University of Alberta

The roles of temperature and host plant interactions in larval development and population ecology of *Parnassius smintheus* Doubleday, the Rocky Mountain Apollo butterfly

by

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For my grandparents.

Abstract

Alpine environments are harsh and unpredictable. Exogenous factors such as weather might therefore be expected to dominate processes affecting population dynamics of alpine organisms, relative to endogenous factors including plant-animal interactions. The alpine butterfly *Parnassius smintheus* Doubleday is a specialist on *Sedum lanceolatum.* I examine the importance of interactions between *P. smintheus* larvae and *S. lanceolatum*, specifically the potential for an induced defense, indicating that host plant/herbivore interactions may play a significant role in *P. smintheus* populations. I also examine the effect of temperature (as a component of climate) on the development and survival of *P. smintheus* caterpillars. Using laboratory and field studies I show that host plant interactions are not important for *P. smintheus* in the field, and instead moderate changes in temperature are more likely to affect *P. smintheus* populations. I suggest that any population-level effects of temperature will likely be indirect and mediated through phenological shifts.

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Chapter 1

Introduction

Endogenous and exogenous factors in populations

The relative strength of endogenous and exogenous processes in regulating populations is a central topic in ecology (Previtali *et al.* 2009, Pickens 2007, McLaughlin *et al.* 2002). Endogenous processes are density dependent, such as competition or predation, while exogenous processes, such as weather or disturbance, operate independently of a population's intra and interspecific interactions (Previtali *et al.* 2009, McLaughlin *et al.* 2002), and as such independent of population density. Insect populations are often considered to be regulated primarily by exogenous factors (Isaac *et al.* 2011, Roy *et al.* 2001, Andrewartha and Birch 1954), but many insect populations are determined by endogenous factors, such as interactions with parasitoids (Turchin *et al.* 2003, Várkonyi *et al.* 2002) or host plants (Underwood 2000, Mook and van der Toorn 1985). Populations are likely driven by a combination of both endogenous and exogenous factors (Nowicki *et al.* 2009, Pickens 2007, Araújo and Luoto 2007, McLaughlin *et al.* 2002). Understanding the relative contribution of each of these processes to a species' population dynamics is particularly relevant with the prospect of global climate change.

Alpine environments feature harsh weather conditions that are highly variable from season to season and even from day to day. Alpine ecosystems are predicted to be among those environments most strongly affected by climate change, both in terms of temperature increase and altitudinal shifts in habitat distribution (Dirnböck *et al.* 2011, Parmesan 2006). Insects in particular are likely to respond quickly to climate change due to their short generation time and heavy reliance on ambient temperature for development (Wilson and Mclean 2011).

They can be affected either directly by climate change through temperature-mediated changes in phenology (Singer and Parmesan 2010), or indirectly through changes in habitat suitability (Ashton *et al.* 2009) or shifts in predator-prey dynamics (Stireman *et al.* 2005).

Insects in cold environments, such as the alpine, are well-adapted to cold weather conditions (Danks 2006). One adaptation that is of particular relevance to population dynamics is flexibility in life cycle length (Danks 1992). Summer is short for arctic and alpine insects and individuals of many species must reach a specific life stage by the end of the growing season in order to survive the winter (*i.e.* overwinter diapause). Insects with flexible life cycles can alter the number of generations per year by a process called "cohort-splitting" (Danks 1992, Ingrisch 1986, Pritchard 1980). Cool years can result in a second diapause stage instead of life cycle completion; the following year would result in an increase in population size relative to the cool year due to the presence of two generations (Ingrisch 1986). This bet-hedging strategy could be particularly beneficial for alpine species if alpine climates become more variable.

Despite the potential for strong weather effects in the alpine, insects are none the less still affected by a variety of biotic interactions. Biotic interactions include, but are not limited to, parasitism (Turchin *et al.* 2003, Várkonyi *et al.* 2002) and interactions with host plants. Host plant quality is highly variable, even within a plant population (Floater and Zalucki 2000, Virtanen and Neuvonen 1999), and can also change with herbivory (Awmack and Leather 2002, Karban and Baldwin 1997). One way in which plant/herbivore interactions known to affect insect populations are chemical defences (Roslin *et al.* 2008, Karban and Baldwin 1997, Heliövaara and Väisänen 1988, Haukioja and Hanhimäki 1985). Plant defences can be either constitutive, whereby they are produced continuously by the plant to deter herbivory, or induced in response to herbivory to prevent further damage (Karban and Baldwin 1997). Induced plant defences are often chemical (but see Massey *et al.* 2007), and can negatively affect larval growth and development (Roslin *et al.* 2008, Hódar *et al.* 2004, Karban and Baldwin 1997). A

rapid induction of a defence over a short time period (minutes to days) prevents further damage by an individual or its cohort, whereas delayed induction, which may take months or years, will negatively affect subsequent herbivore life stages or generations (Karban and Baldwin 1997). Delayed defences can be a source of negative density dependent population growth if the herbivore population overshoots its carrying capacity and is then faced with a lack of high quality food due to the lagged plant defence (Underwood 2000, Berryman 1996). Induced defences can therefore limit herbivore populations at densities lower than competition could by itself because, although there is still foliage, it is unavailable due to the defence. Delayed induced plant defences contribute to the population cycles of several insect species, and often act in conjunction with exogenous factors (Hódar *et al.* 2004) or predator/prey interactions (Haukioja 2005, Baltensweiler *et al.* 1977). The strength of induced defences as drivers of population dynamics differs among insect species, as does relative importance of endogenous and exogenous factors.

An alpine butterfly as a model species

Parnassius smintheus Doubleday (Papilionidae), the Rocky Mountain Apollo butterfly, is a univoltine butterfly inhabiting alpine meadows from the Yukon Territory, Canada, to Colorado, USA. Larvae hatch in late May/early June and, in Kananaskis Country, AB, are largely monophagous on *Sedum lanceolatum* Torrey (Crassulaceae), the lance-leaved stonecrop. *S. lanceolatum* is a yellow-flowered, perennial succulent widely distributed in rocky habitat, (generally mountainous regions) of Western North America, from Northern New Mexico to the Yukon Territory (Clausen 1975). Polyploidy occurs readily in this species, leading to different plant morphs (Clausen 1975). At my study site in Kananaskis Country, AB, the only *Sedum* species that occur are *S. lanceolatum* and *Rhodiola integrifolia* (formerly *S. integrifolium*), which looks quite distinct (*R. integrifolia* has red flowers) (Hallworth and Chinnappa 1997). It is important to note that facultative Crassulacean acid metabolism (CAM) photosynthesis has been shown for other *Sedum* species, whereby plants exhibit CAM photosynthesis in very dry

conditions and C-3 photosynthesis in wetter conditions (Castillo 1996, Gravatt and Martin 1992). Each type of photosynthesis leads to malic acid production at a different time during the day, thus altering the taste of the leaves (Black and Osmond 2003). CAM photosynthetic plants store malic acid produced at night until morning, and therefore taste acidic until then, whereas C-3 plants produce malic acid continually over the day and therefore do not change taste.

