIRCH LEAFMINER (*PROFENUSA THOMSONI*) IN ALASKA

A Dissertation Presented

by

ANNA L. SOPER

Submitted to the Graduate School of the

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DOCTOR OF PHILOSOPHY

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Entomology

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DEDICATION

To my parents,

for your love, laughter, and unwavering support.

ACKNOWLEDGMENTS

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ABSTRACT

BIOLOGICAL CONTROL OF THE AMBERMARKED BIRCH LEAFMINER (*PROFENUSA THOMSONI*) IN ALASKA

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The ambermarked birch leafminer (AMBLM) (*Profenusa thomsoni*) is an invasive leafminer native to the Palearctic from the United Kingdom to Turkey to Japan. It was introduced to the eastern United States in 1921 and has since spread to the mid-western U.S. states and Canadian provinces. This leafminer was introduced to Alaska in 1996, where it has since spread over 140,000 acres, from Haines to Fairbanks. The most severe damage is found throughout the Anchorage bowl, which extends south to Girdwood and North to Wasilla. The damage caused by *P. thomsoni* can be severe, defoliating entire trees.

In 2006, it was noted that urban areas in Alaska experienced higher densities of AMBLM leafminer than adjacent forested areas. To examine the effects of habitat on leafminer densities, twenty permanent plots were established in Anchorage, Alaska in 2006 and were classified as urban and forest (ten each). Temperature records for the twenty permanent sites showed that average daily temperatures and average accumulated degree-days differed significantly between urban and forest sites. In 2007 and 2008, leafminer abundance in each habitat was examined weekly at six plots (three urban and three forest) within the city of Anchorage. Asynchronous emergence, flight, and oviposition times were

observed between leafminers in forests versus urban areas, with peaks of these parameters in forests being about three weeks later than in urban areas.

To control the spread and effects of *P. thomsoni*, a cooperative biological control project was launched in 2003 and the parasitoid wasp *Lathrolestes thomsoni* (Hymenoptera: Tenthredinidae) was selected for release. Parasitized leafminer larvae were collected from the provinces of Northwest Territories and Alberta, in Canada and transferred in soil tubs as pre-pupae to Alaska. From 2004-2008, 3636 adult *L. thomsoni* adults were released in birch tree stands in Anchorage, Soldotna, and Fairbanks, Alaska. Parasitoids have been recovered at all release sites in Alaska and have established populations at most release sites. Currently, AMBLM densities have declined by over 40% in the Anchorage area and the spread of the leafminer throughout the state appears to have slowed.

Throughout the course of the biological control program two additional parasitoids were discovered attacking *P. thomsoni* in Alaska. The first, *Lathrolestes soperi,* an endoparasitoid with similar biology to the released parasitoid *L. thomsoni*, was found to attack early instar larvae within the leaf. The second species, *Aptesis segnis*, is an ecotoparasitoid that attacks pupae and prepupae in their earthen cells in soil. *Lathrolestes soperi* was found to contribute a significant proportion of mortality against the leafminer. The presence of *A. segnis* in the parasitoid guild raised mortality of *P. thomsoni* to 40.3%, showing that the percent parasitism by *A. segnis* was 26%, double that provided by *L. soperi.* This suggests that *A. segnis* is the dominant parasitoid in the guild. It is unknown what effect that the introduced wasp *L. thomsoni* will have on the presumably native *L. soperi* and if one species will outcompete the other over time, or both will coexist. Future work on this system is recommended in five to ten years to see if *L. thomsoni* and *L. soperi* populations remain stable or to see if one parasitoid outcompetes the other and if *A. segnis* maintains its dominant place in the system.

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CHAPTER 1

MECHANISMS AFFECTING *PROFENUSA THOMSONI* (HYMENOPTERA: TENTHREDINIDAE) DENSITIES IN URBAN VERSUS FOREST ENVIRONMENTS IN ANCHORAGE, ALASKA

Abstract

The ambermarked birch leafminer (*Profenusa thomsoni*) is an invasive sawfly that has reached damaging densities in Alaska. Studies in 2006 indicated that *P. thomsoni* densities were higher in urban areas versus forests. Temperature records for twenty permanent sites found that average temperatures and the average degree-days accumulated differed significantly between urban and forest sites. In 2007 and 2008, leafminer abundance in each habitat was examined weekly within the city of Anchorage. Asynchronous emergence, flight, and oviposition times were observed between leafminers in forests versus urban areas, with peaks of these parameters in forests being about four weeks later than in urban areas.

Introduction

Profenusa thomsoni (Konow) is a European sawfly that feeds inside the leaves of several species of birch (*Betula*). The leafminer is parthenogenetic, with populations consisting only of females (Benson 1959) and has five feeding instars in the leaf. The sixth (non-feeding) larval instar drops from the leaf into the soil where it overwinters as a prepupa (Martin 1960; MacQuarrie 2008). The damage to birch trees is primarily aesthetic but is hypothesized to reduce growth and weaken trees, leaving them susceptible to pests or secondary infections (Hoch et al. 2000; Snyder et al. 2007).

Profenusa thomsoni invaded North America at the beginning of the 20th century and was first reported in the eastern United States in 1923 (Ross 1951; MacQuarrie 2008), where it never became a high density pest. In eastern North America, this sawfly occurs in the United States from New England to the Great Lake States and in Canada from the Maritimes to Manitoba. In western North America, this leafminer was first reported in Alberta, which it invaded before 1970 (Digweed 1995) and where it reached high densities in the early 1990s (Digweed 1995). From Alberta, the leafminer spread north and west in Canada and reached Haines, Alaska in about 1991 (Snyder et al. 2007). It was not recognized as invasive in Alaska until 1996 when it was discovered damaging birch trees in Anchorage (Snyder et al. 2007). Possible routes of introduction to Alaska include movement of infested plants (MacQuarrie 2008), movement of adults in vehicles by people (Digweed and Langor 2004), general cargo introduced through the port of Anchorage (Snyder et al. 2007), or perhaps some combination of these routes. However, the actual source of introduction is unknown.

Ambermarked birch leafminer now occurs in birch forests across North America (Digweed 1998); however, populations in such forests do not reach as high density as in many urban sites (Babendreier 2000; MacQuarrie 2008). Digweed and Langor (2004) noted *P. thomsoni* populations in Yellowknife, Northwest Territories, to be highest in urban centers, to decrease toward the urban edge, and to be rare in surrounding forests. In Alaska, Snyder et al. (2007) found that new leafminer infestations were concentrated in population centers and along major roads. High *P. thomsoni* densities have been found in forested areas, but such infestations are rare (Snyder et al. 2007; MacQuarrie 2008). Higher leafminer densities in cities may occur because urban heat islands provide a warmer habitat. In locations at high latitudes such as Alaska, where seasonal heat may be limiting, urban warming may be an important determinant of the success of an invader. Alternatively, urban habitats may support higher insect densities due to lower populations of generalist natural enemies. A better understanding of the role of urban heat effects on pest densities and in promoting invasion success will be useful to improve control methods for invasive species.

This study examined populations of *P. thomsoni* in urban and forested areas in the city of Anchorage. My goal was to quantify differences in pest densities between these habitats and assess the role of heat environments as possible causes for observed differences.

Methods

Definitions of habit types and selection of study sites

The city of Anchorage includes within its boundaries 10,946 acres of municipal parkland, most of which is covered with native forest. Twenty sites within the city were chosen and classified as either forest or urban. Forest sites were ones where sample trees grew in un-mowed vegetation, in a stand of naturally occurring birch, and where the larger setting was mostly forest. Urban sites were ones where sample trees grew in mowed vegetation or a concrete median strip, located more than fifty feet from natural vegetation. At all sites (both urban and forested), the sampled tree was a paper bark birch (*Betula papyrifera* Marsh).

Sites used in the study, in addition to fitting the treatment definitions, were selected because of their accessibility and assurances they would not be sprayed with insecticides. For certain measurements of insect phenology, three urban and three forested sites were selected from the pool of 20 sites, for specific labor-intensive experiments. For some aspects of the study, data were collected annually for 6 years (2006-2011). For studies on effects of heat budgets on phenology of selected leafminer life stages, studies were done only in 2007 and 2008 (further details below).

Overview of insect sampling

From 2006 to 2011, leafminer density samples were taken at all 20 permanent sites. Fifty randomly selected leaves were collected, from each site in the third week of July and again in the third week of August. *Profenusa thomsoni* density was recorded as (1) the percentage of the randomly selected leaves that were mined, (2) the number of larvae per mined leaf, and (3) the relative proportion of all larvae that were in each instar. As a measure of larval survivorship, numbers of fifth and sixth instars and their cast skins were compared between forest and urban sites.

In 2007 and 2008, more detailed observations were made on the phenology of leafminer life stages, including the numbers of adult leafminers, the number of newly laid eggs, and the number of mature larvae dropping from leaves per area per unit of time. These observations were done at three sites of each habitat type and differences in temperature were evaluated as an explanation for differences in leafminer density by habitat type. The following population parameters were measured:(1) emergence cone counts of adult leafminers per unit area per week, (2) leafminer adult catch on sticky cards, (3) leafminer egg recruitment per leaf per week, (4) number of leafminer larvae dropping from leaves per unit area twice per week, and (5) temperature data (once every three hours) within the canopy of each sample tree. Emergence cone trapping, oviposition sampling, and larval drop catch were done at three urban and three forested sites due to the labor-intensive nature of these sampling methods. Other measures (sticky card trapping of adult leafminers and collection of field temperature data) were taken at all 20 sites.

Adult *P. thomsoni* emergence

To see how many adult *P. thomsoni* emerged per unit area of soil surface beneath birch canopies, emergence cones (orange traffic cones (area = 122.7 cm^2 modified to have a clear 2 oz. diet cup on top) were placed at the same 6 permanent sites (three urban and three forest). Ten cones were placed underneath the canopy of each tree (total of 30 per treatment) and left over the exact same patch of soil for the whole season. The cups on top of the emergence cones were replaced weekly once leafminer emergence began. Cups were collected and the number of adult *P. thomsoni* counted.

Profenusa thomsoni sticky card trap catch

To see if fewer adult P. thomsoni were present in forested vs. urban environments, yellow sticky cards (7.6cm x 12.7cm) (BioQuip) were placed in the lower canopy of birch trees at each of the 20 permanent sites. The cards were changed weekly beginning in the fourth week in June and ending in the last week of August. Numbers of adult AMBLM were counted and the sticky cards were preserved in a freezer.

Profenusa thomsoni oviposition rates

To determine the number of eggs laid by the leafminer per leaf per week, 600 birch leaves were individually bagged with conical white organdy bags (10.8 cm by 20 cm**)** (Papermart) before *P. thomsoni* emergence in the third week in June. At the same six study sites, 100 bags were placed over leaves, and, of these, 10 bags were removed weekly at each site. After one week, the newly exposed leaves were collected and a new set of leaves was un-bagged and tagged. The number of eggs laid/leaf was recorded for each collected leaf.

Profenusa thomsoni larval drop

To evaluate differences in total seasonal *P. thomsoni* larval drop between urban and forested sites, the number of larvae dropping per unit area beneath birch canopies was determined. Six tubs (36.8 cm x 20.3 cm x 11.7 cm) (Sterilite) were placed underneath birch trees at each of the same six permanent sites. The tubs were covered with large-cell plastic mesh (Gardeneer) to keep birds and small mammals from eating larvae. The tubs were examined twice weekly and all larvae were counted and discarded.

Heat profiles in research trees

To measure heat profiles over time at all research trees, I-button data loggers (Dallas Semiconductor) were placed on each sample tree (one per tree) at the twenty permanent sites to measure the accumulation of degree-days. Temperature was recorded every three hours at each site for a total of eight samples per site per day. Temperature data were monitored from the third week in June through the first week in September in

2007 and from 1 June until the last week of September in 2008. I-button data loggers were placed in conical white organdy bags (10.8 cm by 20 cm**)** (Papermart) and placed within the tree's canopy out of direct sun on the north side of the tree.

Calculation of day degrees

Degree-day values per day were calculated by taking the temperature (from every three hour intervals) minus a physiological growth threshold of 5°C. The average number of day degrees was calculated for each date. Since no laboratory data were available as to the lower thermal threshold of ambermarked birch leafminer, the value used in analysis was selected based on knowledge of modeling (from examining field distributions) of *P. thomsoni*. In previous work on *P. thomsoni*, models were developed by inference from stage-specific peak densities in field data, using 5°C as the physiological growth threshold (Digweed 1995)**.**

Leaf Drop and Consequences of Late Oviposition in Forests for *P. thomsoni* Mortality

To determine the latest date possible for oviposition to occur and larvae to fully develop before leaf drop, I used modeling of leafminer development by MacQuarrie (2008). Leafminer development values were modeled from leafminer distributions in the field and stage specific development times are shown (Table 1). Development times and accumulated degree-days were compared between the two treatments and the percentage of larvae that fail to develop due to lack of adequate heat was calculated.

Statistical Analysis

All statistical tests were performed in JMP v.9.0 (SAS). Oviposition data, emergence cone data, sticky card data and larval drop data were all log transformed (n+1) and then analyzed. Testing for unequal variance was done using Bartlett's test. For ease of viewing, data in figures were not log transformed. To evaluate that the timing of events was significantly different in our sampling methods (emergence, oviposition, trap catch, and larval drop) in the two habitats a chi-square goodness of fit test was used with $\alpha=0.05$.

Results

Heat profile effects on differences of *Profenusa thomsoni* densities

Using a repeated measure ANOVA, the number of degree-days was examined on sampling dates. In 2007 and 2008, significantly more degree-days were accumulated in the urban environment than the forest environment (2007: $F = 74.58$, df= (1, 1423), $P = <0.0001$); (2008: $F = 62.93$, df =(1, 2055), $P = <0.0001$) (Figures 1 and 2). Additionally, average daily temperatures were observed to be higher in an urban treatment vs. the forest treatment (Figure 3).

Density Sampling

From 2006-2011, there was an overall decline in leafminer densities at both urban and forest sites. In July the percent of leaves mined was significantly different between the two treatments in all years except 2007 and 2009 (Figure 4), which were the two warmest years of the study. In August, urban and forest densities were only statistically significant in the year 2011 (Figure 4).   Larvae per mined leaf only varied significantly in August 2006, with more larvae occurring per leaf in a forest environment (Figure 5). Larval instar distributions varied between the two environments, with the urban environment having more larvae in later instars (4-6th) than the forest habitat (Figure 6).

Adult *P. thomsoni* emergence

Data on adult sawfly emergence per unit area were collected only in 2007 and 2008. In 2007, no statistical difference was found between the urban and forest environments in the number of adult sawflies emerging per unit area of soil (two-tailed t-test: $t = 0.9879$, $df = 52$, $P = 0.327$). However, in 2008, a statistical difference was observed in the number of emerging leafminer adults (two-tailed t-test: $t = 3.81$, $df = 52$, $P = 0.0004$). The number of adult leafminers emerging was significantly different for the urban versus forest treatments on every date except for 7/23/2007 and 6/25/08 (Figure 7). In both 2007 and 2008, adult leafminer emergence peaked on July 2 in the urban environment and on July 31 in the forested environment (Figure 7).

***Profenusa thomsoni* sticky card trap catch**

There was no significant difference in the total (summed over all sample dates) number of adult leafminers caught on sticky card traps in 2007 (two-tailed t-test: $t = 0.005$, $df = 198$, $P = 0.995$), or in 2008 a significant difference was observed between the treatments (two-tailed t-test: $t = 1.99$, $df = 198$, $P = 0.0473$). For individual sample dates, differences between habitats were detected in 2007 on 2 July, 9 July, 16 July, and 30 July 2007 and in 2008, on 9 July (Figure 8).

Profenusa thomsoni oviposition

There were no significant differences found in the total number of eggs oviposited per leaf (summed over all sample dates) in either 2007 (two-tailed t-test: $t = 0.496$, $df = 52$, $P = 0.621$) or 2008 (two-tailed t-test: $t = 1.545$, $df = 58$, $P = 0.064$). However, on particular sample dates the number of eggs laid per leaf was significantly different between treatments in 2007 on 2, 9, 23, and 30 July and in 2008 on 9 July (Figure 9).

Profenusa thomsoni larval drop

In both 2007 and 2008 significantly more mature larvae dropped from leaves to the soil in the urban environment (two-tailed t-test: 2007 $t = 9.77$, $df = 40$, $P = <0.0001$; 2008 $t = 5.3$, $df = 64$, $P = <0.0001$). Furthermore numbers of larvae dropping were found to be statistically significant on all dates in 2007 except on 28 August and 3 September. In 2008 significantly more larvae dropped in the urban environment on 1 September and 9 September (Figure 10).

Leaf Drop and Consequences of Late Oviposition in Forests for *P. thomsoni* Mortality

In 2007 only 2-4% of eggs laid failed to reach development in the forest and all eggs laid in the urban treatment completed development. However, in 2008, which was a significantly colder season than 2007, 19-30% of eggs failed to reach development in the forest compared to 2-4% in the warmer urban environments (Table 2).

Discussion

Most studies on factors influencing insect distributions have focused on large areas rather than local distributions (Bach 1993). Distributions of insect herbivores within local habitats (or microhabitats) tend to have discrete boundaries but the explanation for these tend to be unknown or complex (Crawley 1983; Bach 1993). There are a variety of factors that can influence herbivore density particularly plant characteristics such as host-plant density (Bach 1980), plant age (Price 1991), height of host plants (Fritz 1990), and degree day accumulation (i.e. heat) (Tamiru et al. 2012).

Heat (i.e. the accumulation of degree-days), arguably is one of the major factors affecting insect distributions and in many cases can lead to the success or perhaps lack of success for invaders. In northern latitudes seasonal heat is limited and urban centers supplement local heat budgets, creating to urban heat islands. Thermal biology largely frames where organisms can complete their life cycles and form persistent populations. Thermal characteristics may prevent an organism from living in a location if it is too hot or alternatively does not provide enough heat or growing degree-days to complete development. The latter scenario typically happens in habitats such as northern latitudes and high elevation. Given time, life history strategies should evolve to compensate for such limits. For example, the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), has a life cycle that varies between one generation per year and one generation per two years, depending on degree of host tree resistance and the local heat budget (Cappaert 2005). This life history strategy, however, is only effective if you can enter diapause and resume feeding the next year. A leaf-mining insect is prevented from doing this because the structure needed for development (a leaf) cannot carry over year to year. Therefore, in the case of leafminers, selection acting on the insects in heat-limited environments will favor those individuals with faster development rates able to complete their life cycle in a single season.