Occasionally *P. smintheus* will feed on the closely-related *R. integrifolia*, but it is uncommon. Herbivory of *S. lanceolatum* by any other animal is rare, implying that it maintains either a constitutive or an induced defence (see below). *P. smintheus* larvae are aposematically coloured and are rarely preyed on, a pattern to the sequestration of sarmentosin, a cyanoglucoside (Nishida and Rothschild 1995). Larvae complete five instars before pupating in the soil in late June/early July. Adults are protandrous and fly from mid/late July to late August/early September. Females nectar feed on yellow flowers (including *S. lanceolatum* flowers) and oviposit off of the larval host plant (Fownes and Roland 2002). This behaviour is relatively uncommon in butterflies (Wiklund 1984), but is typical of the genus (Fred *et al.* 2006, Wiklund 1984).

Density and movement of *P. smintheus* populations have been studied on Jumpingpound Ridge in Kananaskis, AB (50°84'N, 114°87'W, ~2200m) since 1995 (Roland and Matter 2007), providing a long-term project within which to conduct my research. *P. smintheus* populations show a two to three year peak and trough pattern of growth rate between 1995 and 2008 (Fig. 1.1). A two-year pattern of growth and decline has been explained for other insect species by endogenous factors generating strong negative density dependent growth (Várkonyi *et al.* 2002, Heliövaara *et al.* 1994) or to cohort-splitting due to exogenous factors like cold temperatures (Danks 1992, Ingrisch 1986).

My research aims to identify the presence and importance of endogenous and exogenous processes in the *P. smintheus* life cycle by exploring the effects of host plant/herbivore interactions and of changes in temperature on larval growth

and development. Results will offer insight into the factors which determine dynamics of an insect in a capricious environment.

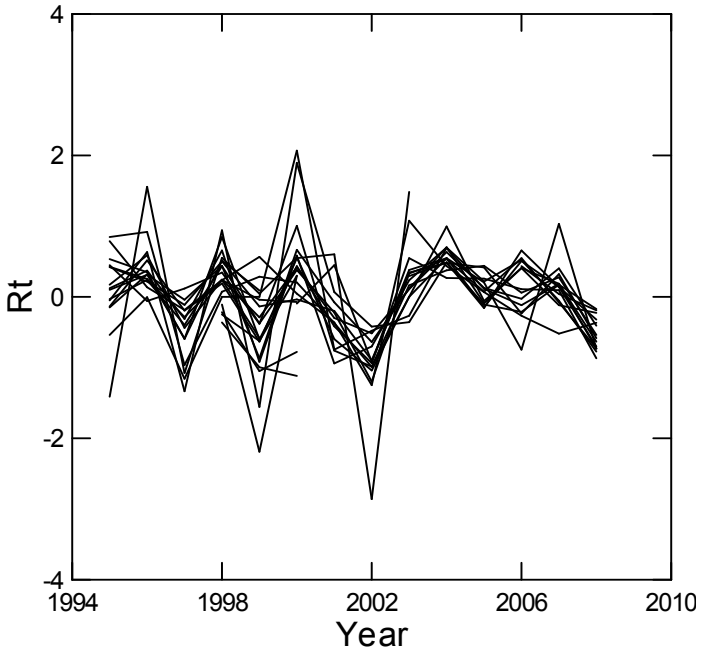

Figure 1.1. Population growth rate for 21 *P. smintheus* subpopulations on Jumpingpound Ridge in Kananaskis, AB from 1995-2008. Each line represents a different subpopulation. From Roland and Matter 2007.

Temperature and Parnassius smintheus

The first mechanism I will address as a potential explanation for *P. smintheus* population dynamics is cohort-splitting (Danks 1992, Ingrisch 1986). Scott (1986) states that a late-instar overwinter diapause stage is possible for *P. smintheus*, which could ultimately lead to two distinct cohorts existing at the same time. If a sufficient number of individuals undergo a second overwintering diapause stage, the following summer would see a significant increase in adult butterfly numbers relative to the year prior. Chapter 2 describes the effect of moderate changes in temperature on the growth and development of late instar *P. smintheus* larvae. I used larvae reared at temperatures slightly above and below the average maximum field temperature to estimate how minor changes in temperature, like those predicted by climate change models (Solomon 2007), impact growth rate and phenology. Of particular interest is the prevalence of an

additional overwintering diapause state among laboratory-reared larvae because of the implications for *P. smintheus* population dynamics.

Host-plant/herbivore interactions of Parnassius smintheus

The other potential explanation that I will address for the two-year pattern of population growth and decline is an herbivory-induced change in host-plant quality. The specialized interaction that *P. smintheus* has with its host may explain the pattern of population growth for several reasons. Firstly, larvae fed previously damaged *S. lanceolatum* in the laboratory show reduced growth compared to larvae fed undamaged plants (Roslin *et al.* 2008), a trend that the authors attribute to an induced defence. Secondly, in addition to this laboratory evidence, patterns of herbivory in the field indicate that herbivory in one year is highest on plants undamaged the previous year (Kurt Illerbrun, *unpub.*). If there is a strong herbivory-induced decrease in host plant quality in this system, knowing the temporal scale on which it is affecting larvae, whether it is lagged and affects subsequent generations or not, is paramount to understanding its implications for population dynamics. Previous research into the effect of host plant damage on *P. smintheus* growth (Roslin *et al.* 2008) was on a very short time scale, 2-5 days, not over multiple generations, used only mechanical damage (not actual herbivory) and was done in a laboratory setting. For these reasons, my research focuses on field experiments evaluating the effect of an herbivory-induced change in host plant quality on larval growth over a relatively long time scale.

In Chapter 3, I describe a series of experiments conducted both in the field and in the laboratory to test the effects of previous herbivory of *S. lanceolatum* on *P. smintheus* larval growth both within a single season and between seasons (*S. lanceolatum* damaged in year one is fed to larvae in year two).

In Chapter 4 I examine adult female *P. smintheus* perception of habitat quality with respect to *S. lanceolatum* herbivory. Ovipositing female butterflies will ideally choose suitable habitat for their relatively immobile larvae (Awmack and Leather 2002, Bergman 1999, Renwick and Chew 1994), and if previous

herbivory is detrimental to larval fitness, females should avoid damaged *S. lanceolatum* plants. Although females do not oviposit directly on *S. lanceolatum*, its presence stimulates oviposition (Fownes and Roland 2002). In Chapter 4, I describe female choice of oviposition site based on *S. lanceolatum* abundance (damaged and undamaged plants) as well as the abundance of nectar resources. Chapter 4 also describes female preference for damaged or undamaged *S. lanceolatum* when ovipositing in a controlled laboratory setting.

My goal is to contribute to the body of knowledge surrounding what drives insect populations in cool climates by exploring the importance of an endogenous and an exogenous factor for an alpine butterfly in an unpredictable environment. The balance of the factors influencing *P. smintheus* population dynamics will lend insight into how this species will respond to a changing world.

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Chapter 2

Temperature, larval development and diapause

Introduction

Insects, like other ectotherms, rely on their environment to provide the heat energy necessary for metabolism (Casey 1993). Global climate change models predict a temperature increase of 1.8°C to 4°C by the end of the century and an overall increase in erratic weather events (Solomon 2007). Because of their relatively short life cycles and generation times, insects are expected to respond quickly to environmental changes through shifts in phenology and distribution (Wilson and Maclean 2011). Alpine environments in particular are likely to undergo significant climatic change (Parmesan 2006). *Parnassius smintheus* Doubleday, the Rocky Mountain Apollo butterfly, currently has stable populations in Kananaskis, AB (Roland and Matter *in review*), but their Eurasian counterparts, *P. apollo* and *P. mnemosyne,* are threatened and are likely to suffer further due to climate change, primarily through changes in host plant distribution (Ashton *et al.* 2009, Araújo and Luoto 2007) . As such, understanding how *P. smintheus* responds to moderate shifts in temperature in the Rocky Mountains benefits our ability to predict what the future may hold for this species and its congeners.

In this chapter I explore two ways in which changes in temperature can directly affect *P. smintheus*, particularly the larvae. Indirect effects of temperature shifts, such as changes in host plant quality or distribution or habitat gain/loss, are left for future studies. The first effect of temperature is the potential induction of an early diapause in larvae under cool temperatures and how this additional life stage could affect population dynamics. If weather becomes more variable, “cool”

years may happen more frequently. *P. smintheus* generally overwinter as pharate first-instar larvae in eggs, but Scott (1986) reports that late instar larvae may overwinter in diapause, particularly in cool years. If significant numbers of larvae enter diapause, it could generate a cohort-splitting effect, where larvae from two generations exist together the following season (Danks 1992), thereby increasing butterfly abundance in alternate years. Cohort-splitting is documented for several species of insects (Danks 1992), including *Tipula sacra* (Diptera: Tipulidae) in Kananaskis, AB that can exhibit a two-year life cycle, but can also have a one-year cohort of quick-growing individuals (Pritchard 1980). Similarly, European Tettigonidae (Orthoptera) exhibit either a one-year life cycle in warm years or a two-year life cycle with two separate dormant stages (Ingrisch 1986). *Oeneis* sp. and *Erebia* sp. (Satyridae) in Northern Fennoscandia have either a two- or three-year life cycle as an adaptation to cool weather (Douwes 1980). This additional diapause affects their population dynamics by altering their periodicity compared to southern populations with two-year life cycles only. Late instar *P. smintheus* larvae have been observed at my study site very early in the spring (mid-May) (Kurt Illerbrun, University of Alberta, *pers.com*.), suggesting the potential for larval diapause in this system. If larval diapause is common, there is potential for a "double cohort" following cool years, which could generate the two-year pattern of population growth and decline (see Fig. 1.1). One of the coolest years in the recent past was in 1999; the average temperature at nearby Nakiska Ridgetop over the summer months (June, July and August) was 9.7°C, compared to the average temperature over those same months from 1999-2008 of 11.7°C (Environment Canada 2009). By rearing larvae at a temperature that is slightly cooler (2°C) than the average temperature of the larval alpine habitat, reflecting a reasonably "cool" season, I will determine what proportion opt not to complete their life cycle and instead enter diapause.

The second effect of temperature I explore is the effect of a small change in temperature on larval growth and development. Given recent climate change predictions, understanding the thermal ecology of *P. smintheus* larvae is instrumental to predicting how the species will respond to potential shifts in

temperature. A relatively small increase in temperature (3°C) significantly increases larval growth rates in the temperate butterflies *Euphydryas editha bayensis* (Nymphalidae) (Hellman 2002) and *Lasiommata maera* (Satyridae) (Gotthard *et al.* 2000), seemingly without negative consequences for the larvae. Conversely, *Boloria aquilonaris* (Nymphalidae) larvae, which are adapted to cool bog habitats, have lower survival rates when ambient temperature increases from 20°C to 25°C (Turlure *et al.* 2010). Larvae have maximum and minimum temperature thresholds for development (Sandhu *et al.* 2010, Tsukagoshi and Higashiura 2009, Golizadeh *et al.* 2007) with an optimal growth temperature nearer the maximum (Casey 1993). By rearing larvae at a range of temperatures surrounding the average temperature in their habitat, I will estimate where ambient temperatures place *P. smintheus* larvae on their growth-response curve and how future changes in temperature might affect their growth. I predict that growth rate will increase with temperature and that development time will decrease. It seems unlikely that larvae are nearing their maximum temperature threshold because *P. smintheus* has an extensive geographic range, from the Yukon to Colorado (Guppy and Shepard 2001, Scott 1986), which includes warmer habitats than my study site.

Methods

Study organism

P. smintheus inhabits alpine meadows where its larval host plant occurs (Scott 1986). At our study site, the larval host plant is *Sedum lanceolatum* (Crassulaceae), the lance-leaved stonecrop, although the preferred host plant varies across the butterfly's geographical range. While generally considered a univoltine species, individuals may overwinter as late instar larvae in arctic environments (Scott 1986). Pharate larvae eclose in early spring (May to early June); the larval stage consists of 5 instars. Larvae bury into the soil to pupate in mid-summer (late June to early July) and adults emerge in mid-July, flying until early September.

Experimental design

Third, fourth and fifth instar *P. smintheus* larvae were collected from Jumpingpound Ridge, Kananaskis Country, AB (50°57'N, 114°54'W, ~2200m) and Powderface Ridge, Kananaskis, AB (50°84'N, 114°87'W, ~2200m) in mid-June of 2009. Larvae were transported to growth chambers at the Biogeoscience Research Institute (University of Calgary) at Barrier Lake, Kananaskis, AB (52°00N, 115°02W, ~1390m) or to the University of Alberta, Edmonton, AB (53°31N, 113° 1W, ~668m). This research was conducted in collaboration with Jeanette Wheeler (Dept. of Mathematics, University of Alberta).