Invasive insects colonizing areas with deficient heat environments (in comparison the climate of the source location) may show evolution to tolerate the new environment. Hemlock woolly adelgid, *Adelges tsugae* Annand, an invasive species in the eastern United States, is able to thrive in the warmer mid-Atlantic states but is hindered by cold winters in northern New England states (Paradis et al. 2008). Climate change could relax those limits by allowing for warmer winters and summers. Eventually, evolution will select for faster development or may select for preference to oviposit in locally available warmer microclimates. Success of such an insect therefore, might vary from year to year and site-to-site based on yearly climate and local microclimates. In certain years, especially cooler seasons, the proportion of a population failing to complete develop may significantly affect the species' population dynamics. In Alaska in 2008, with the third coolest summers on record **(**NOAA 2008**)**, 20-30% of the eggs laid by *P. thomsoni* failed to reach development in the forest.

For the leafminer in this study, there were significant differences in pest density by urban vs. forest habitats in July. However, there was no significant difference between the number of leafminers emerging, flying, or ovipositing in these habitats. There was, however, a significant difference in the timing of these events, with leafminers emerging, ovipositing, and reaching larval maturity much later in the forest. Even though initial, early season, pest densities may not vary significantly between forest and urban sites, in cool summers like 2008 in Anchorage, fewer larvae will be able to complete development in cooler habitats such as forests. Furthermore, since damage to trees is aesthetic, the fact that there are fewer later instar larvae in the forest makes it appear there is less damage to the tree.

Tables

Table 1: Stage specific degree-days needed for *Profenusa thomsoni* to develop in Anchorage, Alaska based on modeling by MacQuarrie (2008).

Stage	Degree days needed to develop
Egg	66.0
1st instar	31.3
2nd instar	24.1
3rd instar	22.1
4th instar	43.7
5th instar	72.8
Total:	260.1

Table 2: Percentage of eggs oviposited by *Profenusa thomsoni* in Anchorage, Alaska that fail to fully develop in 2007 and 2008.

2007	Number of eggs/10 leaves (whole season)	Number of eggs laid too late	% Eggs failing to mature
Urban (sites)			
MedPark	126	0	0
UAA-Library	105	0	0
UAA-Providence	35	0	0
Forest (sites)			
Javier	154	4	2.6
Jewel Lake	64	0	0.0
Sitka	25	1	4.0
2008			
Urban (sites)			
MedPark	104	0	0
UAA-Library	138	0	0
UAA-Providence	143	2	1.40
Forest (sites)			
Javier	139	27	19.4
Jewel Lake	17	5	29.4
Sitka	13	1	7.7

Figures

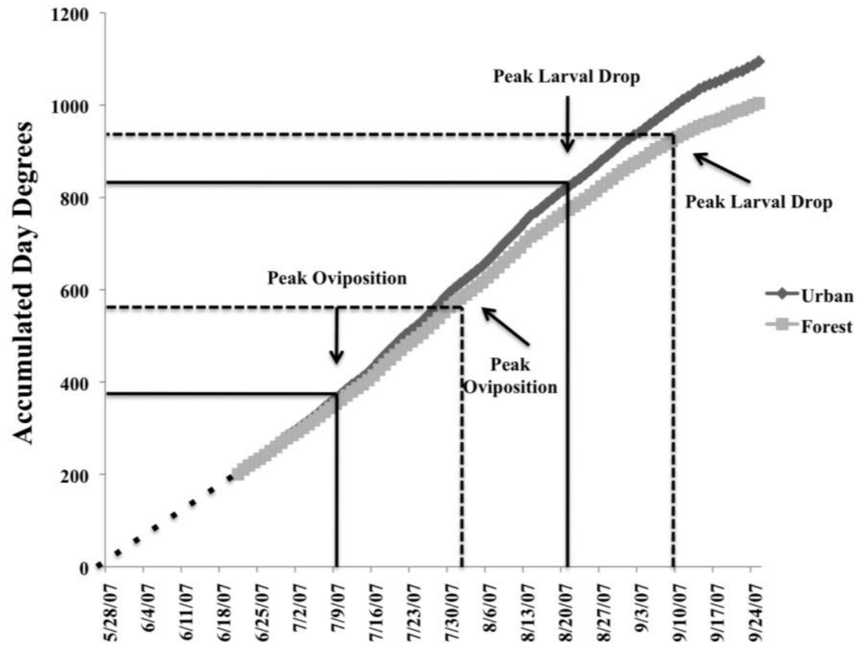

Figure 1: Accumulated degree-days in an urban vs. forest setting in Anchorage, Alaska from May-September 2007. Oviposition arrows mark the dates on which oviposition was first observed at field sites. Larval drop arrows indicate dates when peak larval drop occurred. Temperature data from 28 May -23 June 2007 was obtained from Weather Underground (2007) and was the same for both treatments.

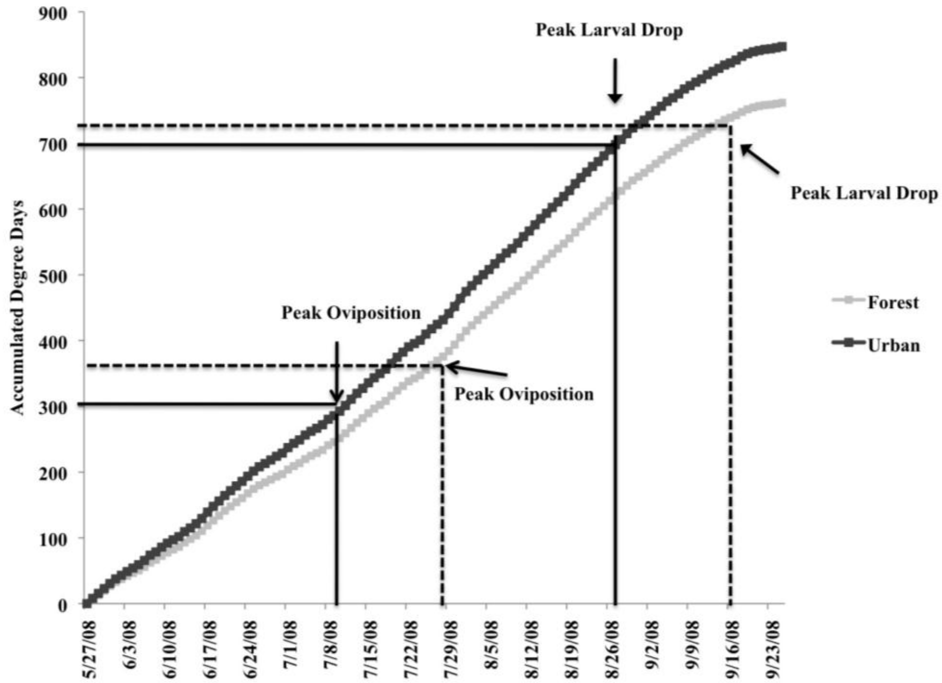

Figure 2**:** Accumulated degree-days in an urban vs. forest setting in Anchorage, Alaska from May-September 2007. Oviposition arrows mark the dates on which oviposition was first observed at field sites. Larval drop arrows indicate dates when peak larval drop is predicted to occur based on stage specific degree day modeling by MacQuarrie (2008).

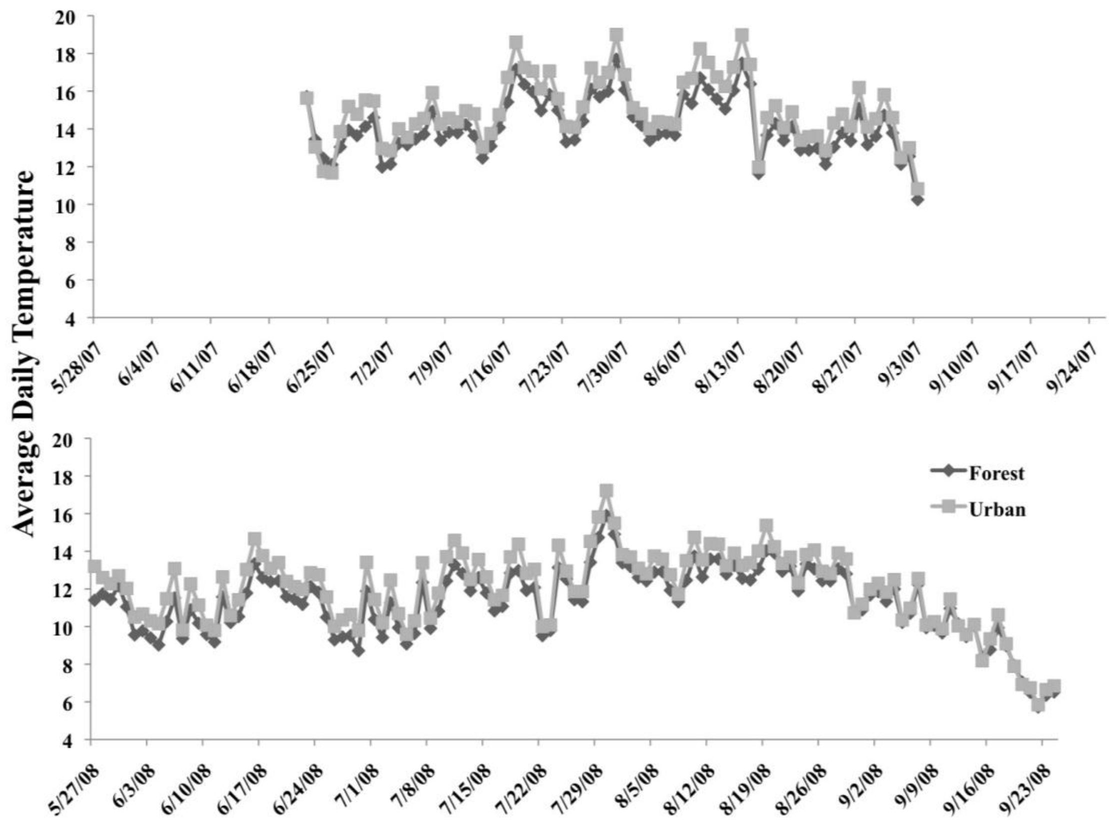

Figure 3: Average daily temperature in an urban vs. forested setting in Anchorage, Alaska from May –September 2007 and 2008.

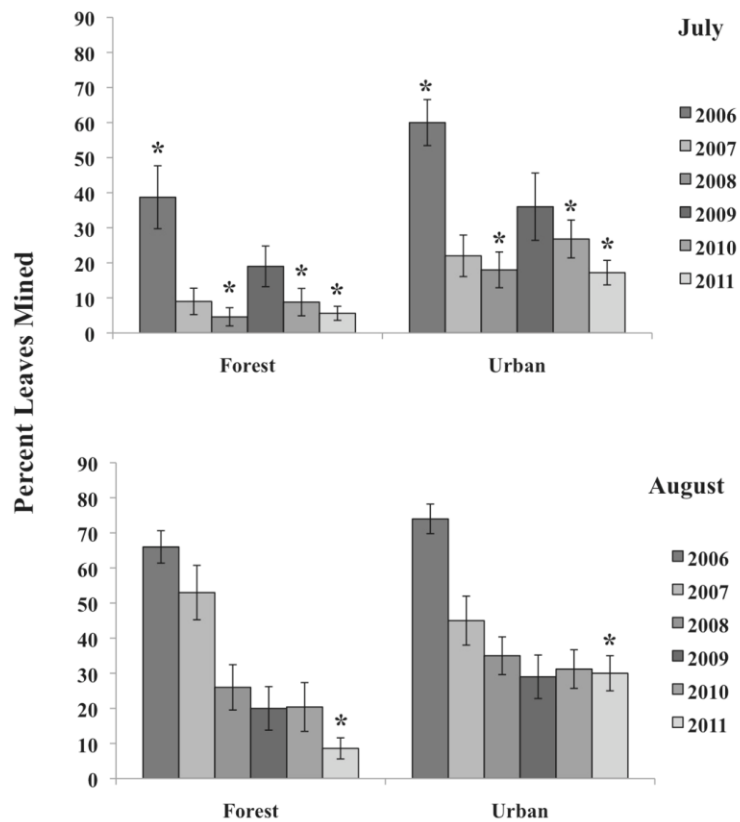

Figure 4: Percent of leaves mined by ambermarked birch leafminer (*Profenusa thomsoni*) in an urban vs. forest setting from paperbarked birch leaves collected in Anchorage, Alaska in the third weeks of July and August 2006-2011. Asterick (*) indicates significantly different results.

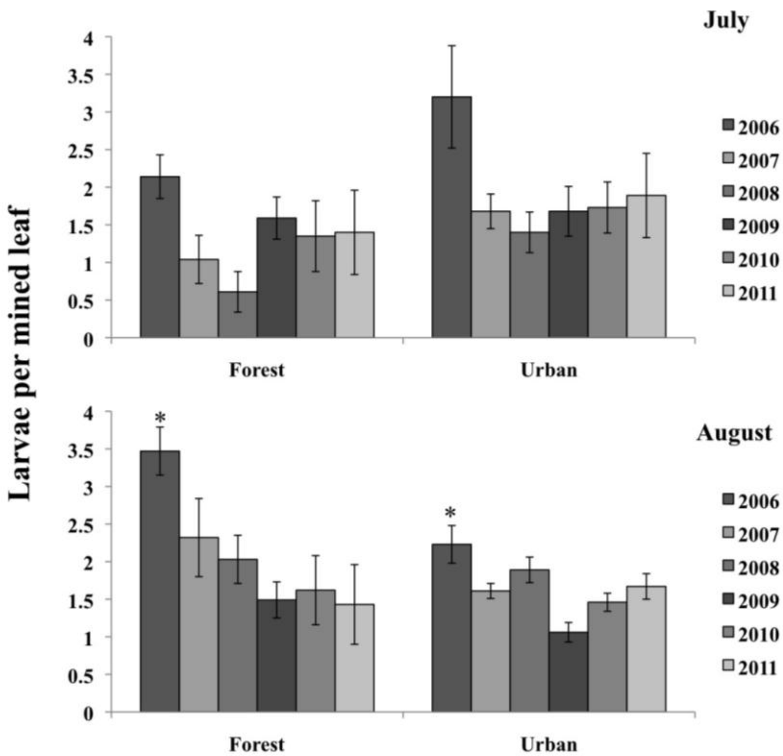

Figure 5: The number of *Profenusa thomsoni* larvae per mined paperbark birch leaf from a sample of leaves collected in Anchorage, Alaska in July and August 2006-2011.

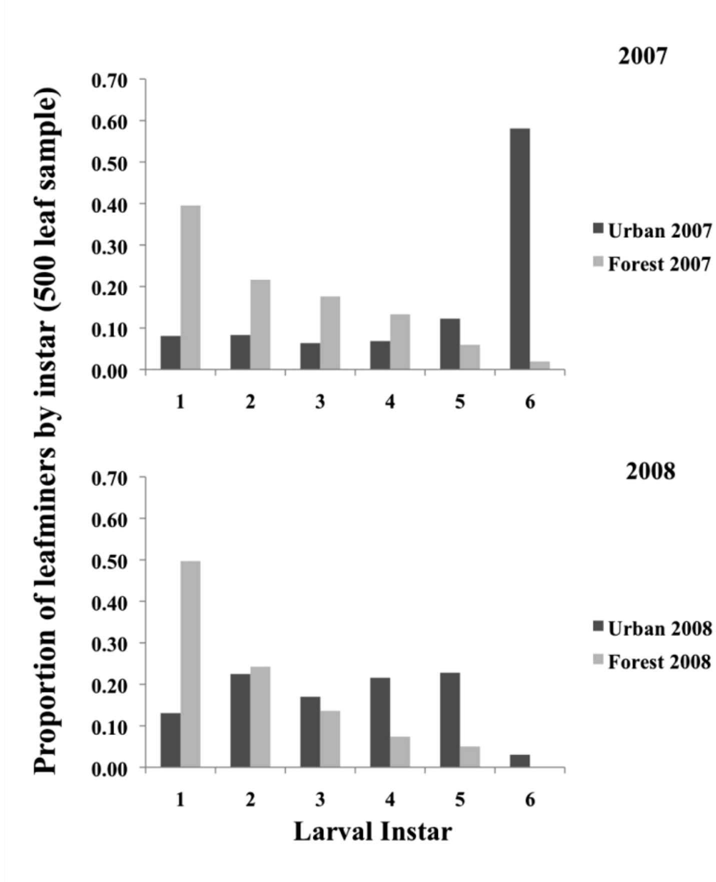

Figure 6: Proportion of *Profenusa thomsoni* larval instars found per sample of 500 leaves in an urban vs. forest environment in Anchorage, Alaska from 2007-2008.

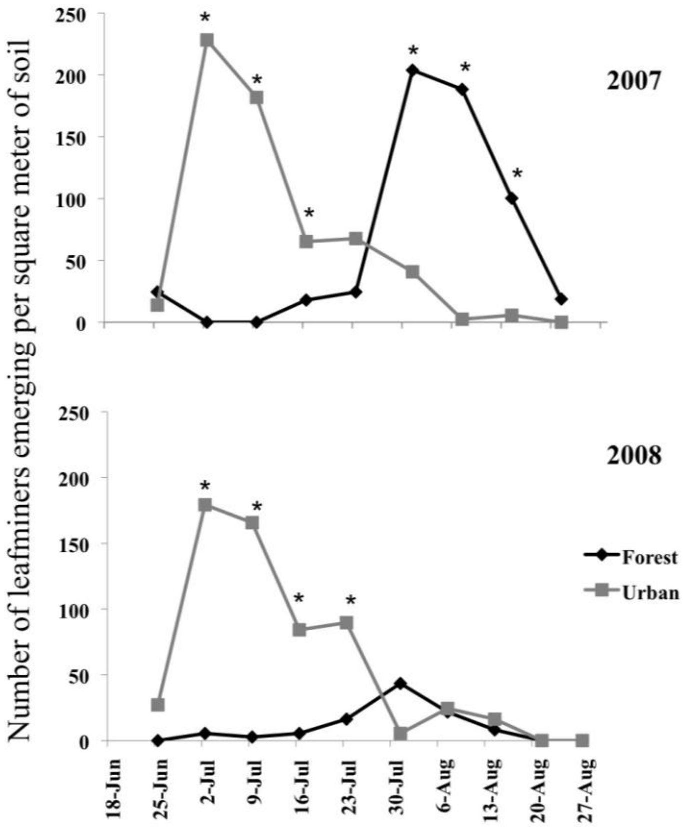

Figure 7: Number of adult leafminers (*Profenusa thomsoni*) emerging per square meter of soil surface in an urban versus forest setting in Anchorage, Alaska from June-August 2007 and 2008.