Larvae were divided into three treatment groups: a "cool" treatment, a "warm" treatment, and a control group. Due to the limited availability of growth chambers, the warm and control groups were held in chambers at the University of Alberta and the cool group was held in a chamber at the Biogeoscience Research Institute. Larvae were placed in their respective temperature treatment chambers within three days of collection. The "control" temperature was calculated as the average weekly maximum temperature over the past ten years (1999-2008) at nearby Nakiska Ridge (50°56 N, 115°11 W ~2543m, Environment Canada 2009). Therefore each weekly "control" group temperature was calculated from the 70 daily maximum temperatures (Table 2. 1). Five degrees Celsius were added to the weekly average to account for the difference between air temperature of the measuring equipment and the ground temperature which caterpillars would experience. Nighttime "control" temperature was calculated the same way, only using the average weekly minimum temperature (calculated from 70 daily minimum temperatures) and without adding the extra five degrees (Table 2.2). Due to problems with one of the growth chambers, the lowest temperature could not fall below 5°C. This limitation was unlikely to affect larval development because the lowest average temperature recorded was 3°C and such temperatures fall below what many consider the larval developmental threshold (Tsukagoshi and Higashiura 2009, Golizadeh *et al.* 2007). The warm treatment group was reared in a chamber 2°C above that of the

control, reflecting the minimum projected increase in temperature in Western North America over the next century (Solomon *et al*. 2007), and the cool treatment group was reared in a chamber 2°C below the control (Tables 2.1&2.2). The cool treatment group, which was also the group used to assess the prevalence of a larval diapause stage in a "cool" year, had less than one third the degree days than did the control group over the 8 weeks (31.5 compared to 101.5). Degree days were calculated as half of the maximum plus the minimum temperatures each day, minus a base temperature of 10°C. All larvae had 16 hours of daytime temperature and light, and 8 hours of nighttime low temperature and dark. Larvae were reared in individual cups with soil collected from Jumpingpound Ridge and fed diets of *S. lanceolatum* collected from Jumpingpound and Powderface Ridges and kept outdoors in large trays. *S. lanceolatum* was replaced as necessary, usually every 1-2 days. Larvae were weighed daily (±0.001g), and moulting and pupation dates were recorded. Larvae were observed until they either entered diapause, pupated or died. There were 13 individuals in the warm treatment group, 12 in the control group, and 20 in the cool treatment group.

Table 2.1: Daytime temperatures (°C) at which *P. smintheus* larvae were reared over an 8 week period in 2009. The control temperature was calculated from the weekly average maximum temperature recorded at Nakiska Ridgetop, Kananaskis Country, AB, averaged from 1999-2008, plus 5°C. The cool treatment temperature is 2°C below the control, and the warm treatment is 2°C above the control.

Week	**Cool Treatment**	**Control**	**Warm Treatment**
1: June 19-25	13	15	17
2: June 16-July 2	15	17	19
3: July 3-9	15	17	19
4: July 10-16	18	20	22
5: July 17-23	18	20	22
6: July 24-30	18	20	22
7: July 31-Aug 6	16	18	20
8: Aug 7- 15	16	18	20

Table 2.2: Nighttime temperatures (°C) at which *P. smintheus* larvae were reared over an 8 week period in 2009. The control temperature was calculated from the weekly average minimum temperature recorded at Nakiska Ridgetop, Kananaskis Country, AB, averaged from 1999-2008. The cool treatment temperature is 2°C below the control, and the warm treatment is 2°C above the control. For technical reasons, 5°C is the lowest temperature at which the growth chambers could be set.

Week	Cool Treatment	Control	Warm Treatment
1: June 19-25	5	5	5
2: June 16-July 2	5	5	5
3: July 3-9	5	5	5
4: July 10-16	5	6	8
5: July 17-23	5	7	9
6: July24-30	5	6	8
7: July 31-Aug 6	5	5	7
8: Aug 7- 15	5	5	7

Data analysis

Fifth instar weights and development times were used for all analyses. There were not enough third and fourth instar larvae per temperature treatment to conduct any meaningful analyses. All analyses were conducted with R (v. 2.9.2, R Development Core Team 2009).

I had two main research questions: 1. Can larvae enter diapause as means of delaying emergence as adults the next year? and, 2. How does temperature affect larval growth and development? Changes in temperature may affect population structure through changes in phenology or survival, even if there is no effect of cold-induced early diapause. Data addressing the first question required no analysis because I was only observing what proportion of the larvae entered a cold-induced early diapause to determine whether it is a sufficiently common phenomenon to affect population dynamics.

The second question was divided into two parts for analysis. The first was how small shifts in temperature, would affect *P. smintheus* growth rate. To answer this question, I used a linear mixed effects model to model weight attained as a

function of time. The mixed effects models allowed me to account for repeated measures on individuals (Crawley 2007). Each larva's weight was truncated at the maximum weight attained because larvae lost weight at the end of the fifth instar, regardless of whether they died or pupated. Larval weight (g) is modeled by temperature treatment and number of days in fifth instar as fixed effects. The random effects, however, are Julian date (not days in fifth instar) nested within each individual caterpillar. Julian date is included as a random effect because the temperature regime changed weekly. Because of this weekly change, larvae that began their fifth instar early in the trial were subjected to a different temperature regime than those who started in later weeks, regardless of treatment. No terms were dropped from the final model because all are pertinent to the research question. The significance of each model term was estimated with an F-ratio test (α=0.05).

Pupal weight is often used as a measure of potential fecundity (Berger *et al.* 2008). Therefore, I also analysed final larval weights (prior to pupation) as a surrogate for pupal weight. To compare mean final larval weights between temperature treatments I used a Kruskal-Wallis rank sum test. I compared the weights of larvae that pupated only, not those that died. There were 9 individuals in the warm treatment group, 5 in the control group and 9 in the cool treatment group.

The second analysis assessed the effect of temperature on development time. To model time to pupation from the first day of the fifth instar as a function of temperature treatment, I used survival analysis. Censored larvae were those who died during the trial (n=22) or went missing (n=1). I chose a non-parametric Cox proportional hazards model because the sample size in each treatment was relatively low and I did not need to extrapolate beyond the last pupation event (Crawley 2007). Temperature treatment and the first day of fifth instar (Julian date) are the main fixed effects. Julian date is included in the full model because the temperature regime changed weekly, and larvae that were fifth instars in the first week would have been subject to a different set of temperatures than were those moulting into fifth instar in the third week. The best fit model was chosen

using backwards stepwise regression. There were 13 individuals in the warm treatment group, 12 in the control group, and 20 in the cool treatment group.

In addition to the above analyses, I used logistic regression to determine whether temperature affected the probability of an individual dying.

Results

Larval diapause

No larvae in any treatment group entered diapause by the end of the trial. All larvae either pupated or died. After a larva stopped feeding and moving, it was kept in its cup until it had either desiccated or was infected by fungus as a precaution against falsely considering a larva dead if it had in fact entered diapause.