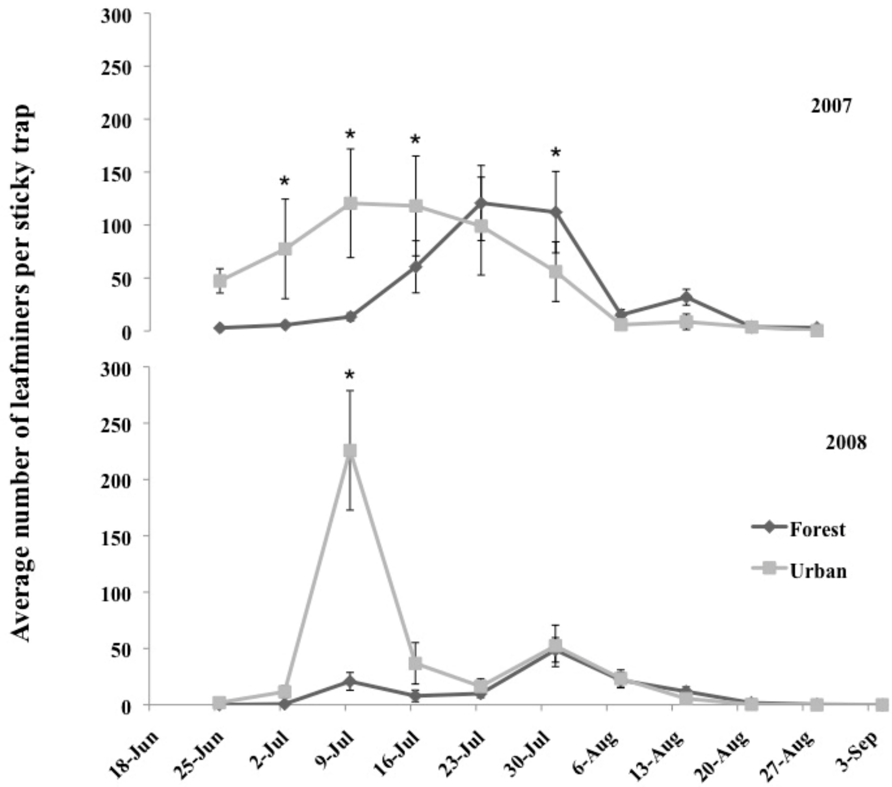

Figure 8: Number of ambermarked birch leafminers (*Profenusa thomsoni*) found per sticky trap in an urban vs. forested setting in Anchorage, Alaska from June-August 2007 and 2008.

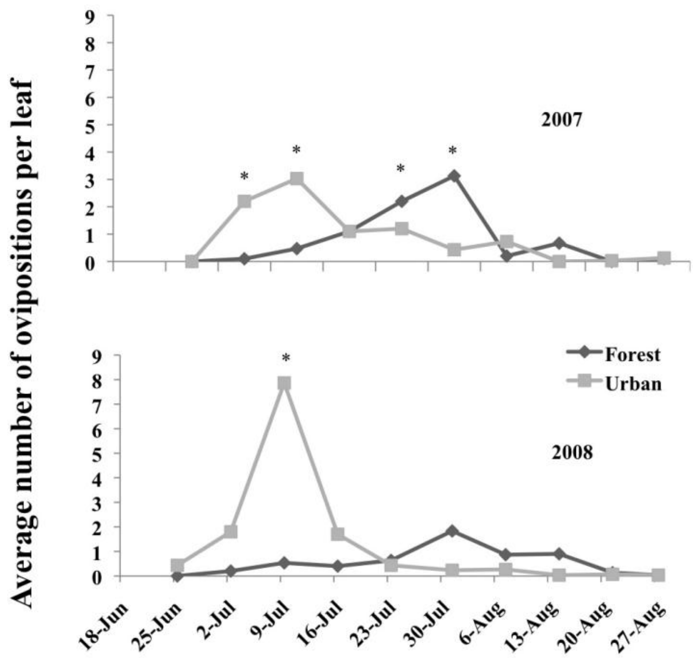

Figure 9: Average number of ovipositions by ambermarked birch leafminer (*Profenusa thomsoni*) found on paper bark birch leaves in an urban vs. forest environment in Anchorage Alaska from June-August 2007 (top) and 2008 (bottom).

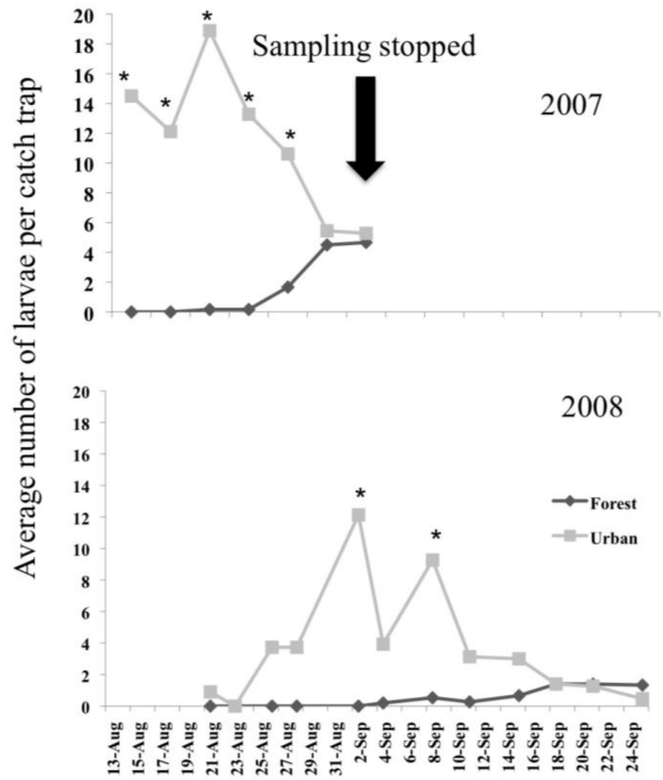

Figure 10: Average number of dropping *Profenusa thomsoni* larvae caught per tub in an urban vs. forest setting in Anchorage, Alaska from August-September 2007-2008.

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CHAPTER 2

INTRODUCTION, RELEASE, AND RECOVERY OF *LATHROLESTES THOMSONI FOR* BIOLOGICAL CONTROL OF THE AMBERMARKED BIRCH LEAFMINER, *PROFENUSA THOMSONI* (HYMENOPTERA: TENTHREDINIDAE), IN ALASKA

Abstract

The ambermarked birch leafminer, *Profenusa thomsoni* Konow, was first discovered in Haines, Alaska in 1991 but was not accurately identified until 1996 when the leafminer invaded Anchorage, Alaska and became a widespread pest. A biological control project was launched in 2003 and the parasitoid wasp *Lathrolestes thomsoni* (Hymenoptera: Tenthredinidae) was selected for release. Parasitized leafminer larvae were collected from the Northwest Territories and Alberta, Canada and transferred in soil tubs as pre-pupae. From 2004-2008, 3636 adult *L. thomsoni* adults were released in birch tree stands in Alaska. Parasitoids have been recovered at all release sites in Alaska and have established populations at most sites. The current status of the pest and its introduced and indigenous natural enemies is discussed.

Introduction

The ambermarked birch leafminer, *Profenusa thomsoni* Konow (Hymenoptera: Tenthredinidae), is a Palearctic sawfly native to Europe, central Asia, and Japan (Benson 1959; Smith 1971; MacQuarrie 2008). *Profenusa thomsoni* is considered a minor pest of birch trees (*Betula* spp.) in Europe and is found only at low densities (Benson 1959). *Profenusa thomsoni* invaded North America in the early 1900s and was first recorded in

Connecticut in 1923 (Ross 1951; MacQuarrie et al. 2007), where it never reached outbreak levels. By the 1950s, this leafminer was found throughout eastern Canada (Ontario) and the mid-western United States (Benson 1959; MacQuarrie et al. 2007). *Profenusa thomsoni* was found in Alberta before 1970 (Digweed et al. 1997), where it became a significant pest until it came under biological control in the early 1990s (Digweed et al. 2003). *Profenusa thomsoni* invaded Haines, Alaska in 1991, but the pest was not correctly identified until 1996 when many birch trees showed symptoms of damage in Anchorage (Snyder et al. 2007). The first surveys for damage by this pest in Alaska were conducted from 2004-2006 (Snyder et al. 2007), when *P. thomsoni* was found to be widespread throughout the south central region of Alaska, as well as in the Fairbanks area. Aerial flight surveys conducted in 2003 estimated that the damage affected over 12,800 hectares in the Anchorage bowl and extended into the Matanuska-Susitna (Mat-Su) Valley (Whittwer 2004; Snyder et al. 2007)

History of the Biological Control Program

In 2003, a classical biological control program was initiated with the USDA Forest Service, the Canadian Forest Service, and the University of Alberta with the goal of introducing to Alaska a highly specialized parasitoid known at the time as *Lathrolestes luteolator* Gravenhost (Hymenoptera: Tenthredinidae) (now correctly recognized as *Lathrolestes thomsoni* Reshchikov). This parasitoid had been found attacking *P. thomsoni* populations in Edmonton, Alberta, where it was associated with the collapse of the leafminer population (Digweed et al. 2003; MacQuarrie 2008). The first releases of this parasitoid in Alaska were made in 2004 and 2005 by Chris MacQuarrie, principally at one site, a city park called Balto Seppala (61°11’26N, 149°56’37W, Wisconsin Street and W. 32nd Ave.). In 2006, the University of Massachusetts Amherst joined the biological control program and continued the project until the end of 2011. In August 2007, Dr. Andrew Bennett, an ichneumonid Research Taxonomist with the Canadian National Collection of Insects, determined that the parasitoid being released in Alaska was not *L. luteolator* and that previous publications had applied that name incorrectly to material from Canada. In 2009, Dr. Alexey Reshchikov of the University of St. Petersburg revised the Nearctic members of the genus *Lathrolestes* and described new species as needed. Based on his results, it was determined that there were no records of the European species *L. luteolator* from North America and that the parasitoid being released for biological control of *P. thomsoni* in Alaska was determined to be an undescribed species. Reshchikov (2010) described the species being released in Alaska as *Lathrolestes thomsoni* Reshchikov to reflect its host association.

Evidence of Native Parasitism

In 2006, in Anchorage, we witnessed evidence of this leafminer being attacked in the field by presumably native parasitoids. Parasitism of *P. thomsoni* larvae was obtained by dissecting leafminer larvae at permanent sites (see Chapter 1) that were geographically separated from release sites (13 km), at rates higher than would be expected from native generalist parasitoids. Because sampling was based on detection of immature parasitoids found in host dissections rather than adults from rearings (which is difficult because of a diapause that requires a 10-month rearing period), other means were sought to identify the species causing the observed parasitism. Extraction of DNA and amplification of the COI mitochondrial gene was performed on the immature parasitoid larvae and sequences were obtained and compared to adult *L. thomsoni* wasps and other wasp species collected from emergence cones placed beneath birch trees at permanent plots. One wasp species, in particular, was determined to be the cause of the parasitism of larvae in leaf mines at forest sites. Adults of this species (matched via DNA to larvae in hosts in mines) were sent to Alexey Reshchikov for identification and were determined to be yet another undescribed Lathrolestes (Ichneumonidae) species. A species description was written and the wasp designated as *Lathrolestes soperi* Reshchikov (Reshchikov et al. 2010). The origin (native or previously introduced) of *L. soperi* has not been determined as there are no other reports of its occurrence.

Biology of *Profenusa thomsoni*

Life history information and life tables for Alaska populations of *P. thomsoni* were developed by MacQuarrie et al. (2010). In Alaska, *P. thomsoni* has one generation per year. Adults emerge from late June to August and deposit their eggs singly on the central midrib of birch leaves (Martin 1960; Digweed 2006). Leaves receive multiple ovipositions from either the same or different females. In late July and August, larvae feed on the inner tissues of the leaf and convert the leaves into brown, crinkled mines. In mid-to-late August, larvae mature and drop to the soil, where they form an earthen cell and overwinter as prepupae (Digweed 2006). The population is parthenogenetic and no males are known (Benson 1959; MacQuarrie 2008). The immediate damage to affected birch trees is aesthetic. Long-term effects of leafminer damage on birch trees are unknown but may include reduced tree growth (MacQuarrie 2008) and increased tree susceptibility to secondary infections (Hoch et al. 2000; Snyder et al. 2007). In North America, *P. thomsoni* attacks *Betula aleghaniensis* Britton, *Betula glandulosa* Michx., *Betula lutea* Michx., *Betula neoalaskana* Sarg., *Betula occidentalis* Hook., *Betula papyrifera* Marsh., and *Betula populifolia* Marsh. (Martin 1960; Cheng and LeRoux 1964; Digweed et al. 1997; MacQuarrie 2008).

Biology of Parasitoids

In Alaska, both *Lathrolestes thomsoni* and *L. soperi* are univoltine, koinobiont endoparasitoids that lay their eggs in developing *P. thomsoni* larvae.

Adults of *L. thomsoni* attack early (1^{st} to 3^{rd}) instar larvae. A single egg is laid in each host larva. The egg of *L. thomsoni* hatches soon after being deposited, but the larva does not feed until *P. thomsoni* drops to the ground and forms an earthen cell (MacQuarrie 2008). The parasitoid then completes most of its larval development within the host's prepupa in its cocoon in the soil (Digweed et al. 2003), as happens with other species of birch leafminer sawflies in the genus *Lathrolestes* (Pschorn-Walcher and Altenhofer 1989; Digweed et al. 2003). After consuming the host, the wasp overwinters as a late instar larva (MacQuarrie 2008) and pupates shortly before adult leafminer emergence the following year.

Lathrolestes soperi biology was unknown before this study. Females attack early instars (1^{st}-3^{rd}) and lay one or several eggs per host. Such superparasitism may be a means to avoid encapsulation by the host, as this may be a new host for this parasitoid. Encapsulation was witnessed frequently in dissection of *P. thomsoni*. The rest of the life history of *L. soperi* is unknown but is believed to be similar to that of *Lathrolestes thomsoni*.

Materials and Methods

Acquisition and Release of Parasitoids

Releases in 2006. Rearing of adult parasitoids for release in Alaska in 2006 was done in Alberta in 2005 by S. Digweed, C. MacQuarrie and others of the Canadian Forest

Service, Edmonton, and details on rearing methods and collection are in MacQuarrie (2008). In brief, parasitized *P. thomsoni* larvae were collected in July and August of 2005 in Hay River and Fort Smith, NWT and were placed in clear plastic tubs 30cm x 45cm x 15cm (LxWxD) where they were allowed to burrow into a soil medium. In September 2005, half (five) of the tubs with overwintering larvae were dug into the ground by the Canadian Forest Service in Edmonton, Alberta. The other five tubs were shipped to Anchorage (via commercial airline) for overwintering there. This was done to determine which system might best synchronize adult parasitoid emergence with leafminer stages in Anchorage. In May 2006, the tubs that had been overwintered in Alberta were flown by commercial airline to Anchorage and sunk into the ground at two outdoor emergence locations.

Emergence cages (0.5 m x 0.4 m x 0.6 m) were screened with mesh fabric (no-seeum netting and had a sleeve for access to the inside. Emergence cages were checked daily from 20 June to 28 August 2006 for adults of *P. thomsoni* and *L. luteolator*, which were collected with an aspirator. Adult leafminers were counted, killed, and disposed of. Adult parasitoids were counted, sexed and transferred to containers with water-soaked dental wick (Absorbal) and streaks of honey. Approximately 20 wasps were held per container in a refrigerator at 5°C, until released.

Releases in 2006 were made at a single site in July and August, in Anchorage, Alaska (see details below). A total of 168 (98 males, 70 females) adult *L. thomsoni* from Alberta were released at one release site (site 1 on the University of Alaska campus in Anchorage) in July or August 2006. An additional 188 adult wasps (157 males, 31 females) collected as adults in Edson, Alberta by C. MacQuarrie were released on 20 July 2006 and 67 additional field-collected adults (43 males and 24 females) from Hay River, NWT, collected by S. Digweed and A. Soper, were released on 10 August 2006. All wasps collected in 2006 were released immediately (or within a few days) after their emergence or field-collection as adults at one site on the University of Alaska-Anchorage campus (61.1903ºN, 149.828ºW). This site was selected because of access and an agreement that the trees would not be sprayed. It was also selected because leafminer larvae were relatively more advanced (in terms of instar) compared to other sites in the city, which was an initial concern (since the minimum instar acceptable for oviposition was not known at the time of site selection and the assumption was that oviposition occurred in older instars).

Parasitoids were either freely released on the foliage, or placed in sleeve cages positioned over branches with mined leaves. In the case of free release, parasitoids were allowed to crawl out of the container onto leafminer-infested leaves. In nearly all the free-style releases, oviposition by female wasps was immediately observed. In sleeve cage releases, six males and three females were placed in organdy sleeve cages (60 cm dia. x 115 cm l) enclosing branches with approximately 10-15 leaves, most of which were mined by several larvae each. A laminated, yellow plastic card streaked with honey and a water source was included in each cage. Sleeve cages were kept closed for three days of good weather and then removed. This method was not used in subsequent years because oviposition was witnessed immediately when wasps were free released onto foliage and sleeve cages were deemed unnecessary.

Releases in 2007. In the spring of 2007, twenty-six tubs of soil containing overwintering parasitized leafminer larvae collected in 2006 in Edmonton and Edson (Alberta) or Fort Smith and Hay River (NWT) were shipped by truck from Edmonton, Alberta to Anchorage, Alaska. All tubs had been overwintered in Alberta. Once in Alaska, they wasps were treated in the same manner as in 2006. MacQuarrie (2008) showed that overwintering parasitized leafminers in Alberta produced more emerging parasitoids and thus no tubs were overwintered in this year in Alaska, as had been done in the previous year.

Once in Alaska in the spring, tubs were sunk into the ground at the same locations as used in 2006 (Zogas and Rose) plus at one additional location (the home of Steve Patterson, USDA-FS) (GPS coordinates). All tubs were then covered with emergence cages as in 2006. Emergence cages were checked daily from 1 July to 24 August 2007. Adult *P. thomsoni* were counted, killed, and preserved in 70% EtOH. Adult *L. thomsoni* wasps were counted, sexed, fed honey and gel water (Cricket Crystals), and held in groups for an average of three days to ensure mating and to assemble adequate numbers for release.

At the peak of emergence, parasitoids were released daily due to the large number emerging. At other times, parasitoids were held for up to a week in a refrigerator at 5°C before they were released due to lower numbers emerging per day. Daily, parasitoids were taken out of the refrigerator for approximately one hour and warmed up to allow them to drink water and eat honey. Additionally, parasitoids for release were obtained by collecting adult parasitoids in the field in Edmonton, Alberta (53.2635°N, 113.3015°W) in later July (details below) Adult parasitoids was collected from the undersides of birch leaves and the target species (*L. thomsoni*) was recognized in the field by the distinctly yellow clypeus. Wasps were aspirated directly from birch trees (*B. pendulum* and *B. papyrifera*) and then transferred into white opaque containers (6 cm high x 9.5cm dia.) with a translucent domed lid, containing gel water (Cricket Crystals) and streaks of honey. Approximately 20 wasps were held per container in an iced cooler until they could be transferred to a refrigerator. Anna Soper, Chris MacQuarrie, and Scott Digweed collected a total of 441 males and 142 females in this manner on19-21 July 2007. In addition to wasps collected in Edmonton, Alberta, Scott Digweed collected a total of 415 males and 87 females in Hay River, Northwest Territories, which were also transferred to Anchorage, Alaska for release. These wasps were hand carried by A. Soper to Anchorage, Alaska on 21 July.

Parasitoids collected in 2007, either from emergence cages in Anchorage or in the field as adults in Edson, Alberta, were released at four new locations, not previously used for parasitoid releases. A total of 2266 (1134 males and 932 females) wasps were released at four new sites (Table 3). The first of these four sites was located on the campus of the University of Alaska, Anchorage (site 2 on campus) (61.1129°N, 149.50159°W**),** approximately 0.5 miles from the 2006 release site. Only one release was made at this location. The second release site was Javier de la Vega Park (61.1009°N, 149.5511°W). Six releases were made at this site. Due to the large number of emerging wasps obtained in this year, it was decided that additional release sites would be made outside of Anchorage. The third release site was in Soldotna (60.2915°N, 151.0319°W), a small city located on the Kenai Peninsula approximately 150 miles from Anchorage. Parasitoids were released in the Fred Meyer parking lot (60.291, 151.031) in a stand of eight landscape paper bark birch trees. One release was made at this site. The fourth release location was the Eielson Air Force Base near Fairbanks, Alaska (64º40933, 147º04574) in a stand of three landscaped trees in a residential housing development. One release was made at this location.

All parasitoids were free-released onto birch foliage. In all releases, oviposition by female wasps was immediately observed. Parasitoids were typically seen ovipositing in first, second and third instar larvae, which was an important observation because it was previously supposed that older instars were preferred or even necessary for oviposition (Digweed et al. 2003).

Releases in 2008. In May of 2008, twelve tubs of soil containing *P. thomsoni* larvae, collected in the summer of 2007 in Edmonton, Alberta and the Northwest Territories, Canada by Scott Digweed and Chris MacQuarrie were taken to Anchorage, Alaska after having been over-wintered in Edmonton, Alberta, Canada at the Canadian Forest Service (by Chris MacQuarrie). Tubs containing *P. thomsoni* larvae were moved by car from Edmonton, Alberta to Anchorage Alaska in May 2008 by Richard Reardon (USDA FS). Once in Anchorage, tubs were dug into the ground as before at the homes of Steve Patterson and Corlene Rose (both former outdoor emergence cage locations) and covered with emergence cages, as in previous years. Emergence cages were checked daily from 1 July to 1 September 2008, for adults of leafminers and parasitoids, which were collected with an aspirator. A total of 64 *P. thomsoni* and 1101 *L. thomsoni* (566 males and 535 females) were collected in this manner. Adult *P. thomsoni* were counted and preserved in 70% EtOH. Adult *L. thomsoni* were counted, sexed, fed honey and water (as soaked dental wicking [Absorbal]), and held in groups for an average of three days to ensure mating and to assemble adequate numbers for a release. At the peak of emergence, parasitoids were released daily. At other times, parasitoids were held for up to a week before they were released due to lower numbers emerging per day.

Parasitoids collected in 2008, from emergence cages in Anchorage, were released at two new locations, not previously used for parasitoid releases. A total of 13 releases were made at these new release sites. The first of these release sites was located at the home of Corlene Rose (UAF, Cooperative Extension) (which was also an outdoor emergence cage location) (61.1205686°N, 149.552333°W). Only one release was made at this location. The second new release site was the home of Ken Zogas (USDA FS) (a former outdoor emergence cage location) (61.063188°N, 149.540886°W). Twelve releases were made at this site. All parasitoids were free-released onto birch foliage and oviposition was witnessed immediately into early instar larvae.

Monitoring for Establishment.

Dissection of larvae and use of DNA markers. Determining the identity of parasitoid larvae obtained leafminer hosts from samples of mined leaves collected to measure leafminer parasitism in the field is difficult because *Lathrolestes* larvae have few distinctive morphological characters and none that are diagnostic to species (Fig. 11). Dissection alone could not distinguish between larvae of the released wasp *L. thomsoni* and the pre-existing (perhaps native) wasp *L. soperi*. Therefore, DNA extraction and sequencing for the COI gene was employed to identify specimens to exact species by extracting DNA from parasitoid larvae. To estimate parasitism rates, samples of mined leaves containing *P. thomsoni* larvae were collected at all 2006, 2007, and 2008 release sites. One hundred mined leaves were collected per site weekly for 8 weeks. Sampling at a given site began as soon as larvae were present. Larvae were placed in alcohol and later dissected to detect parasitoid eggs, larvae, and encapsulated eggs.

To identify specimens recovered by dissection to exact parasitoid species, a molecular method was used. DNA was extracted using a method developed by Nathan Havill. Larvae were placed in 10µl AE elution buffer (Qiagen) and 1µl Proteanase K (Qiagen). They were incubated for at least 2 hours at 55^{o}C and then at 95ºC to denature the proteanase K enzyme and break down the cells further. PCR reactions were carried out using two separate methods (or occasionally three) to ensure that DNA sequences were obtained from most parasitoid larvae. The first PCR reactions were carried out in 25 µl reactions using a PCR master mix of 12.5 µl EconoTaq® Plus Green 2x Master Mix (Lucigen, Madison, WI), 10 µl water, 1 µl genomic DNA and 1 µl each primer. PCR primers for all reactions appear in Table 4. All primers were used to amplify a short region of the mitochondrial cytochrome oxidase c subunit I (COI). All reactions were carried out in an MJ Research Inc., programmable thermocycler. An initial denaturation at 94 °C for 1 min. was followed by five cycles of 94 °C for 1 min., 45 °C for 1.5 min, and 72 °C for 1.5 min; 35 cycles of 94 °C for 1 min., 50 °C for 1.5 min. and 72 °C for 1 min; 72 °C for 5 min. DNA presence was visualized on an agarose gel.

Instead of sequencing all specimens, a less expensive, quicker approach was used based on the use of restriction enzymes. The SAC I enzyme has been found to differentiate these two species on an agarose gel that visually displays two bands for *L. soperi* but just one band for *L. thomsoni* (Figure 12). All single bands were sequenced to confirm the identity of putative *L. thomsoni* recoveries. Subsets of individuals producing double bands were sequenced to confirm specimens as *L. soperi*.

The second type of PCR reaction contained species-specific primers developed by A. Soper to increase amplification success. Each PCR reaction contained the same chemical profile as outlined previously, but used a different heat profile. Initial denaturation at 95 ˚C for 3 min. occurred, followed by 35 cycles of 95 ˚C for 30 sec., 58.9 ˚C for 1 min. and 72 ˚C for 1 min., with a final extension of 72 ˚C for 10 min. DNA presence was visualized on an agarose gel and all successful reactions were sequenced. PCR products were purified prior to sequencing using ExoSAP-IT® (U.S. Biochemicals, Cleveland, OH) as per manufacturer's instructions. PCR products were sent to the Genomics Center at The University of Massachusetts-Amherst. Sequences were edited in Sequencher 4.2 (Gene Codes Corporation) and aligned in MacClade.

In rare situations, the 28s gene was used to identify wasp species. The 28s gene was used because it has a high rate of amplification success. Wasps that would not yield a PCR product in the COI region were subject to the same PCR chemical profile as the previous wasps; however, a primer pair modified by Morse and Normark (2006) but developed by Dowton and Austin (1998) and Whiting et al. (1997) and a touchdown program developed by Morse and Normark (2006) was used. An initial annealing temperature of 54 °C was decreased by 3 °C every 5 cycles until reaching 49 °C and decreased by 2 °C every 5 cycles until reaching 45 °C, which was held for 30 cycles. After an initial denaturation for 60s at 95 °C, each cycle denatured at 95 °C for 30 s, annealed at the appropriate temperature according to the touchdown procedure for 30 s, and extended at 72 °C for 60 s. The program finished with a final extension at 72 °C for 10 min (Gwiazdowski et al. 2006). The sequences were then compared to adult wasps of the respective species. This gene was not often used, because it only displays one unique base change between the two species.

To understand the actual rate that leafminers were being attacked and evaluate overlap in parasitism by both species of *Lathrolestes*, the marginal rate of attack was calculated based on the proportional hazards assumption (Elkinton et al. 1992).

Sweep sampling. In 2010, quantitative sweep sampling was used to detect the released biocontrol agent over time at release sites. Three sets of 20 sweeps were taken in both the canopy and at ground level at all release sites, except Eielson Air Force Base (due to the geographical distance). Release sites were sampled weekly from 18 June 2010 until 24 September 2010. Since *L. thomsoni* has a distinct yellow clypeus, field identification was easy and rapid. Therefore, once wasps were counted, all *L. thomsoni* wasps were liberated at the collection site. *Lathrolestes soperi* wasps were collected and preserved to confirm identity.

Post-release sampling for parasitoid spread. Qualitative sweep sampling was employed at all release sites to determine the spread of the released wasp *L. thomsoni*. In the last week of July and the first week in August, trees close to release sites were sampled in all four cardinal directions, moving outward from the original point of parasitoid release. This process was repeated four times for each site to maximize the likelihood of locating wasps.

Permanent monitoring plots. In June 2006, twenty trees, one at each of twenty sites, were located for repeated observations of leafminer density and parasitism in Anchorage. These sites were established to assess future impacts of *L. thomsoni* on *P. thomsoni* in Anchorage. At all permanent sites, the native paper bark birch (*B. papyrifera*) was chosen for sampling. Sampling of leafminer density at release sites used the same methods used at the permanent study plots. Sampling at release sites was begun once a site was established and the first release had been made (in 2006, 2007, or 2008). Density at release sites was then measured again in 2011 to evaluate the effect of the parasitoid. Sampling was not performed annually so as to not disturb the establishment of the wasps at these sites.

Results

Monitoring for Establishment.

Dissection of larvae and use of DNA markers. Detection of healthy parasitoid eggs in dissection samples was rare. Parasitoid larvae and encapsulated eggs or larvae were the stages most often found. Parasitoid larvae were white to clear, distinctly segmented, and ranged in size from 0.68-1.2 mm (Figure 11). Encapsulated eggs or larvae were dark brown, oval, and were ca 0.4 mm long**.** Using the molecular method described above, the released parasitoid *L. thomsoni* was detected at all release sites except for Soldotna Release 2007. Only one *L. thomsoni* larval wasp was found at Eielson AFB. This is the only evidence of establishment at this location. In the 2009 and 2010 at UAA-Release 2006 release site, the highest percentage of *L. thomsoni* parasitism was detected; however, the majority of parasitism found at all release sites in both years was attributed to *L. soperi*. For percent parasitism rates at all release sites see Table 5 and Figure 13. Low rates of parasitism at the Soldotna and Eielson AFB sites can be explained by the fact that *L. soperi* (the pre-existing parasitoid) was not found at either of those locations.

Sweep sampling. *Lathrolestes thomsoni* wasps were found in sweep net samples at all release sites, supporting the view that the species is now established at these locations (Figure 14). In addition, sweep sampling measured the phenology of parasitoid flight over the sampling season. *Lathrolestes thomsoni* was the most abundant wasp at release sites, peaking at seven wasps per 20 sweeps at the UAA-2006 site. Conversely, *L. soperi* wasps were found to fly later in the season from 18 June until 3 September at some sites (Figure 15).

Sweep sampling for spread. The farthest that wasps were detected from a release site was 367 meters. This occurred at the 2006 release site. However, the UAA-07 release site is approximately 1 km away so it is difficult to determine if the wasps came from the UAA-06 site or the UAA-07 site. For the farthest distances at which the released wasp *L. thomsoni* was recovered at each release site, see Table 6.

Profenusa thomsoni Density at Permanent Density Plots

The percentage of leaves mined by *P. thomsoni* larvae at 20 permanent study sites in Anchorage declined from 2006 to 2011 (Figure 16). In July samples, from 2006 to 2011, the percentage of leaves mined dropped from 50 to 11%. In August samples, over the same period, the drop was from 70% to 19%. Since the newly introduced parasitoid was not present at these permanent plots during much of this period, it is most likely that this decline was due to *L. soperi*.

Profenusa thomsoni Density at Release Sites

Profenusa thomsoni densities declined at all release sites except Eielson AFB (Figure 17), where densities increased by 10%. This increase was likely due to the fact that the Eielson AFB was under construction for two years (2009-2010), which could have possibly caused the wasps to disperse due to dust, rather than stay on the specific release trees. The Zogas 2008 site saw the largest decline in density, going from 52% of leaves mined to 6% of leaves mined (Figure 17).

Discussion

At least five species of birch leafminers (*Heterarthrus nemoratus* [Fallén], *P. thomsoni*, *Fenusella nana* [Klug], *Fenusa pumilla* Leach, and *Scolioneura vicina* Konow) that are native to the Palearctic have been introduced to North America in the 20th century (Digweed et al. 2009). These leafminers have undergone population explosions in some instances and not others in patterns that can be explained by their association or separation from key natural enemies. At least three of these species (*H. nemoratus, P. thomsoni,* and *F. pumilla*) have been brought under successful biological control and in Canada, *F. nana* and *S. vicina* are considered good candidates for future biological control efforts (Digweed et al. 2009). Natural enemies that provided control of *F. pumila* and *H. nemoratus* were imported from Europe, but the origin of *L. thomsoni,* the parasitoid that controlled *P. thomsoni* in Canada, is unknown, as no specimens have been recorded outside of North America. It is possible that *L thomsoni* coevolved with *P. thomsoni* in its native Palearctic range because this pest exists there only at low population densities (Benson 1959). However, *L. thomsoni* has never been recorded in Europe.

In Massachusetts, *L. thomsoni* is found attacking *P. thomsoni* (A. Soper unpublished), where population densities are low and populations patchily distributed. These populations would easily go unnoticed unless sought out by a specialized observer. Additionally, the ectoparasitoid *Aptesis segnis* Gravenhost has been found attacking *P. thomsoni* populations in both Massachusetts and Alaska. *Aptesis segnis* attacks *P. thomsoni* prepupae in their earthen cells in the soil. It is unknown if *A. segnis* attacks *P. thomsoni* in Alberta; however, if it is found there, it very likely attacks the ambermarked birch leafminer. If *A. segnis* does attack this leafminer in Alberta, the collapse of the ambermarked birch leafminer population in the city of Edmonton that was attributed to *L. thomsoni* may have been caused in whole or in part by *A. segnis* because it could easily have been entirely overlooked, attacking the host as it does in the soil.

Alaska is unusual in that it has a third parasitoid, *L. soperi*, attacking *P. thomsoni* and the interactions among these three parasitoids (see Chapter 3) will become of increasing importance as time passes since the introduction of *L. thomsoni*, which may displace one or the other of the pre-existing parasitoids. *Lathrolestes soperi*'s origin is unknown as it is not recorded anywhere except Alaska (Reshchikov et al. 2010). As this appears to be the first parasitoid to interact with the leafminer, it is likely not a coevolved species. *Lathrolestes soperi* may originally have been associated with another native Alaskan leafminer or other tenthredinid sawfly (free feeder or leafminer) and opportunistically started to attack *P. thomsoni* when the leafminer invaded. It may also have been introduced, although there is no evidence for this. Attempts were made to locate hosts with this parasitoid on other host trees in Alaska were unsuccessful (Chapter 3).

If *L. thomsoni* is a coevolved parasitoid, it is possible that over time it will overtake populations of *L. soperi* through competitive displacement. The theory of competitive displacement is well documented in several biological control systems, including California red scale, *Aonidiella aurantii* [Mask] (Murdoch et al. 1996), the imported cabbage worm, *Pieris rapae* (L.) (Van Driesche 2008), and imported fire ants (LeBrun et al. 2009) . In the case of California red scale, the parasitoid *Aphytis lingnanensis* Compere provided inadequate control of red scale in California. When *Aphytis melinus* DeBach competitively displaced *A. lingnanensis,* satisfactory biological control was achieved within a few generations (Murdoch et al. 1996). If this were to happen in our study system, the effect would probably take much longer to occur, as ambermarked birch leafminer and its parasitoids are all univoltine in Alaska.

In Anchorage, *L. soperi* appears to be a major cause of mortality and DNA methods identified it as the largest proportion of parasitism, at least during the above ground portion of the host life cycle. However, even though leafminer densities appear to be declining at permanent and release sites in Anchorage, this has not yet happened on a broader scale in

Alaska (pers. observ.). Furthermore it is unclear how much mortality in the ambermarked birch leafminer life system is being added by the subterranean pupal parasitoid, *A. segnis*.

Lathrolestes thomsoni is widely established on *P. thomsoni* populations throughout Anchorage and a few individuals have been found in Soldotna and Eielson Air Force Base. While it appears that *L. thomsoni* has established at these last two release locations, parasitoid populations there remain very low and based on host density counts before (2007) and after (2011) the release at Eielson, the wasp is clearly not yet having any impact. It is difficult to ascertain which of the three parasitoid species ultimately may cause the largest amount of mortality. However, *L. thomsoni* has been shown to effectively control ambermarked birch leafminer populations in Alberta and Massachusetts, suggesting it may have the same effect on pest populations in Alaska. Further study of this system is necessary after a lapse time of five to ten years to assess the degree to which *L. thomsoni* dominants the system.

Tables

Table 3: Release sites and numbers of *Lathrolestes thomsoni* (Hymenoptera: Ichneumonidae) released in Alaska from 2004-2008 for the biological control of the invasive leafminer *Profenusa thomsoni* (continued onto next few pages)

Release Sites			**Dates**	**Total *L. thomsoni* released[1]**		**Source[2]**
Name	**Lat.**	**Long.**		**Males**	**Females**	
Anchorage						
Balto Seppala Park	61.1910	-149.943	7.vii-9.viii.2004	15	42	Reared
			2.vii-10viii.2005	75	71	Reared
			6.viii.2005	7	5	Collected
UAA-2006	61.1903	-149.829	20.vii.2006	48	15	Reared
			20.vii.2006	157	31	Collected (Edson, AB)
			24.vii.2006	25	18	Reared
			1.viii.2006	21	22	Reared
			1.viii.2006	43	24	Collected (Hay River, NWT)
			10.viii.2006	3	12	Reared
			21.viii.06	1	3	Reared
UAA-2007	61.1883	-149.836	23.vii.2007	28	17	Reared
			23.vii.2007	489	190	Collected (Edmonton, AB)

Javier de la Vega Park	61.1695	-149.919	27.vii.2007	219	122	Reared
			31.vii.2007	165	112	Reared
			1.viii.2007	40	72	Reared
			3.viii.2007	78	88	Reared
			14.viii.2007	10	50	Reared
			20.viii.2007	6	12	Reared
Rose 2008	61.2017	-149.923	20.vii.2008	127	41	Reared
Zogas 2008	61.1090	-149.902	27.vii.2008	120	53	Reared
			1.viii.2008	67	80	Reared
			4.viii.2008	26	73	Reared
			6.viii.2008	14	30	Reared
			8.viii.2008	7	14	Reared
			11.viii.2008	9	30	Reared
			12.viii.2008	13	13	Reared
			13.viii.2008	18	22	Reared
			15.viii.2008	30	39	Reared
			18.viii.2008	12	32	Reared
			21.viii.2008	9	22	Reared
			24.viii.2008	4	22	Reared
				1886	**1377**	**Anchorage Grand Total**

Interior						
Eielson Air Force Base	64.674	-147.073	15.vii.2004	0	2	Reared
Eielson Air Force Base	64.6833	-147.077	10.viii.2007	62	179	Reared
				62	**181**	**Interior Grand Total**
Kenai Peninsula						
Soldotna	60.4900	-151.051	6.viii.2007	40	90	Reared
				1988	**1648**	**Grand Total**

[1]Releases made in 2004 and 2005 were by Chris MacQuarrie, University of Alberta-Edmonton

[2] "Reared" refers to adults that emerged from rearing tubs in Alaska; "Collected" refers to adults collected by an aspirator directly from trees in Canada and hand carried to Alaska

Table 4: PCR primers used for mitochondrial and nuclear (28s) DNA amplifications.