Growth rate

Not surprisingly, all larvae in all treatment groups gained weight over time ($F_{1, 41}$=329.52, P<0.001). Larvae in the warm treatment grew more quickly than did those in the other two treatments, as indicated by the significant interaction between treatment and time (Fig. 2.1, Table 2.3). Pre-pupal larval weight did not differ among treatment groups (Kruskal-Wallis K=2.80, df=2, P=0.25)

Table 2.3. Mean growth rates of larvae (g/day ± 95% confidence interval) reared at three temperature regimes: cool (2°C below control), control, and warm (2°C above control).

Temperature treatment	**Growth Rate (g/day ± 95%C.I.)**
Cool	0.0117 ±0.00381
Control	0.0133 ±0.00543
Warm	0.0281 ±0.00566

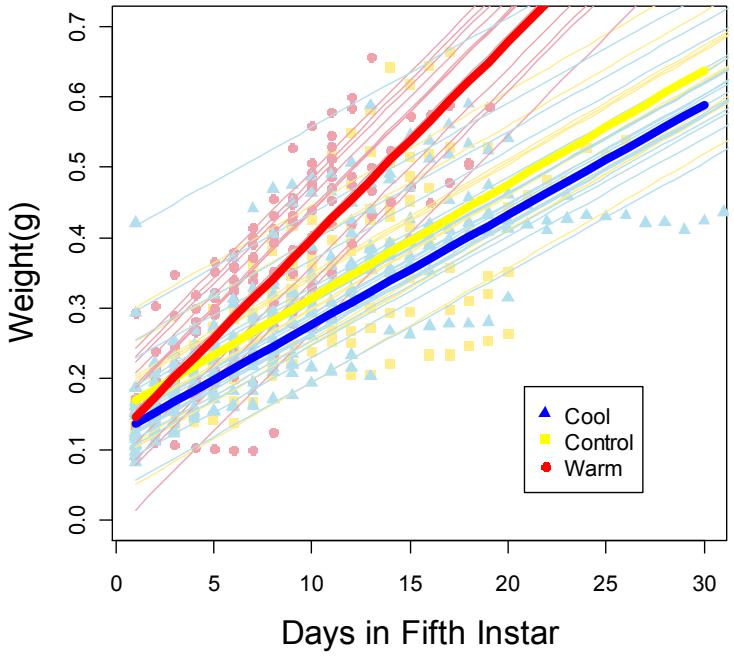

Figure 2.1: Growth curves for larvae reared at three temperatures: cool (2°C below control; blue triangles and lines), control (yellow squares and lines), and warm (2°C above control; red circles and lines). Data were truncated at the maximum weight attained for each individual. Curves for individual caterpillars are indicated with pale colours.

Development time

Temperature treatment was the only variable in the final model as it significantly affected the time taken for larvae to pupate (χ^2= 13.00, df=2, P=0.0015). In the warm treatment larvae pupated sooner than did those in the control group (z=3.43, P<0.001, Fig 2.2), but larvae in the cool treatment pupated at the same time as those in the control group (z=1.03, P=0.30, Fig 2.2). The mean number of days to pupation (±S.E.) was 20.9 (±2.31) in the cool treatment group, 26.4 (±3.52) in the control group, and 18.9 (±1.21) in the warm treatment group.

Temperature did not affect the probability of an individual dying, despite the differences in time to pupation (χ^2=3.58, df=2, P=0.17).

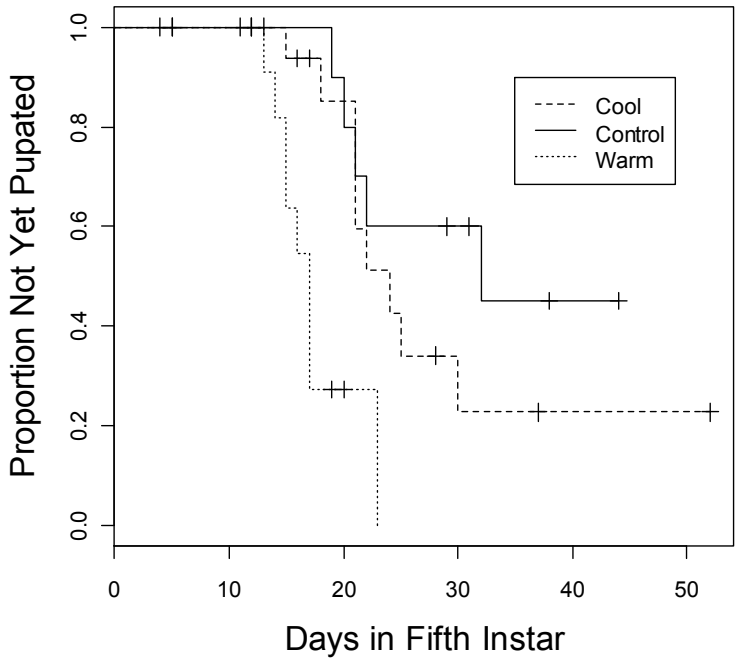

Figure 2.2: Cox Proportional Hazards model used to estimate the proportion of larvae pupating during the fifth instar. Larvae were reared at three temperatures: cool (2°C below control), control, and warm (2°C above control).

Discussion

Early diapause and cohort-splitting

To better understand the potential effect of larval diapause stages on *P. smintheus* population dynamics, larvae were reared below the mean average temperature. I wanted to determine whether some individuals enter diapause as larvae before completing their life cycle, instead of pupating, resulting in two simultaneous larval cohorts the following season. However, no larvae entered diapause during this study, even in the coolest temperature treatment. The trials lasted well into August, far later than the typical larval growth period in the field, implying that larvae rarely, if ever, prolong their life cycle by entering diapause at our site. Consequently, a double cohort of larvae after a particularly cool year is

unlikely to contribute to the two-year oscillation in *P. smintheus* population growth rates. Although my findings are contrary to what Scott (1986) reports, larval diapause could be a rare occurrence in extremely cold years, and was simply not captured in my study.

Double cohorts generally occur in cool climate-adapted insects whose resources are limited or highly unpredictable (Danks 2006, 2002); because *P. smintheus* larvae do not appear to be affected by host plant quality (see Chapter 3) and because their host plant is abundant and available as soon as snow melts, bet-hedging by delaying the life cycle is perhaps an unnecessary strategy for larvae. Instead, relatively late instar larvae observed at my study site early in the growing season likely developed in warm microclimates such as insolated black soil patches where snow melts occurs. Similar choice of warm habitat patches occurs for *Andrena fenningeri* Viereck (Hymenoptera: Andrenidae) colonies in the early spring, which are located in red soil that heats up more quickly than the surrounding area (Batra 1999). It may also be that my study sites are too far south for larvae to have adopted a second diapause which might occur only in more northern populations with a more adverse climate. *Oeneis* sp. and *Erebia* sp., for example, exhibit both two- or three-year life cycles in the northern part of their range, but only two-year cycles in the south (Douwes 1980).