Gene	Primer	Direction	Sequence	Reference
COI	LCO 1490	Forward	5'-GGTCAACAAATCATAAAGAT ATTGG-3'	(Hebert et al. 2003)
COI	HCO 2198	Reverse	5'-TAAACTTCAGGGTGACCAAAA AATCA-3'	Hebert et al. 2003
COI	FLath59	Forward	5'-CTACAAATCATAAGGATAT CGG-3'	this paper
COI	Rlath697	Reverse	5'-GTCTCCTCCTCCATTAGGY-3'	this paper
28s	S3660	Forward	5'-GAG AGT TMA ASA GTA CGT GAA AC-3'	Dowton and Austin 1998
28s	A335	Reverse	5'-TCG GAR GGA ACC AGC TAC TA-3'	Whiting et al. 1997

Table 5: Estimated rates of parasitism of *Profenusa thomsoni* by *Lathrolestes* species at *Lathrolestes thomsoni* release sites in Anchorage, Alaska, sampled in 2009 and 2010. Continued onto next few pages.

				Parasitoid Split Ratio		Calculated % Parasitism		Marginal Rate of Attack	
Date	Release Site/ Year of Release	Total Percent Parasitism	No. of Successful DNA Amplifications	*L. soperi*	*L. thomsoni*	*L. soperi*	*L. thomsoni*	*L. soperi*	*L. thomsoni*
9-Jul-09	UAA-Release 2006	43	26	0.42	0.58	18	25	21	28
15-Jul-09	UAA-Release 2006	16	13	0.47	0.53	7	9	7	9
22-Jul-09	UAA-Release 2006	27	19	0.32	0.68	5	12	6	13
6-Aug-09	Javier Release 2007	14	11	0.91	0.09	13	1	13	1
6-Aug-09	Rose Release 2008	23	16	0.81	0.19	26	6	26	7
6-Aug-09	UAA-Release 2006	13	13	0.85	0.15	11	2	11	2

6-Aug-09	UAA-Release 2007	14	12	0.75	0.25	11	4	11	4
6-Aug-09	Zogas Release 2008	39	27	0.89	0.11	35	4	36	5
11-Aug-09	Eielson (AFB) 2007	1	1	0	1	0	1	0	1
12-Aug-09	UAA-Release 2007	24	18	0.83	0.17	20	4	20	4
13-Aug-09	Zogas Release 2008	29	24	0.92	0.04	27	1	27	1
20-Aug-09	UAA-Release 2007	9	9	0.89	0.11	8	1	8	1
20-Aug-09	Zogas Release 2008	25	20	0.9	0.1	23	3	23	3
27-Aug-09	UAA-Release 2007	5	3	0.67	0.33	3	2	3	2

27-Aug-09	Zogas Release 2008	13	11	0.91	0.09	12	1	12	1
3-Sep-09	Zogas Release 2008	23	19	0.89	0.11	21	2	21	2
6-Aug-10	Javier Release 2007	43	38	0.97	0.03	42	1	42	1
6-Aug-10	Rose Release 2007	10	5	0.4	0.6	4	6	4	6
6-Aug-10	UAA Release 2006	37	23	0.65	0.35	24	13	26	15
13-Aug-10	Javier Release 2007	29	29	0.97	0.03	28	1	28	1
13-Aug-10	UAA Release 2006	41	32	0.75	0.25	30	10	32	12
13-Aug-10	UAA Release 2007	34	59	0.92	0.08	31	3	32	4

13-Aug-10	Zogas Release 2008	64	39	0.97	0.03	62	2	63	3
18-Aug-10	UAA Release 2006	42	21	0.86	0.14	36	6	37	7
20-Aug-10	UAA Release 2007	36	23	0.96	0.04	34	2	34	2
27-Aug-10	UAA Release 2006	20	17	0.88	0.12	18	2	18	2
27-Aug-10	UAA Release 2007	14	19	0.95	0.05	13	1	13	1
3-Sep-10	UAA Release 2006	13	12	0.92	0.08	12	1	12	1
10-Sep-10	UAA Release 2007	29	27	0.93	0.07	27	2	27	2
16-Aug-11	Javier Release 2007	39	33	0.91	0.09	35	4	36	5

16-Aug-11	Rose Release 2007	30	24	0.79	0.21	24	6	25	7
16-Aug-11	UAA-Release 2006	53	46	0.7	0.3	37	16	41	20
16-Aug-11	UAA-Release 2007	23	17	0.71	0.29	16	7	17	8
16-Aug-11	Zogas Release 2008	47	25	0.88	0.12	41	6	43	8

Table 6: The greatest distances *Lathrolestes thomsoni* wasps were found from their release sites in a given direction at sites in Anchorage, Alaska.

	Distance from release site (m)			
Site:	North	South	East	West
UAA-Release 2006	28	108	367	45
UAA-Release 2007	116	35	69	46
Javier Release 2007	Not found	46	15	156
Zogas Release 2008	22	33	36	12
Rose Release 2007	109	20	Not found	Not found
Soldotna Release 2007	Not found	Not found	59	10
Balto Release 2005	15	59	32	162

Figures

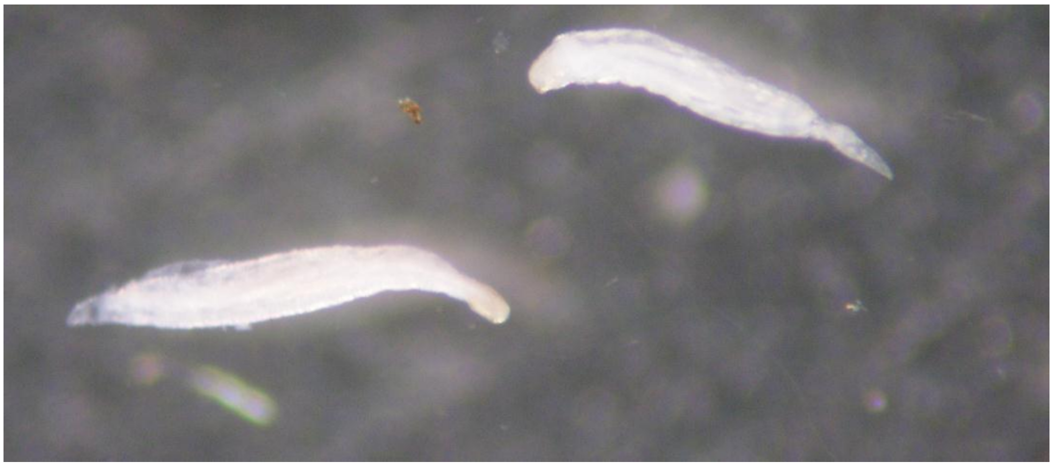

Figure 11: Parasitoid larvae (*Lathrolestes* sp., Ichneumonidae) dissected from *Profenusa thomsoni* larvae from Balto Seppala release site in Anchorage, Alaska in August 2006.

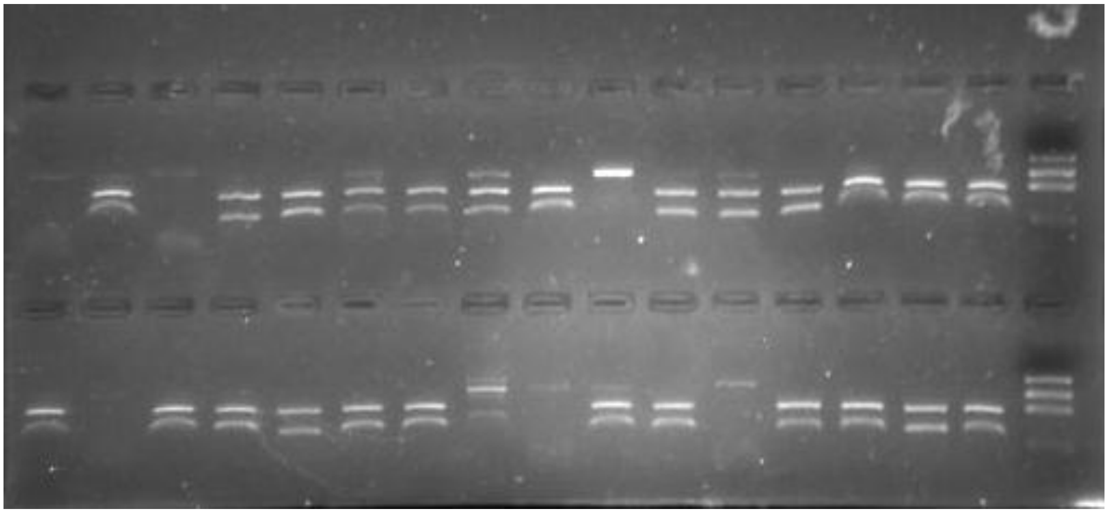

Figure 12: Picture of an agarose gel displaying double bands (*Lathrolestes soperi*) and single bands (*Lathrolestes thomsoni*).

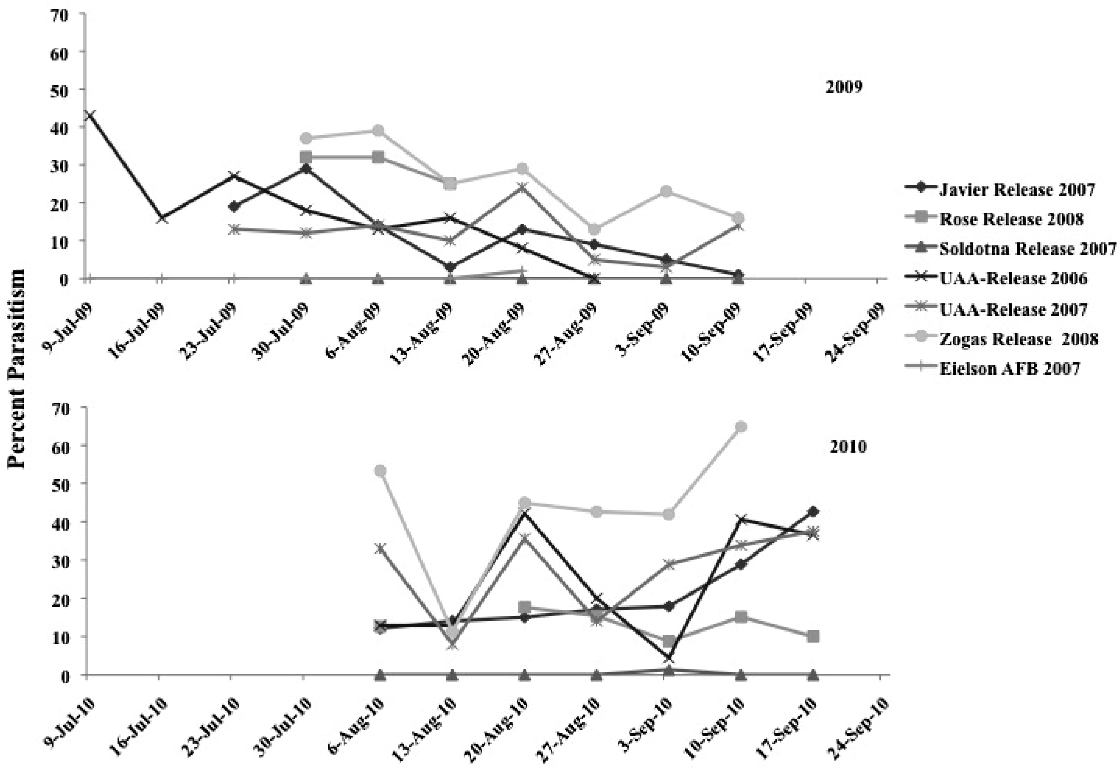

Figure 13: *Lathrolestes* spp. parasitism of ambermarked birch leafminer (*Profenusa thomsoni*) larvae collected at *Lathrolestes thomsoni* release sites from July-September 2009 and 2010 in Alaska.

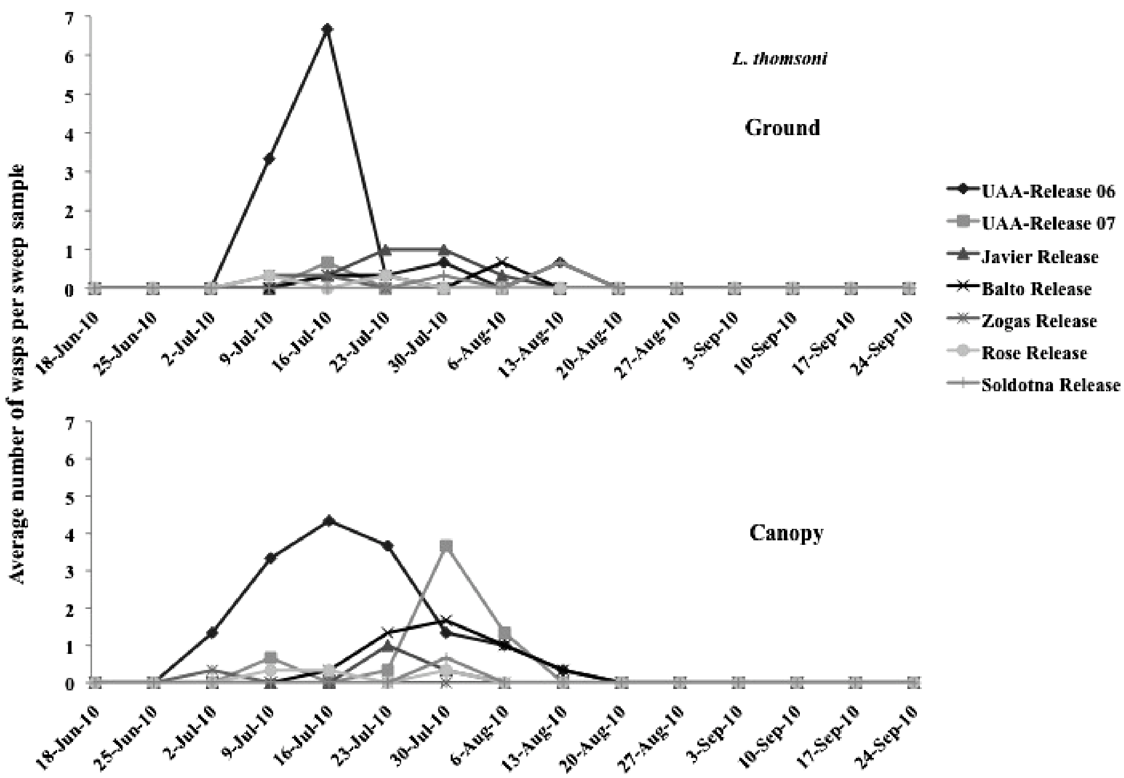

Figure 14: Average number of *Lathrolestes thomsoni* wasps found per sweep sample at release sites on the ground and in the canopy in Alaska from June to September 2010

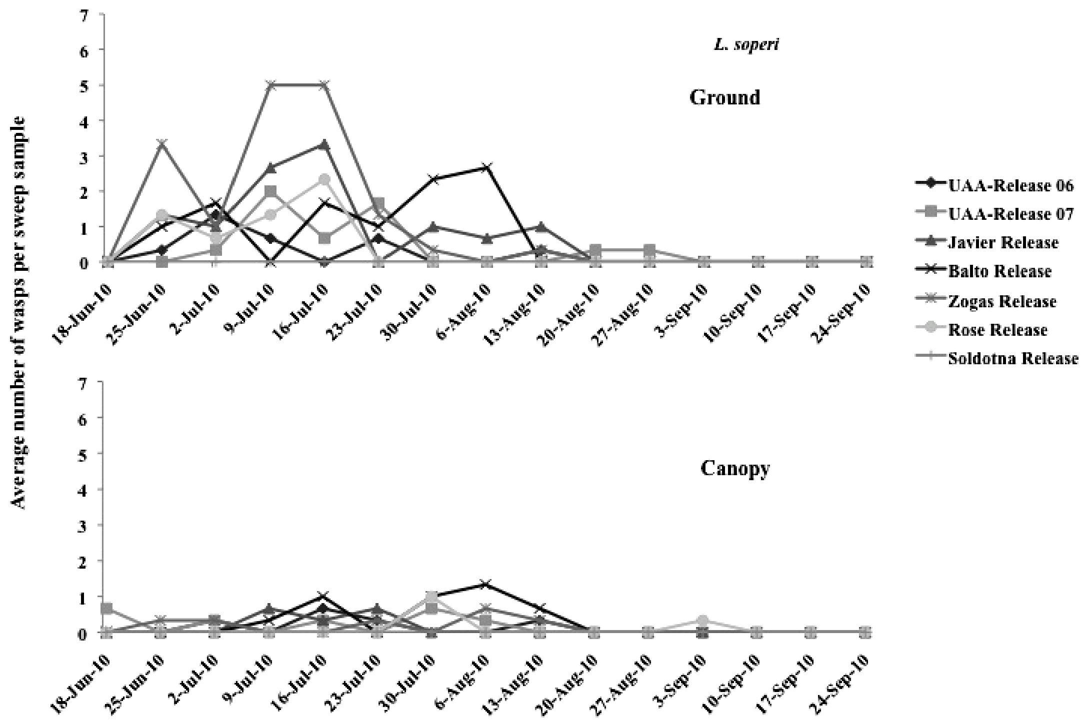

Figure 15: Average number of *Lathrolestes soperi* wasps found per sweep sample at release sites on the ground and in the canopy in Alaska from June to September 2010

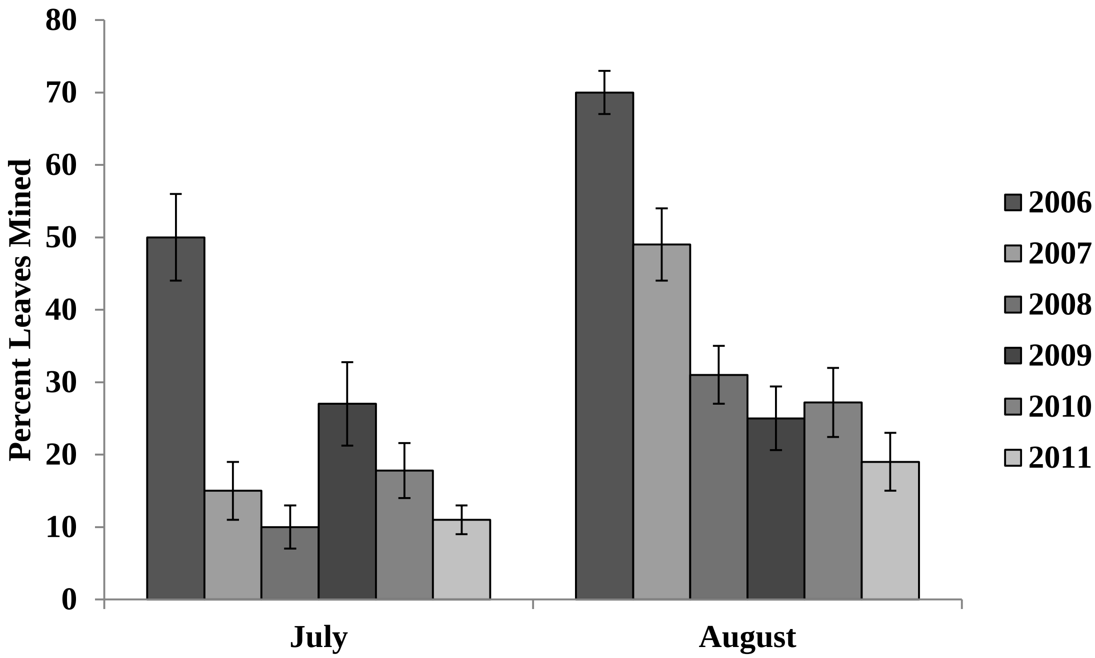

Figure 16: Percent of leaves mined by ambermarked birch leafminer (*Profenusa thomsoni*) on paper bark birch in Anchorage, Alaska in July and August 2006-2011.

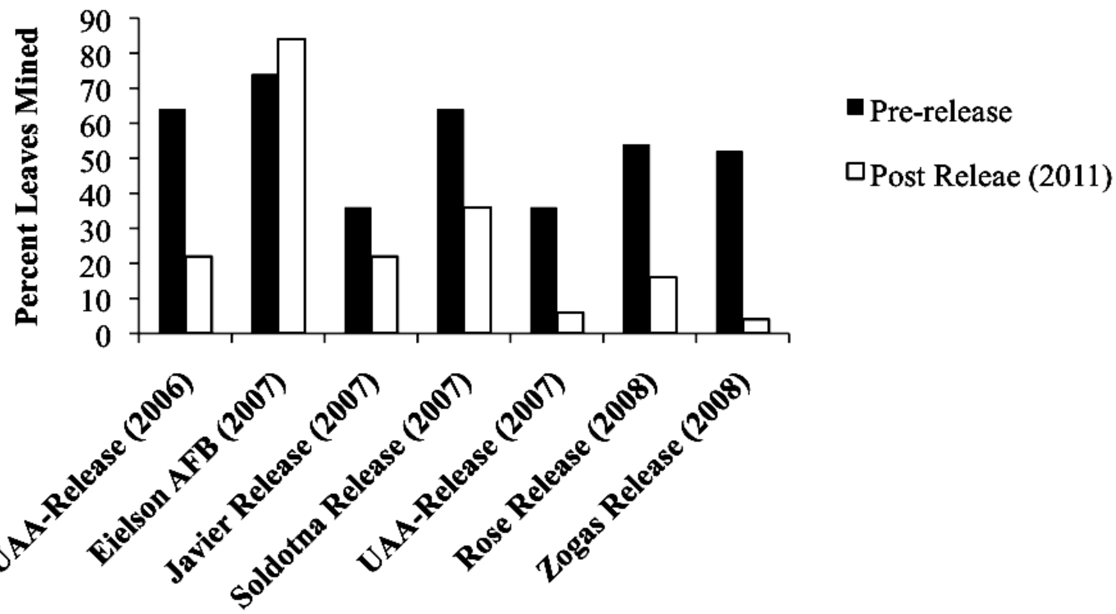

Figure 17: Percent of leaves mined by ambermarked birch leafminer (*Profenusa thomsoni*) on paper bark birch at all *Lathrolestes thomsoni* release sites; samples collected in July of 2011and the year of release (from 2006 to 2011), in Alaska.

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CHAPTER 3

THE PARASITOID COMPLEX OF THE AMBERMARKED BIRCH LEAFMINER, *PROFENUSA THOMSONI* KONOW (HYMENOPTERA: TENTHREDINIDAE), IN ANCHORAGE, ALASKA AND EACH SPECIES' ROLE IN BIOLOGICAL CONTROL

Abstract

Three parasitoids are known to attack *Profenusa thomsoni* in Alaska. *Lathrolestes thomsoni* and *Lathrolestes soperi,* both of which are endoparasitoids and have similar biology, attack early instar larvae within the leaf. The third species, *Aptesis segnis*, is an ecotoparasitoid that attacks pupae and prepupae in their earthen cells in soil. This study focuses on sampling throughout the growing season in several years in Alaska to assess the relative importance of each of these parasitoids and their interaction.

Introduction

Leaf-mining sawflies are well known for their well developed parasitoid guilds, particularly in their native ranges (Pschorn-Walcher and Altenhofer 1989). Leaf mines are conspicuous and easy for natural enemies to locate and penetrate (Pschorn-Walcher and Altenhofer 1989). Host larvae are less able to escape or defend themselves than are free feeding sawfly larvae (Benson 1950; Pschorn-Walcher and Altenhofer 1989).

Despite extensive research on leaf-mining sawflies in Europe, very little is known about the parasitoid communities of the ambermarked birch leafminer, *Profenusa thomsoni*

Konow (Hymenoptera: Tenthredinidae) (Digweed et al. 2009), a European species that is an invasive, damaging pest in North America (Benson 1959). *Profenusa thomsoni* invaded eastern North America in the early 1900s (Ross 1951; MacQuarrie et al. 2007). It has since spread to the Midwestern United States (Benson 1959), Ontario (MacQuarrie et al. 2007), Alberta (Digweed 1998), the Northwest territories, Canada (Digweed et al. 2009), and Alaska (MacQuarrie 2008). In the early 1990s, *P. thomsoni* was brought under biological control in Alberta (Digweed et al. 2003) and in 2004 attempts were set in motion to do so in Alaska (MacQuarrie 2008). This study was part of the biological control program in Alaska that focused on introducing *Lathrolestes thomsoni* Reschikov a species of unknown origin to control *P. thomsoni*.

In 2006 and 2007, two parasitoid wasps were found in abundance in birch tree plots in Anchorage, Alaska. The first wasp, an ichneumonid, koinobiont, endoparasitoid, was found parasitizing *P. thomsoni* larvae in leaf mines. This wasp was identified by Alexey Reschikov as an undescribed species of *Lathrolestes* that was subsequently described as *Lathrolestes soperi* Reschikov (Reshchikov et al. 2010). In 2007, a second wasp was trapped in abundance in cones placed on the ground beneath birch trees infested with *P. thomsoni* larvae. Andrew Bennett of the Canadian National Collection identified that species as the ichneumonid *Aptesis segnis* Provancher.

***Aptesis segnis* biology**

Very little is known about *Aptesis segnis* and most inferences come from notes on specimens in museum collections and old agriculture reports. Parrott and Fulton (1915) observed *A. segnis* (given at the time as *Pezoporus tenthredinarum* Rohwer) attacking the hawthorn leafminer *Profenusa canadensis* Provancher in cherry orchards in New York state.

Aptesis segnis wasps have also been collected in Saskatchewan, Alberta, and Quebec (Krombein et al. 1979). In the United States, *Aptesis segnis* wasps have been recorded in California, Colorado, Massachusetts, Maine, New Hampshire, New York, South Carolina, and Wyoming (Krombein et al. 1979). No life history information has been published on *A. segnis*, but based on its placement in the genus *Aptesis,* it was believed to be an ectoparasitoid that parasitized leafminer pupae or their parasitoids in their pupal cells in the soil. In 2010, I observed several *A. segnis* wasps flying low over the soil in late May and early June. This leads me to believe that in Anchorage, wasps of this species emerge early in the season (mid-late May) before *P. thomsoni* emerges and attack leafminer pupae in their earthen cells, in the soil, where they overwinter.

Aptesis spp. are known to attack several species of sawflies, including pine sawfly (*Neodiprion sertifer* Geoff.) (Griffiths 1961), European apple sawfly (*Hoplocampa testudinea* Klug) (Hymenoptera: Tenthredinidae) (Babendreier 2000), spruce sawfly (*Lygaeonematus albientinus* Christ)(Valenta et al. 1976) and a few species of Lepidoptera including winter moth (*Operophtera brumata* L.) (Sechser 1970). The European apple sawfly is parasitized by both the primary parasitoid *Lathrolestes ensator* Brauns and the facultative hyperparasitoid *Aptesis nigrocincta* Gravenhorst (Babendreier and Hoffmeister 2003). This species attacks either leafminers or larvae of *L. ensator* but only does so in the leafminer's cocoon in the soil, which is what we hypothesized the biology of the *Aptesis* species to be as well. Babendreier and Hoffmeister (2003) considered *A. nigrocincta* to be a possible biological control agent causing a significant proportion of the mortality affecting the host.

***Lathrolestes thomsoni* biology**

Adults of *Lathrolestes thomsoni* attack early instars (1st to 3rd) of *P. thomsoni* and lay a single egg in each larva. The egg hatches soon after being deposited, but the parasitoid larva does not feed until the *P. thomsoni* larva drops to the ground and forms an earthen cell (MacQuarrie 2008). The parasitoid then completes most of its larval development within the host's prepupa in the cocoon in the soil (Digweed et al. 2003), as happens with other species of parasitoids in the genus *Lathrolestes* that attack various birch leafmining sawflies (Pschorn-Walcher and Altenhofer 1989; Digweed et al. 2003). After consuming the host, the parasitoid overwinters as a late instar larva (MacQuarrie 2008), which pupates shortly before emergence the following year.

***Lathrolestes soperi* biology**

The biology of *Lathrolestes soperi* was previously unknown and details are taken from observations made in the course of this study. Females attack early instars (1st-3rd) and lay one or several eggs per host (A. Soper pers. obs.). Such superparasitism may be a means to avoid encapsulation by the host, as this may be a new host for this parasitoid. The rest of the life history of *L. soperi* is unknown but is believed to be essentially the same as for *L. thomsoni*.

Study objectives

The goal of this study was (1) to confirm that *A. segnis* was indeed attacking the ambermarked birch leafminer larvae, (2) to determine the relative importance of *A. segnis* and *L. soperi* as sources of mortality for ambermarked birch leafminers and (3) to better understand the biology of *A. segnis* and how this species interacts with *L. soperi*. Furthermore, we sought to understand if *A. segnis* acts as a facultative hyperparasitoid (similar to the *Aptesis* species attacking European apply sawfly), parasitizing *L. soperi* larvae or pupae and thus reducing that parasitoid's efficacy. Finally, we sought to measure what contribution *A. segnis* makes to total mortality of the ambermarked birch leafminer

Materials and Methods

Soil sampling of ambermarked birch leafminer earthen cells, 2009-2011

To determine if *A. segnis* attacks *P. thomsoni*, soil cores were collected from each of the same six permanent sites where emergence cones were placed to monitor insect emergence from soil (Chapter 1). Soil cores were collected from July to September in 2009. Because few earthen cells were found at the six sites sampled in 2009, I selected four new sites for sampling in 2010. Based on insights gained in sampling in 2009 and 2010, it seemed necessary to take soil cores earlier. Therefore, in 2011, soil cores were collected only once, in early May before emergence of *A. segnis, P. thomsoni,* and *L. soperi*.

In 2009, two 12-cm (depth) soil cores per site per week were taken using a long handle bulb planter (Vigoro). In 2010, six soil cores per site per week were taken using the same method and in 2011, 20 soil cores were taken per site, once in May. The soil cores were bagged and frozen immediately to prevent further development of any organisms in the soil. To locate earthen cells in the soil, an apparatus developed by Charles Vincent, Agriculture and Agri-Food Canada was employed. Soil cores were placed on a screen with large enough mesh to let earthen cells pass through. The screen was positioned over a bucket and the material was manipulated by hand to break up the soil. A hose was then placed over the screen and water used to flood the bucket and force the soil through the screening. Most of the soil and other particulate matter sunk to the bottom while the earthen cells floated and could then be collected off the water surface. Earthen cells were placed in 100% ethanol for later dissection.

Parasitism of *P. thomsoni* by *L. soperi* at non-*L. thomsoni* release sites in Alaska

From 2006-2011 leafminer larvae were collected at the 20 permanent plots (as detailed in Chapters 1 and 2) in the third week of August. From 2008-2010, *P. thomsoni* larvae were collected weekly at these twenty sites. All larvae collected were placed in 70% ethanol for later dissection. Dissected *P. thomsoni* larvae were examined for parasitoid eggs, larvae or encapsulated eggs/larvae (which stages are difficult to separate when encapsulated). Parasitoid larvae are white to clear, distinctly segmented, and ranged in length from 0.32 to 1.6 mm. Encapsulated eggs are or/larvae were dark brown, oval and were 0.32-0.41mm in length.

Adult *A. segnis* and *L. soperi* trap catch on birch in Alaska

To determine the timing of adult flight by *L. soperi* and *A. segnis* at permanent sites, yellow sticky cards (7.6cm x12.7cm) (BioQuip) were placed in the lower canopy of birch trees (one card per site) at each of the 20 permanent sites in 2007, 2008, and 2009. The cards were changed weekly beginning in the first week of July and ending in the second week in September in all three years.

Adult *A. segnis* and *L. soperi* emergence

To determine the timing of wasp emergence, cones (orange traffic cones; area=122.7 cm^2; modified to have a clear 2 oz. trap-cup on top) were placed beneath six permanent sites each year from 2007 to 2010. Ten cones were placed underneath the canopy of each six trees (total of 60) and left over the same patch of soil for the whole season. The cups on top of the emergence cones were replaced weekly once wasp emergence began. Cups were collected and the number of adult *L. soperi, A. segnis*, and *P. thomsoni* was recorded.

Alternate hosts of *L. soperi* and *A. segnis*

To determine if either of these parasitoid had alternate hosts, various other tree species were examined for other species of leafminers that *L. soperi* or *A. segnis* might attack. Yellow sticky cards were placed in the tree canopy of five different species deciduous trees in Anchorage in 2010. One yellow sticky card (3" x 5") (BioQuip) was placed in the canopy of five trees of each of the following species: alder (*Alnus* spp.), cottonwood (*Populus* *balsamifera* [L.]) willow (*Populus tremuloides* Michx.), bird cherry (*Prunus padus* [L.]), and hawthorn (*Crataegus* spp.), for a total of 25 cards total. Bird cherry and hawthorn are both non-native tree species in Alaska. Hawthorn is found in landscape plantings in and around Anchorage. Bird cherry was originally planted as a landscape tree but has since invaded the natural forests in the Anchorage and Fairbanks area (Viereck and Little 2007). Sticky cards were changed weekly from July 17 to August 15. *Aptesis segnis* and *L. soperi* wasps caught on cards were counted. The count data were log transformed in order to meet the assumption of normality.

Sweep sampling to locate *A. segnis* and *L. soperi* in Alaska

To determine where *A. segnis* and *L. soperi* were present in the state of Alaska, sweep samples were taken weekly from 17 July to 20 August in birch leafminer infested habitat. Sampling took place north to Wasilla, Alaska and south to Soldotna, Alaska. Along the highways connecting these locations, I took a group of six sweep samples (each sample consisting of 20 sweeps) at sites every 10 miles. . If no birch leafminers were observed at the selected point, sweep sampling still occurred. Sweep samples were collected into plastic bags (Ziploc®), and placed on ice to prevent spiders and other predators from consuming anything. The samples were placed in a freezer upon return. GPS coordinates were recorded, wasps of the target species were counted, and their distributions mapped.

Levels of mortality causing by *A. segnis* to *L. soperi* and *P. thomsoni*

To understand if *A. segnis* functions as a facultative hyperparasitoid (similar to the European apply sawfly system) and parasitizes *L. soperi*, functionally reducing the parasitoid's effect as a biological control agent, the percent parasitism rates caused by *L. soperi* to leafminer larvae in the last seasonal sample (August 15) of the previous year were compared to the percentage that *L. soperi* wasps comprised of all insects (*L. soperi, P. thomsoni, A. segnis*) caught in emergence cones at the same site the following year, when summed over the whole season.

Additionally, to understand how much mortality *A. segnis* attributes against the leafminer in addition to *L. soperi* parasitism, percent parasitism of the leafminer (by *L. soperi*) on 15 August of the previous year was regressed against the total parasitism (*L. soperi* plus *A. segnis*) in spring of the following year (again, after summing emergence for the whole season.

Comparison between *P. thomsoni* earthen cell availability and *A. segnis* flight time

To understand when *A. segnis* attacks leafminers in the soil, *A. segnis* flight times were compared from sticky card data to the estimated proportion of leafminer larvae available in the soil from emergence cone data. The total number of leafminers emerging per trap was summed over the entire season and then subtracted from each date they emerged to present the amount of available leafminer left in the soil. *A. segnis* wasp flight activity was compared to the number of available leafminers in the soil in 2007, 2008, and 2009.

Statistical Analysis

All statistical tests were run in JMP® 9.0.2 (Inc. 2010). Testing for homogeneity of variance was done in JMP and was not violated in any tests performed.

Results

Soil sampling of ambermarked birch leafminer earthen cells, 2009-2011

Soil collections were the only method to definitively show that *Aptesis segnis* attacked *Profenusa thomsoni* as hypothesized. The first evidence of such attack was found in 2009 where one earthen cell was found to have an adult *A. segnis* wasp. Also in 2009, of 307 dissected earthen cells from 120 soil cores, four earthen cells were found containing *L. soperi*. The remaining cells contained *P. thomsoni* adults or evidence of *P. thomsoni* (molted sixth instar skins, etc), contained unidentifiable materials, or were empty (Table 7). Fifteen cells contained empty wasp pupal exuviae. Wasp pupal exuviae were found encasing adult *A. segnis* and *L. soperi* wasps, when they were found inside earthen cells. It is unknown if either wasp species consumes its exuvia upon emergence or if the exuvia disintegrates, which can happen in some species of wasps. In some cases, the wasp pupal exuvia was shriveled up and resembled that of a 6^{th} instar leafminer cast skin. Because of these complexities, wasp exuviae were not assigned to exact species.

In 2009, the small number of recoveries of *L. soperi* and *A. segnis* were likely due to the effects of the sample date. Soil core collections in July and August were perhaps too late and *A. segnis* wasps had already emerged.

In 2010, of 486 dissected cells from 540 soil cores collected at six sites, 19 were found that contained *A. segnis* remains and 12 earthen cells were found containing *L. soperi*. Twenty-two cells were found containing evidence of wasp pupal exuviae. Evidence of *P. thomsoni* (as adults or prepupae) was found in 87 cells. The remaining cells we were unable to identify or were empty (Table 8).