Temperature is commonly used as a diapause cue for insects in cool environments, either as the dominant cue, as for *Heleomyza borealis*, an arctic Dipteran (Worland *et al.*2000) or as a main cue in combination with photoperiod, as for *Phaedon brassicae*, a chrysomalid beetle (Wang *et al.* 2007). Host plant senescence, can also act as a cue along with temperature, such as for *Danaus plexxipus* (Nymphalidae) (Goehring and Oberhauser 2002). It is important to note, however, that some species, such as *Pieris brassicae* (Pieridae) (Spieth 2002) and *Hydromedion sparsutum*, a subantarctic Dipteran (Haderspeck and Hoffman 1991), use only photoperiod, with minor or no influence of temperature, as a diapause cue. Although unlikely, if *P. smintheus* does use photoperiod exclusively as their diapause cue, the study design used in my experiment would be

inadequate to address the question because photoperiod remained constant instead of shortening at the end of season. The photoperiod was 16 hours of light over the entire experiment, but by the end of the lab trials in August, photoperiod would have been closer to 14.5 hours.

Temperature-dependent growth and development

Not surprisingly, larvae grew most quickly and pupated soonest in the warmest treatment group. Although the optimal development temperature for *P. smintheus* is not known, that of the closely-related *P. apollo* is 24-33°C, with an upper developmental threshold of 45°C (Nakonieczny *et al.* 2006). The upper threshold for caterpillar development is often around 30-35°C (Sandhu *et al.* 2010, Tsukagoshi and Higashiura 2009, Golizadeh *et al.* 2007). Although the upper temperature threshold is not known for *P. smintheus*, it is likely that the warmest temperatures used in my study, and those attained in the field, are still below the upper boundary. Few larvae experience their maximum temperature-dependent development rate (Casey 1993) and my study implies that *P. smintheus* larvae are no exception. If, in my study, larvae were nearing the upper threshold for growth, the warmest treatment group would likely have shown increased mortality, which it does not. In contrast to *Boloria aquilonaris* (Nymphalidae) larvae, which are adapted to cool bog habitats and face greater mortality rates above the relatively low temperature of 20°C (Turlure *et al.* 2010), it appears there is ample opportunity for *P. smintheus* to thrive at temperatures higher than those in my study. Faster growth at higher temperatures is attributed to a faster food consumption rate in *Papilio glaucus* (Lepidoptera: Papilionidae) (Scriber and Lederhouse 1983), and is a plausible explanation for the higher growth rate of *P. smintheus* larvae in the warm treatment group compared to the cool and control groups. Unfortunately, I did not monitor the rate of *S. lanceolatum* consumption, although larvae likely ate similar total amounts of *S. lanceolatum* given that all groups attained similar final pre-pupal weight. Pupal weight is a strong indicator of female fecundity (Berger *et al.* 2008), which probably is not directly affected by minor differences in temperature for *P. smintheus*.

Interestingly, there was no difference in development rates between the control and cool temperature groups. This pattern seems counterintuitive if *P. smintheus* larvae are indeed developing well below their maximum temperature threshold. One explanation could be a "plateau" in the temperature-growth curve commonly displayed by ectotherms, whereby metabolic rate remains constant over a range of temperatures (*i.e.* is independent of temperature). Such a plateau acts to moderate energy expenditure over a range of optimal temperatures (reviewed by Casey 1993, Cossins and Bowler 1987). This range generally coincides with the normal air temperature of the organism's environment. If the cool and control treatments both fell within this range of temperatures for *P. smintheus* over most of the trial, we may not see temperature-dependent growth. Because all other metrics were held constant (*i.e.* food, light, habitat choice, *etc.*), larvae in the cool and control groups could not maximize temperature-independent growth, leading to a lower growth rate than those in the warmest treatment group.

Another possible explanation for the similar growth rates in the two coolest treatment groups is that larvae in the cool and control groups were developing at the extreme low end of their range of suitable temperatures and that the variability of individual growth rates at each temperature asymptotes as growth rate approaches zero (Gilbert 1984). It seems unlikely, however, that such relatively high temperatures (minimum daytime temperature of 13°C) would be the lower threshold for development in an alpine species, but perhaps with appropriate behavioural adaptations, larval body temperature is substantially higher than is air temperature in field conditions.

One such behavioural adaptation to cold is basking behaviour (Casey 1983). Guppy and Shepard (2001) report that *P. smintheus* larvae, which are black in colour, rely on basking to thermoregulate, as do other early spring larvae, such as *Hemileuca lucina* (Saturniidae) (Stamp and Bowers 1990) and *Euphydryas aurinia* (Nymphalidae) (Porter 1982). If larvae are highly dependent on heat from basking to feed, body temperatures attained in the laboratory could

have been artificially low given an inability to bask in sunlight. A basking larva in sunlight can raise its body temperature by 5°C to 35°C above air temperature, significantly affecting feeding efficiency (Stamp and Bowers 1990, Porter 1982). *P. apollo* larvae thermoregulate by moving from cooler, shrubby areas to bare ground, where the temperature is approximately 3°C warmer than ambient air temperature, selectively choosing microhabitats between 20°C -28°C (Ashton *et al.* 2009). Although *P. apollo* are found in warmer climates than is *P. smintheus* at my site, and are thus likely have higher temperature ranges, my warmest temperature may more closely approximate ideal microhabitat temperature for *P. smintheus* than do the other two temperature treatments. The degree to which *P. smintheus* larvae rely on basking behaviour or on shifts in microhabitat use warrant further study. Understanding these behaviours in *P. smintheus* would be valuable to our understanding of their thermal ecology.

Consequences of variable development rates

Although survivorship did not differ between temperature treatment groups, longer development times, like those observed in the cool and control groups, can increase the risk of predation or parasitism in the field due to longer exposure (van Nouhuys and Lei 2004, Fordyce and Shapiro 2003, Benrey and Denno 1997). Predation of *P. smintheus* larvae is rare, which is is attributed to the sequestration of sarmentosin, a deterrent cyanoglycoside (Nishida and Rothschild 1995). However, parasitoid eggs are seen on late-season *P. smintheus* larvae (*pers. obs*.). Little is known about parasitism in this species; in only two instances has a Tachinid (*Chetogena* sp.) parasitoid ever been reared from *P. smintheus* larva in a laboratory (Matter *et al.* 2011, John Stireman, Wright State University, *pers. com*.) or from *P. smintheus* anywhere. Although parasitism is presumed to be a low source of mortality for *P. smintheus* (Matter *et al.* 2011), research into the synchrony between developing larvae and the timing of attack by parasitoids provides an avenue for future research into both the general ecology of *P. smintheus* and the potential risks associated with slower development in cooler weather.

In addition to reducing the risk of parasitism late in the season, accelerated development may benefit larvae in early spring when temperatures drop below the threshold for feeding and movement (Ashton *et al.*2009, Fordyce and Shapiro 2003). If larvae enter a "chill coma", where they are too cold to eat but still have active metabolism, they deplete energy reserves, which can be fatal. Larger larvae have a better chance of surviving periods of cool weather than do smaller larvae. For example, small *Battus philenor* (Papilionidae) larvae develop more slowly and are vulnerable to potentially fatal chill comas for longer than are larger larvae (Fordyce and Shapiro 2003). *P. smintheus* larvae have been observed feeding on Jumpingpound Ridge in mid-May (K. Illerbrun, *pers. com.*), despite a mean maximum temperature below 7°C over the past ten years and a minimum temperature just below zero (Environment Canada 2009) for that seasonal interval. Larvae that hatch during a brief warm spell could use up energy reserves if temperatures then become too cool to feed. Ashton *et al.* (2009) consider "false springs" a potential threat to *P. apollo* populations in Spain, especially if such events were to become more common. Although I only have growth data for large (fifth instar) larvae, it stands to reason that younger larvae also grow more quickly with an increase in temperature, decreasing the risk associated with early-season cold spells.

Climate change and predictions for Parnassius smintheus

P. smintheus larvae develop and pupate more quickly at warmer temperatures but this accelerated development does not affect larval survival. This study does not address the full extent to which a shorter development time could alter *P. smintheus* phenology over time, but the trend towards a shorter larval stage is apparent. Similar changes in phenology, earlier first flight sightings for butterflies, in particular, occur in response to recent climate change (reviewed by Parmesan 2006). Survivorship of larvae reared at higher temperatures in the laboratory is the same as those reared at control temperatures, and the benefits of a shorter development time in the field include reduced exposure to predators and fatally cool temperatures (Fordyce and Shapiro 2003). Because adult butterflies

nectar-feed on a broad variety of wildflowers (Matter *et al*. 2009), there is a low chance of phenological asynchrony with nectar plants if adults emerge sooner in the season (Matter *et al.* 2011). Although *P. smintheus* may face other broad scale climate-related challenges, such as rising treeline affecting local population dynamics (Roland *et al.*2000) or egg mortality with particularly harsh winters (Roland and Matter *in review*, Matter *et al.* 2011) it appears that larvae are not directly negatively affected by a slight increase in temperature. It should be noted, however, that theoretical models indicate that temperature increases may lead to greater asynchrony between males and females in this protandrous species (Wheeler 2010).

Conclusions and future research

No larvae had entered diapause by the end of the temperature trials, indicating early diapause is not a common life history choice in *P. smintheus* and is unlikely to have any impact on population dynamics in our populations. Larval growth responded to an increase in temperature only (not to a decrease), suggesting that larvae at our field site are exposed to conditions that result in development at the cool end of their growth response curve, or possibly that they experience a temperature-independent growth plateau at the ambient temperature of their environment. This study did not measure metabolic activity (*i.e.* oxygen consumption) at different temperatures, which future research could address to better understand the mechanism behind the observed growth pattern. Larvae grew more quickly and pupated sooner in the warmest treatment group compared to the control and cool groups. This difference in development rate did not, however, affect larval survival nor pre-pupal weight, indicating that any negative effects of a slower development time on dynamics would likely be indirect and mediated interactions between weather and predators or weather and host plants. Future studies should address temperature-dependent growth and survival of larvae in the field where larvae can exhibit their natural thermoregulatory behaviours, such as basking, a phenomenon which would render climate change less of a threat.

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Chapter 3

Effect of prior host plant herbivory on larval growth and development

Introduction

Exogenous and endogenous factors are often considered the two main drivers behind insect population dynamics, although the relative importance of each varies by system (Nowicki *et al.* 2009, Pickens 2007, McLaughlin *et al.* 2002, Watt and Woiwood 1999). For example, *Euphydryas editha bayensis* (Lepidoptera) population dynamics are best described by climate and microclimate variation, while density dependent effects play a secondary role (McLaughlin *et al.* 2002, Dobkin *et al.* 1987). In contrast, density dependent effect of competition for food plants and ant hosts explains more than 60% of year to year variation in *Maculinea* sp. (Leptidoptera) population fluctuations (Nowicki *et al.* 2009). Knowing the strength of endogenous, or density-dependent, processes in influencing population growth provides insight into the impact that a changing environment might have on the future of a species.

Strong, lagged density-dependent competition explains, or contributes to, the dynamics of several insect species (Hódar *et al.* 2004, Underwood 2000, Karban and Baldwin 1997, Berryman 1996). Reduced host plant quality due to insect herbivory, and the potential for increased intraspecific competition can generate strong density dependence in insect populations and produce population cycles (Hódar *et al.* 2004, Underwood 1999, Heliövaara and Väisänen 1988). For example, *Retinia resinella* (Tortricidae) fluctuations in Finland are thought to be driven by an herbivory-induced defence in *Pinus sylvestris*, preventing the establishment of larvae the following season (Heliövaara and Väisänen 1988).

Previous herbivory can decrease plant quality for herbivores in several ways. Herbivory can change the plant's allocation of nutrients (carbon and nitrogen) and sugars within the plant (Awmack and Leather 2002), the nutrient or water content of the plant tissue (Prior and Hellman 2010), or by changing plant defensive compounds (Karban and Baldwin 1997). Induced defences are activated by the plant when fed upon, preventing further damage (Karban and Baldwin 1997, Levin 1976). Such a defence against insect herbivores is present in *Betula* species (Hartley and Lawton 1987, Haukioja *et al.* 1985), members of the Solanaceae family (Brunissen *et al.* 2010, Musser *et al.* 2002, Van Dam *et al.* 2001), *Pinus* species (Hódar *et al.* 2004, Heliövaara and Väisänen 1988), *Quercus* sp. (Schultz and Baldwin 1982) and others (Massey *et al.* 2007, Agrawal 2000). Despite the number of studies focusing on herbivory-induced decreases in host plant quality, the importance of this interaction in insect population dynamics is still a topic of debate (Underwood 2010, Klemola *et al.* 2008, Haviola *et al.* 2007, Haukioja 2005). Short-term decreases in host plant quality, such as induced defences acting within minutes or days, for example, tend to stabilize populations (Karban and Baldwin 1997). In contrast, long-term, lagged decreases in host plant quality that reduce plant quality for future generations can destabilize populations if the negative effects on herbivores is sufficiently strong, thereby contributing to cyclical dynamics (Haukioja 2005, Underwood 1999, Karban and Baldwin 1997).