In 2011, 517 dissected cells were located in 140 soil cores. Adult *A. segnis* wasps were found in 54 earthen cells and *L. soperi* wasps were found in 22 cells inside their pupal exuviae. Of the cells examined, 104 contained evidence of *P. thomsoni* either as prepupae or as adults, 201 cells were found to be empty and the contents of 37 cells were unidentifiable. An additional 24 cells contained wasp pupal skin but no wasps inside of them (Table 9).

Parasitism rates of *P. thomsoni* by *L. soperi* at non-*L. thomsoni* release sites in Alaska

Parasitism rates in samples taken in the third week of August were compared among 2006, 2007, 2008, 2009, and 2010 (Figure 18). In 2006, at permanent plots, percent parasitism rates averaged around nine percent. In 2011, percent parasitism rates climbed to 30 percent, with some sites seeing greater that 50% parasitism (Figure 18). A one-way ANOVA was used to test for differences in parasitism rates between years. Parasitism rates for years differed significantly across the years, $F= 6.0297$, $df=2,19$ $p <0.0001$. Testing for homogeneity of variance was done using Bartlett's test and no assumptions were violated ($F= 1.7520$, $df = 5$. $p = 0.1190$). Using the Tukey-Kramer HSD test, pair-wise comparisons were made between the years and 2011 parasitism rates were significantly higher than 2006, 2007, 2008, and 2009 (Table 10). All results of the Tukey-Kramer tests are detailed in Table 10.

Weekly parasitism rates from 2008 to 2010 were compared among samples (Figure 19). A one-way ANOVA was used to test for differences in parasitism rates between years. Weekly parasitism rates for each year differed significantly ($F = 3.2862$, $df = 2, 221$, $p=0.0392$). A Tukey-Kramer HSD pair-wise comparison test found that parasitism rates were significantly higher in 2010 as compared to 2009 ($p = 0.0342$) and not significant between 2010 and 2008 ($p = 0.0641$) (Table 11).

Adult *A. segnis* and *L. soperi* trap catch in Alaska

The timing of peak flight varied between wasp species, with *L. soperi* having a later flight time (Figure 20). The lower density of wasps in 2009 may have been due to the extremely cool temperatures in 2008, which may have not allowed all wasps time to complete their development.

Adult *A. segnis* and *L. soperi* emergence

The timing of emergence between the two wasps was similar in all four years sampled (Figure 21).

Alternate hosts of *L. soperi* and *A. segnis*

A one-way ANOVA showed no significant difference between the log (n+1) of *A. segnis* ($F=1.2380$, $df=4,120$, $p=0.298$) or *L. soperi* wasps ($F = 1.8116$, $df=4,120$, $p=0.1310$) found per sticky card in any tree species in Alaska (Figure 22). Sampling more trees in a wider geographic area during a longer sampling period would likely be able to provide more information regarding what other leafminers or sawflies exist in Alaska as well as host trees for those sawflies.

Sweep sampling to locate *A. segnis* and *L. soperi* in Alaska

No wasps of either species were found in locations where *P. thomsoni* did not co-occur. In locations where the host did occur, both wasps exhibited a slightly different distribution. *Lathrolestes soperi* was found widespread throughout Wasilla, Palmer, and the Anchorage bowl. It was found south of Anchorage to Girdwood, Alaska. No *L. soperi* wasps were found on the Kenai Peninsula in Cooper Landing or Soldotna, Alaska (the major urban centers on the Kenai peninsula). *Aptesis segnis* was found in all sweep sample locations containing *P. thomsoni*, suggesting a much larger distribution (Figure 23).

Levels of mortality causing by *A. segnis* to *L. soperi* and *P. thomsoni*

There was a significant relationship between the percent parasitism of *L. soperi* wasps in leafmines of one year and the proportion of *L. soperi* wasps of all insects (*L. soperi*, *A. segnis*, and *P. thomsoni*) trapped in emergence cones the following year ($R^2 = 0.252$, $p = 0.0146$) (Figure 24). For sites examined, the average fall parasitism was 13.8%. The following season, the average proportion of *L. soperi* wasps emerging from emergence cones was 13.3%, suggesting that *A. segnis* wasps have very little impact on the effectiveness of *L. soperi*.

The presence of *A. segnis* in the parasitoid guild raised mortality of P. thomsoni to 40.3%, showing that the percent parasitism by *A. segnis* was 26%, double that provided by

L. soperi (Figure 25). This suggests that this parasitoid, a heretofore overlooked species, is in fact the dominant parasitoid in the guild.

Comparison between *P. thomsoni* earthen cell availability and *A. segnis* flight time

Aptesis segnis wasps continue to fly even after the majority of *P. thomsoni* have emerged (Figure 26). This suggests to us that some proportion of wasps attack the leafminer after it has completed development and dropped back into the soil. If this is occurring, we expect some low-level of facultative hyperparasitism by *A. segnis.*

Discussion

Understanding leaf-mining sawfly parasitoid communities is important as several sawfly species are invasive throughout the world (Gilbert et al. 2004; Digweed et al. 2009) and several have been brought under successful biological control (Cassagrande et al. 2009; Digweed et al. 2009). Extensive parasitoid complexes of leaf-mining sawflies are well documented in their native range (Pschorn-Walcher and Altenhofer 1989) and some hosts can have as many as 10-17 parasitoids per species. *Fenusa pumila* Leach has a parasitoid complex of seventeen wasps, most of them generalists (Pschorn-Walcher and Altenhofer 1989). Currently, classical biological control recognizes that several natural enemies may be required to control an invasive species (Van Driesche et al. 2008), as long as the introduced species do not have any damaging non-target effects.

In Europe, most species of leaf-mining sawflies in the tribe Fenusini are attacked by a single species of the endoparasitic genus *Lathrolestes* (Pschorn-Walcher and Altenhofer 1989). The only previously reported exception is *Messa glaucopis* Konow (poplar sawfly) and *Messa hortulana* Klug (poplar and maple sawfly) (Çalmaşur and Özbek 2004), both of which are attacked by two species of *Lathrolestes* (Pschorn-Walcher and Altenhofer 1989). In addition to one specialized *Lathrolestes* sp., sawflies are often attacked by some ectoparasitoid, which in Europe largely comes from the genus *Grypocentrus* (Ichneumonidae) (Pschorn-Walcher and Altenhofer 1989) or in some cases by a species of *Aptesis*. Babendreier (2000) showed that *A. nigrocincta,* a bivoltine ectoparasitoid of apply sawfly, was able to parasitize 12 to 39% of its hosts per generation, thereby, contributing the greatest degree of mortality (Babendreier 2000).

Attack on invasive insects by native parasitoids is common but usually only occurs at low rates. (Aebi et al. 2007; Klug et al. 2008). For example, in Italy, sixteen native parasitoids attacked the invasive chestnut gall wasp; however, their attack rates were between 0.5-1.6% (Aebi et al. 2007). In the case of *L. soperi* parasitism rates are high (greater than 50% at some sites) and this species is acting as a natural biological control agent in Anchorage, Alaska. Certainly, some new host/parasitoid associations do exist and are suppressive of the host (Van Driesche et al. 2008). Furthermore, *A. segnis,* another parasitoid that seems not to have evolved with *P. thomsoni*, is responsible for significant mortality of ambermarked birch leafminer.

It is difficult to study soil-dwelling arthropods and evaluate their effects on other species. It is likely that *A. segnis* attacks other species of leafminers in North America, but because its attacks occur underground, they could easily overlooked. In particular, if studies are based on collecting larvae from leafminer (larvae that have never been exposed to soil-acting parasitoids), it is very likely that species such as *A. segnis* would go unnoticed. It was not previously known if *A. segnis* facultatively attacks both *L. thomsoni* and *L. soperi* or was a primary parasitoid of the leafminer. However, based on our study, it seems unlikely that *A. segnis* does act as a hyperparasitoid, because it emerged earliest in the season of all the relevant species (personal observ.) and attacked *P. thomsoni* pre-pupae or pupae in their earthen cells before they emerge. No evidence of adult *A. segnis* wasps was found at the end of the growing season (September or October), either as free-flying wasps or in soil samples.

There were however, free flying wasps *A. segnis* wasps occurring in the system after peak *P. thomsoni* emergence. One scenario is that the majority of *A. segnis* wasps emerge early in the season and attack *P. thomsoni* as prepupae in the soil and those wasps that emerge too late don't get the opportunity to attack *P. thomsoni* and they fail to continue. The second scenario is that *A. segnis* wasps emerge early in the season (mid-May) until mid-August. Most *A. segnis* wasps attack *P. thomsoni* larvae in the soil before they emerge (as pre-pupae). However, some of those *A. segnis* wasps that exist later in the season would also have the opportunity to attack *P. thomsoni* larvae as they drop into the soil to overwinter. In the second scenario there would likely be some level of facultative hyperparasitism of *Lathrolestes* wasps as it would be difficult for the wasp to distinguish those that are parasitized and those that are not. This is possible but the empirical data don't show much of a shift such that *Lathrolestes* parasitism would decline between the two seasons. Since they appear not to reduce the *Lathrolestes* impact we infer that they do make the distinction but we do not have any direct observations to support that. More research is necessary to tease apart these interactions.

In general, sampling in leafminer habitats either by sweeping, emergence cones, or sticky cards would be valuable to search for specialist parasitoids. More attention to soil dwelling parasitoids could be useful in biological control projects. This is the first record of *A. segnis* attacking *P. thomsoni*. Two of the three *P. thomsoni* parasitoids found in Alaska occupy the same niche (attacking host larvae in the leaf) and while one species may outcompete the other over time there is potential for stability with three parasites in the system as witnessed in similar sawfly parasitoid communities by Pschorn-Walcher and Altenhofer (1989). Because *A. segnis* attacks the leafminer in the soil, it seems unlikely that Lathrolestes parasitoids will have any effect on it. Future work on this system is recommended in five to ten years to see if *L. thomsoni* and *L. soperi* populations remain stable or to see if one parasitoid outcompetes the other and if *A. segnis* maintains its dominant place in the system.

Tables

Table 7: Evidence from dissections of *Profenusa thomsoni* earthen cells from soil samples collected in Anchorage, Alaska at six permanent sites from July-September 2009.

Site:	***A. segnis***	***L. soperi***	***P. thomsoni***	**Empty**	**Cast skins**	**Un-known**	**Wasp Pupal Skins**
Sitka	0	0	3	1	2	0	0
Jewel Lake	0	3	10	16	42	1	11
Javier	0	1	28	25	10	3	4
UAA-Providence	0	0	23	14	9	1	0
UAA-Library	0	0	39	12	37	4	0
MedPark	1	0	2	2	1	2	0
Total	**1**	**4**	**105**	**70**	**101**	**11**	**15**

Table 8: Evidence from dissections of *Profenusa thomsoni* earthen cells from soil samples collected in Anchorage, Alaska at six permanent sites from May-September 2010.

Site:	***A. segnis***	***L. soperi***	***P. thomsoni***	**Empty**	**Cast Skin**	**Un-known**	**Wasp Pupal Skin**
Balto	1	2	7	14	22	18	1
Javier	10	8	50	65	46	37	17
Lutheran Church	2	0	6	1	4	7	1
Oceanview	1	0	0	0	4	0	0
Pt. Woronzof	1	1	3	4	3	1	1
UAA-Library	4	1	21	27	37	33	2
Total	**19**	**12**	**87**	**111**	**116**	**96**	**22**

Table 9: Evidence found from dissections of *Profenusa thomsoni* earthen cells from soil samples collected in Anchorage, Alaska at six permanent sites and one release site in May 2011.

Site:	***A. segnis***	***L. soperi***	***P. thomsoni***	**Empty**	**Cast Skins**	**Un-known**	**Wasp Pupal Skin**
Balto	2	0	5	8	4	1	1
Javier	14	9	49	52	9	23	4
Lutheran Church	2	1	1	11	2	0	0
Oceanview	0	0	1	4	3	1	0
Pt. Woronzof	0	0	2	2	3	3	3
UAA-Library	5	5	20	16	16	0	0
UAA-Release 2006	31	7	26	108	38	9	16
Total:	**54**	**22**	**104**	**201**	**75**	**37**	**24**

Table 10: Tukey-Kramer statistical analysis displaying pair-wise comparisons and p-values between years (2006-2011) of parasitism by *Lathrolestes soperi* against *Profenusa thomsoni* collected at permanent plots in Anchorage, Alaska in the third week of August.

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
2011	2006	23.078	4.701	9.414	36.74136	<.0001*
2011	2007	20.579	4.701	6.9151	34.24252	0.0004*
2011	2008	17.491	4.899	3.2533	31.72779	0.0071*
2011	2009	16.514	4.701	2.8499	30.17733	0.0086*
2010	2006	11.802	4.701	-1.8622	25.46522	0.1311
2011	2010	11.276	4.899	-2.9611	25.51337	0.2032
2010	2007	9.303	4.701	-4.361	22.96639	0.3619
2009	2006	6.564	4.495	-6.501	19.62902	0.6901
2010	2008	6.214	4.899	-8.0228	20.45165	0.8013
2008	2006	5.587	4.701	-8.0766	19.25081	0.8414
2010	2009	5.238	4.701	-8.4262	18.90119	0.8745
2009	2007	4.065	4.495	-8.9998	17.13018	0.9446
2008	2007	3.088	4.701	-10.5754	16.75197	0.9861
2007	2006	2.499	4.495	-10.5662	15.56382	0.9935
2009	2008	0.977	4.701	-12.6868	14.6406	0.9999

Table 11: Tukey-Kramer statistical analysis displaying pair-wise comparisons and p-values between years (2008-2010) of parasitism by *Lathrolestes soperi* against *Profenusa thomsoni* collected at permanent plots in Anchorage, Alaska weekly from July to September 2008-2010.

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
2010	2009	0.0739323	0.0294745	0.004382	0.1434826	0.0342
2010	2008	0.0662204	0.0293313	-0.002992	0.1354326	0.0641
2008	2009	0.007712	0.0202132	-0.039985	0.0554086	0.9229

Figures

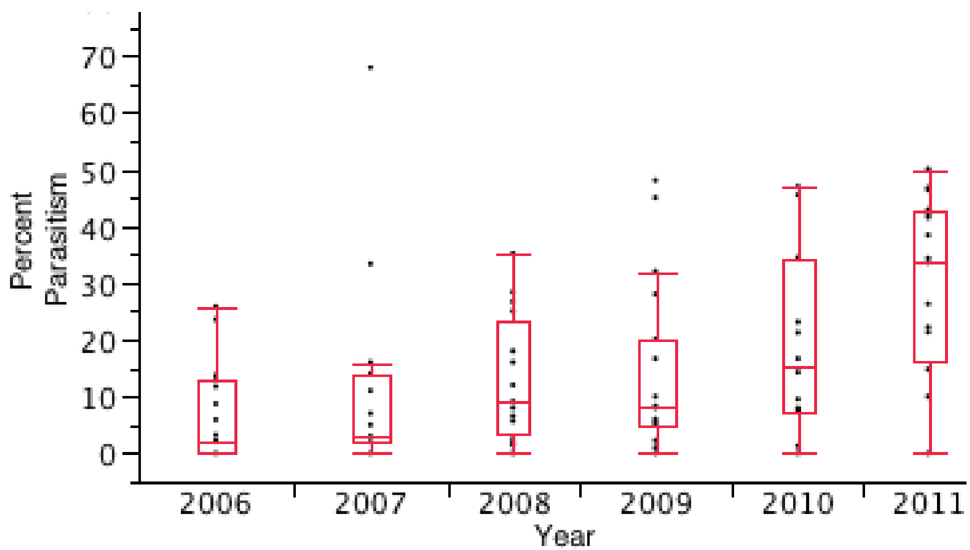

Figure 18: Parasitism of the ambermarked birch leafminer (*Profenusa thomsoni)* larvae at permanent plots in Anchorage, Alaska in the third week of August 2006-2011.

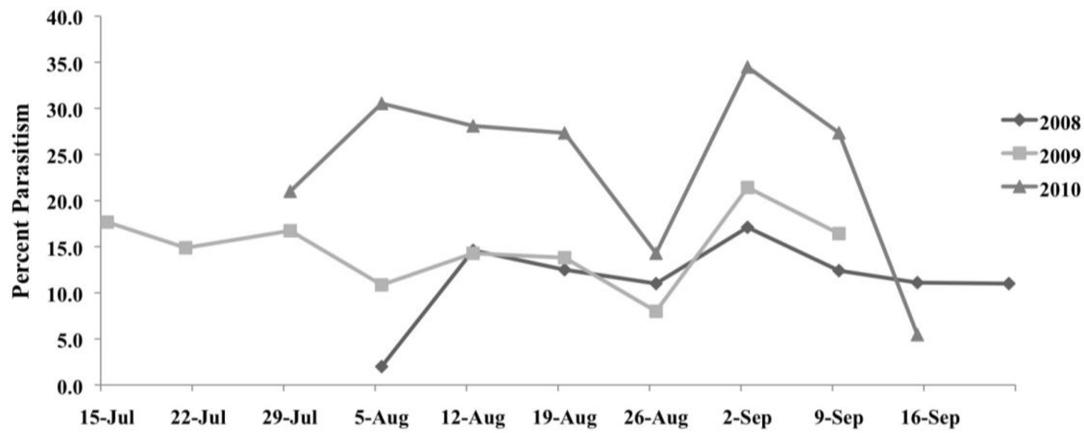

Figure 19: Parasitism of ambermarked birch leafminer (*Profenusa thomsoni*) larvae by *Lathrolestes soperi* at permanent plots in Anchorage, Alaska from August-September 2008-2010.

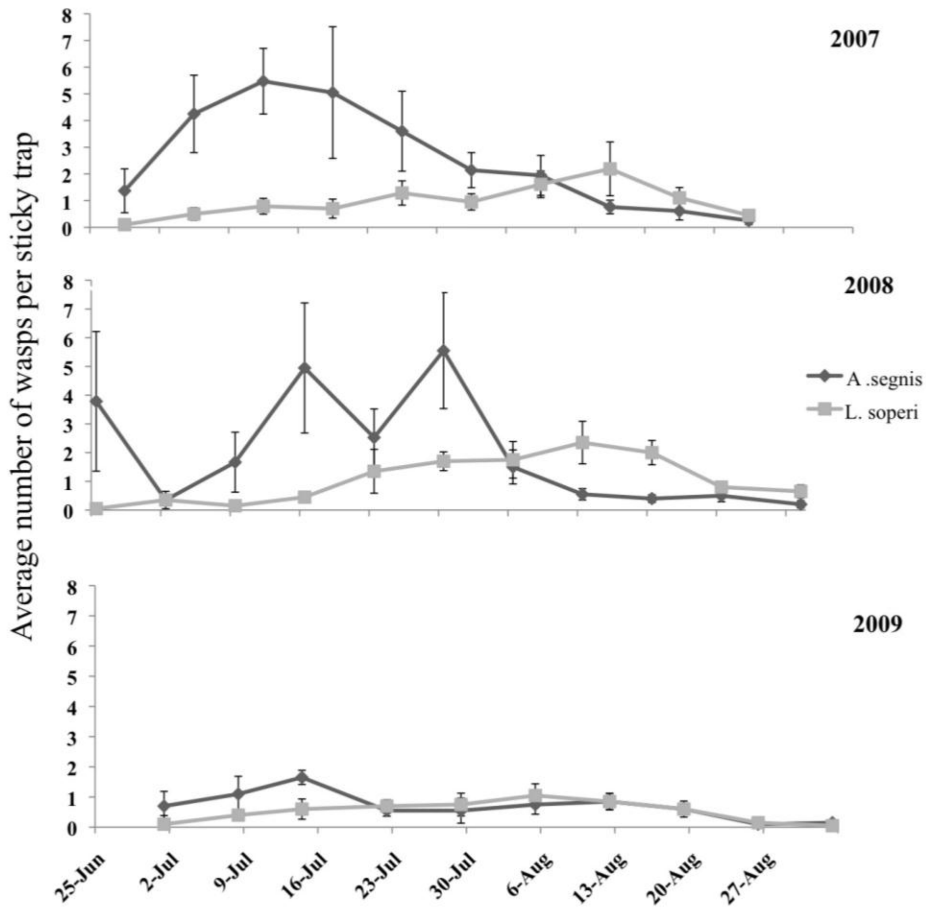

Figure 20: The average number of *Aptesis segnis* and *Lathrolestes soperi* adults caught on yellow sticky cards in Anchorage, Alaska from June-September 2007, 2008 and 2009 at permanent plots.

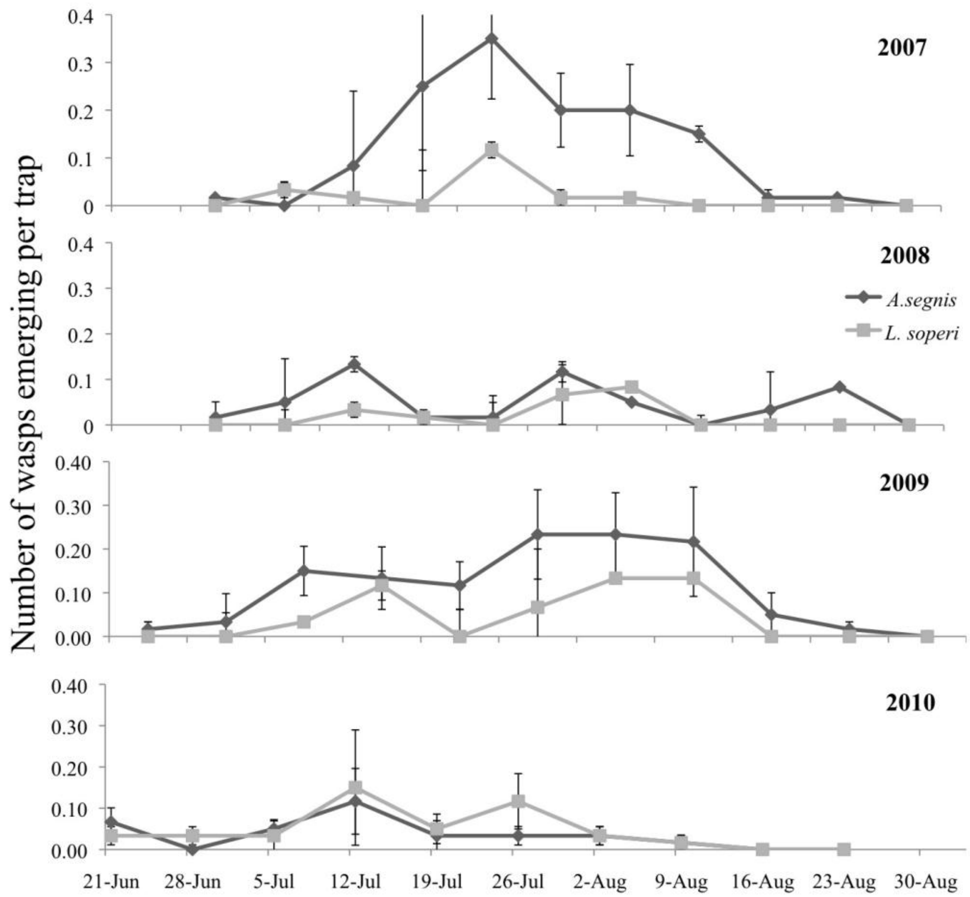

Figure 21: The average number of *Aptesis segnis* and *Lathrolestes soperi* adults caught in emergence cones in Anchorage, Alaska from June-September 2007, 2008 and 2009 at permanent plots

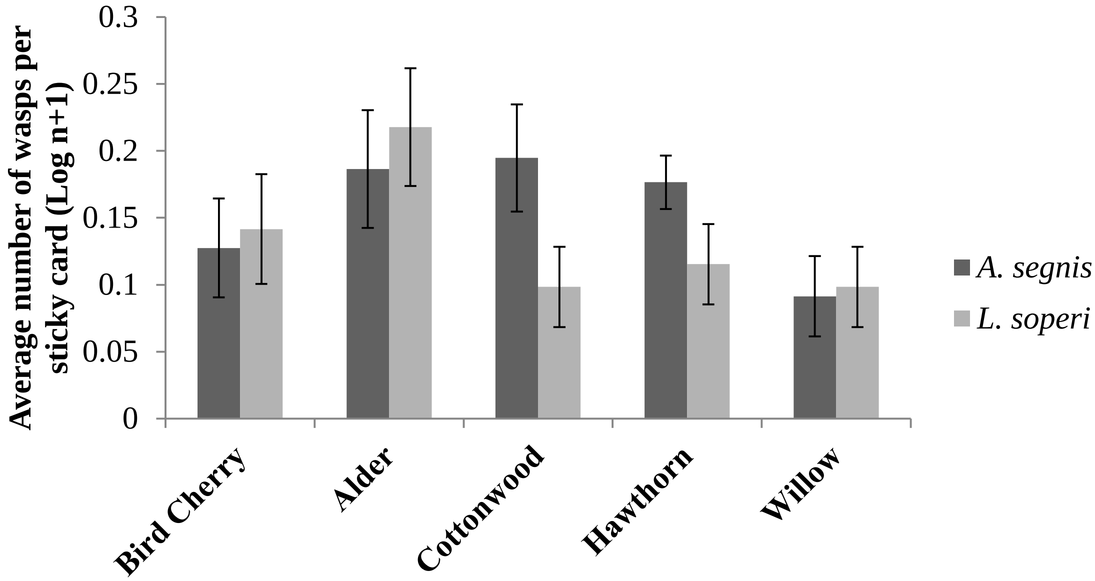

Figure 22: Average log (n+1) of *Lathrolestes soperi* and *Aptesis segnis* wasps found per sticky card in different tree species in Anchorage, Alaska collected from July 17-August 15 2010.

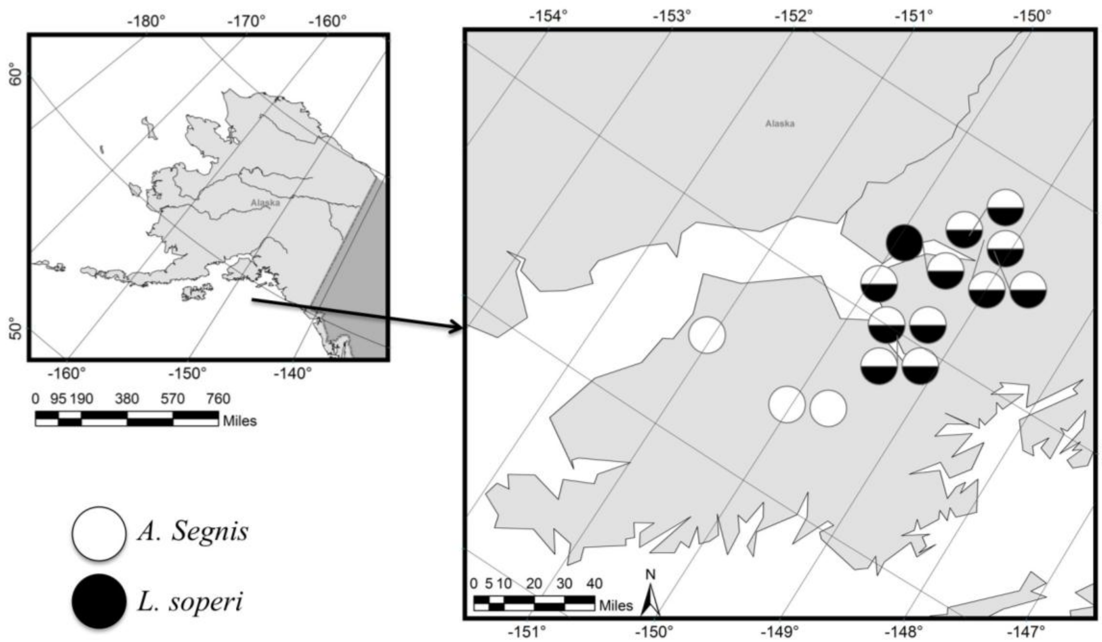

Figure 23: Map of south-central Alaska displaying *Aptesis segnis* and *Lathrolestes soperi* distributions as found from sweep sampling July-August 2010.

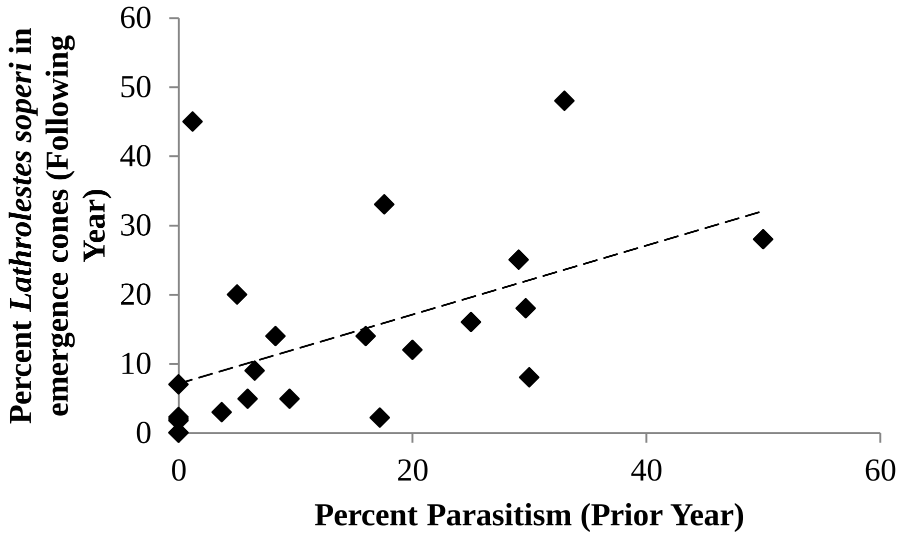

Figure 24: Linear regression of the proportion of *Lathrolestes soperi* adults emerging from emergence cones summed over the entire season vs. the percent parasitism of *L. soperi* against *Profenusa thomsoni* at permanent plots in Anchorage, Alaska on August 15 of the previous year.

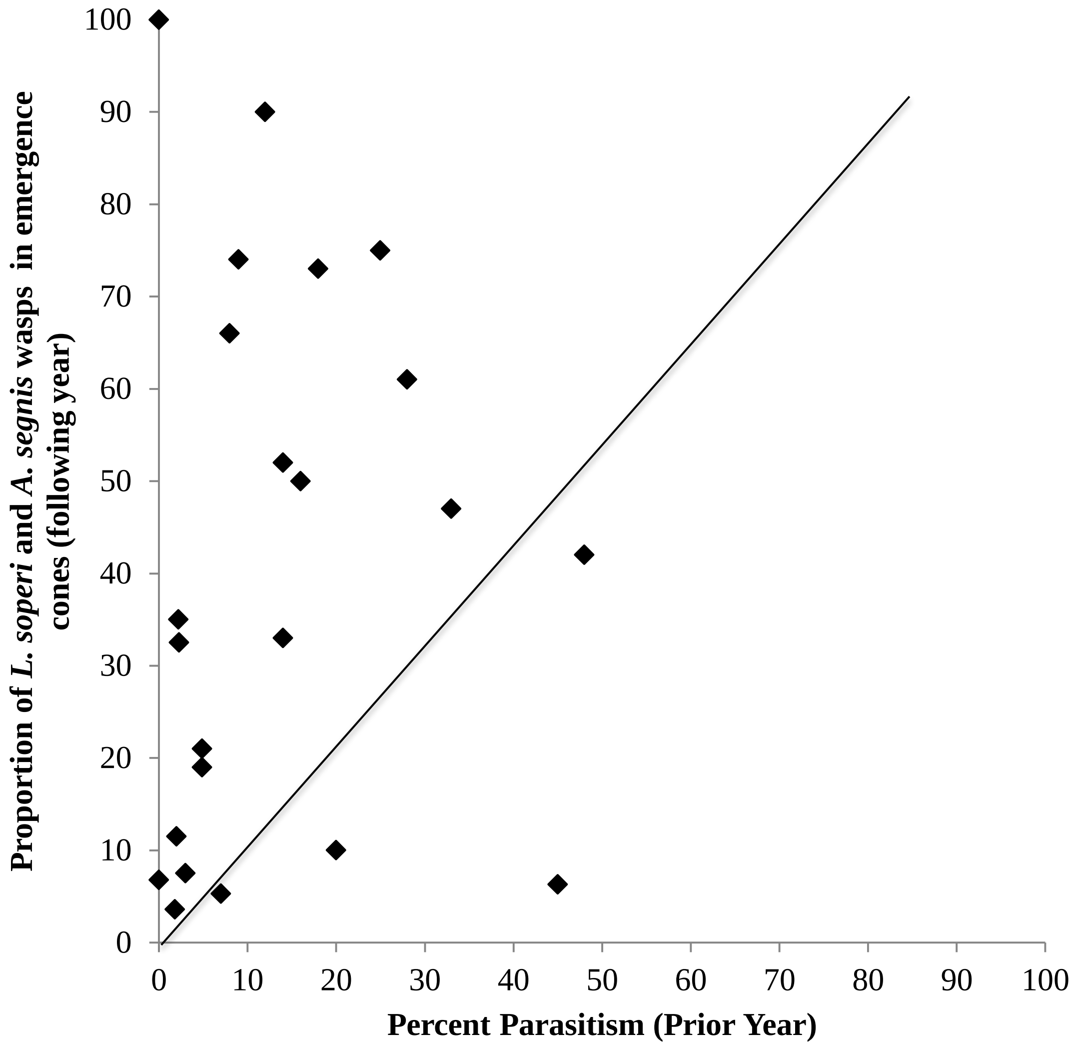

Figure 25: Linear regression of the proportion of *Aptesis segnis* and *Lathrolestes soperi* adults emerging per per unit area of soil (122 cm^2) summed over the entire season vs. the percent parasitism of *L. soperi* against *Profenusa thomsoni* at permanent plots in Anchorage, Alaska on August 15 of the previous year.

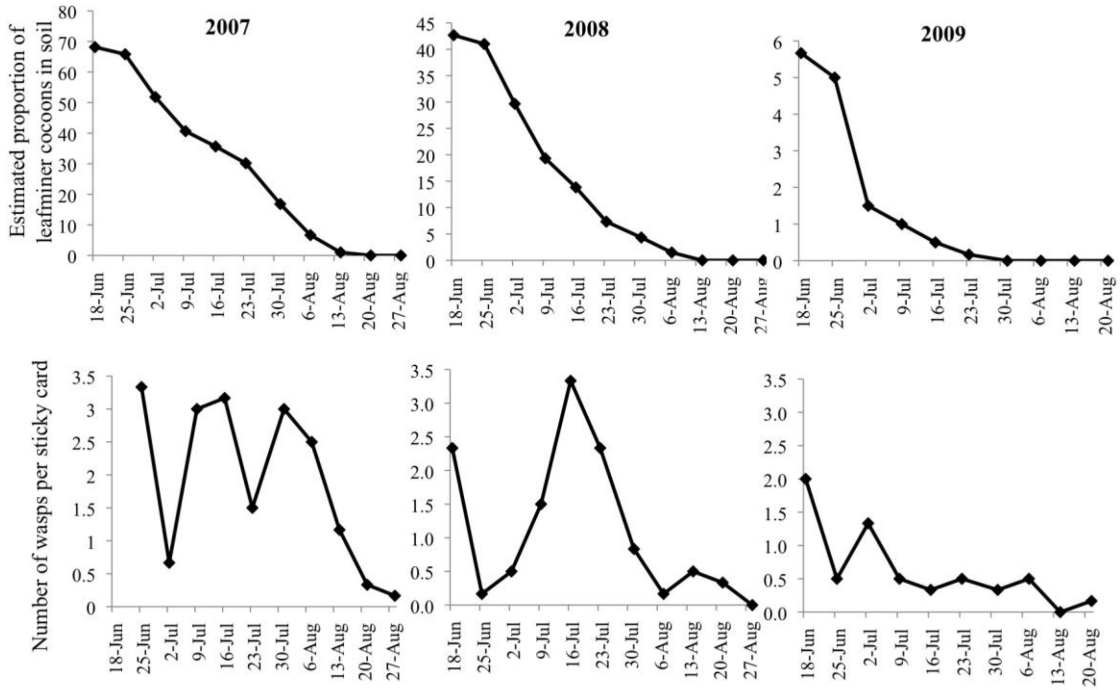

Figure 26: Comparison between *A. segnis* seasonal flight time and the estimated proportion of *P. thomsoni* cocoon availability in permanent plots in Anchorage, Alaska from 2007-2009.

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APPENDIX

SUPPLEMENTARY TABLE

Site	Latitude	Longitude	Habitat
15th Avenue School	61.208531	-149.890033	Urban
Anchorage High	61.200525	-149.915011	Urban
Balto Seppala	61.190808	-149.946917	Forest
Delaney Park	61.212994	-149.907669	Urban
Earthquake Park	61.198953	-149.991081	Forest
Extension	61.196467	-149.838633	Urban
Forsythe	61.119125	-149.773325	Forest
Gooselake	61.196389	-149.824511	Forest
Javier	61.169017	-149.917806	Forest
Jewel Lake	61.141414	-149.959789	Forest
Lutheran Church	61.148267	-149.892531	Urban
MedPark	61.195936	-149.839792	Urban
Oceanview	61.099083	-149.858419	Urban
Orca Avenue	61.207547	-149.854936	Urban
Pt. Woronzof	61.199356	-150.021631	Forest
Sitka Park	61.206044	-149.847053	Forest
UAA-Extra	61.188303	-149.833919	Urban
UAA-Library	61.190919	-149.816572	Urban
UAA-Providence	61.188553	-149.835269	Urban
Westchester Lagoon	61.202544	-149.918394	Forest

Table 1: GPS coordinates of permanent plots in Anchorage, Alaska.

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