Parnassius smintheus populations in Kananaskis, AB, exhibit a peak/trough pattern of growth approximately every two years (Fig. 1.1). At this site, *P. smintheus* larvae feed almost exclusively on *Sedum lanceolatum* (Crassulaceae), a perennial succulent. Herbivory of *S. lanceolatum* by other animals is rare, suggesting the presence of either a constitutive or induced defence in the plant. *P. smintheus* larvae are aposematically coloured and are rarely preyed upon. This pattern is attributed to the sequestration of sarmentosin, a deterrent cyanoglucoside (Nishida and Rothschild 1995). Although sarmentosin is not induced (Weber 2010), larvae fed damaged *S. lanceolatum* plants in laboratory

trials showed reduced growth compared to those fed undamaged plants, a pattern the authors attribute to the induction of some other chemical (Roslin *et al.* 2008). Roslin *et al.* (2008) found that larval growth rates were lowest when fed *S. lanceolatum* damaged two to five days previously. Growth rates were marginally reduced when larvae were fed plants damaged only one day previously. In addition to this evidence of a short-term induced defence, or other decrease in host plant quality, there is evidence that herbivory pressure is highest on *S. lanceolatum* plants (Illerbrun, *unpub*.) undamaged the previous year, although this pattern could be due simply to larval avoidance of the previous year's feeding damage.

I conducted experiments to explore how previous herbivory affects *P. smintheus* larval growth to determine whether host plant-herbivore interactions could generate a sufficiently strong effect over a sufficiently long time scale to impact adult *P. smintheus* population dynamics. Roslin *et al.* (2008) found that growth was reduced in larvae fed damaged plants in a laboratory setting, but the experiment looked at the effect of herbivory on larval growth only within five days of damage. I conducted similar feeding trials in the field using *S. lanceolatum* plants damaged within a few days, within a growing season and between years in order to determine the time scale over which changes in host plant quality affect larval performance, if at all. I used actual larval feeding damage instead of mechanical damage (as used by Rosin *et al.*2008) in all trials because plants do not always respond the same way to mechanical damage as they do herbivory (Schultz 1988). As such, I wanted my experiment to be as realistic as possible. Such an experiment allows me to understand the relative importance of endogenous host plant/herbivore interactions when larvae are facing exogenous, climatic stressors. Based on the findings by Roslin *et al.* (2008), larval herbivory patterns between years (Illerbrun, *unpub*.), and population growth patterns (Roland and Matter 2007), I predicted that larval growth would be affected within a season, but that a greater effect would be apparent a year after herbivory.

Methods

Study locations and organisms

Feeding trials for both the within-year and between-year effects of herbivory on host plant quality were conducted in the field at Powderface Ridge in Kananaskis, AB (50°84'N, 114°87'W, ~2200m), using larvae collected from the nearby Jumpingpound Ridge (50°57'N, 114°54'W, ~2200m). Both ridges consist of dry, open alpine meadow habitat (Roland *et al.* 2000). Larvae collected from Jumpingpound Ridge were held in growth chambers at the nearby Biogeoscience Research Institute (University of Calgary) at Barrier Lake, Kananaskis (52°00N, 115°02W, ~1390m) until the start of the feeding trials. In 2009, growth chamber temperature was set at the weekly average daily high and nightly low temperature for a 2200m elevation with 16h:8h light:dark schedule. The laboratory study evaluating the effect of within-year herbivory on host-plant quality was also conducted in 2009 at the Biogeoscience Research Institute, and used the same chambers and temperature regime. In 2010, the chambers were used to hold caterpillars only and were set at room temperature (approximately 21°C) during the day and 5°C at night with 16h:8h light:dark schedule.

Experimental design

I used four separate experiments to test the effect of previous herbivory on larval growth. Three of these experiments tested the effects of within-season herbivory on larval growth (one laboratory and two field studies), and one field study tested the effect of between-year herbivory (Table 3.1). All field trials were located within ~200m of each other on a dry, South-facing slope of Powederface Ridge. None of the *S. lanceolatum* plants used for the feeding trials were located in wet areas, thus minimizing the possibility of plants switching between CAM and C3 photosynthesis and hence on plant quality for the larvae. Care was taken to use plants of the most common morph (smaller, redder plants, as opposed to large, bright green) to avoid any possible effects of ploidy on plant quality or plant response to herbivory.

Table 3.1: Summary of feeding trial experiments used to determine the effect of herbivory on *P. smintheus* larval growth. Included in the summary is the year the study was done, the location of the study (field or laboratory), the herbivory time scale the study encompasses (time since herbivory), and the number of larvae used for data analysis per treatment group.

Year	Location	Time since herbivory	Treatments	Larvae/treatment
2009	Lab	Within-year; 2-3 days	Herbivory	22
			Control	24
2009	Field	Within-year; 6-23 days	Early season	9
			Late season	9
			Control	12
2010	Field	Within-year; 2-10 days	0 days since herbivory	10
			2 days since herbivory	11
			5 days since herbivory	9
			10 days since herbivory	10
2010	Field	Between-year; 1 year	Herbivory	15
			Control	15

Within-year herbivory effects

Laboratory feeding trials 2009

In collaboration with Kurt Illerbrun (University of Alberta), 63 third, fourth and fifth instar *P. smintheus* larvae were reared in a growth chamber at the Biogeoscience Research Institute in the summer of 2009. Larvae were subjected to one of two treatments: an herbivory treatment or a control treatment. In the herbivory treatment, larvae were reared on *S. lanceolatum* fed upon two or three days previous by the larvae in the control group (Roslin *et al.* 2008). Plants were moved from the control group to the herbivory group after 10-20% of the plant was eaten. This amount of damage is higher than the average amount of damage observed in the field (Roslin *et al.* 2008), but I wanted to ensure that, if there was an induced defence, it would be activated. The control group was fed undamaged plants. All plants were collected with their root systems intact from Jumpingpound Ridge and Powderface Ridge as needed and held outdoors in large

trays at the Biogeoscience Research Institute. Roslin *et al.* (2008) used the same plant storage technique. Plants were transferred into the experiment growth chamber several days before they were used as food. Larvae were held in individual cups and given new plants when plants were more than half eaten, usually every 1-2 days. Larvae were weighed daily to the nearest 0.001g (error: ±0.010g; My Weigh GemPro 250 Precision) until pupation.

Field feeding trials 2009

In May and June 2009, 90 *S. lanceolatum* plants on Powderface Ridge were enclosed by lawn-edge fencing to prevent herbivory prior to the experiment. All plants remained undisturbed at their original location but were enclosed in plastic cylinders 30cm in diameter with open tops and bottoms. Enclosures were 12 cm above ground and were inserted approximately 3 cm into the ground. Enclosures functioned as a barrier to unwanted larval herbivory and as a "cage" for larvae during the feeding trial. Plants were randomly divided into three treatment groups to estimate the effect of herbivory over the larval season: early season herbivory, late season herbivory, and control (no herbivory). Herbivory treatment plants were treated by placing 1-3 fourth and fifth instar "feeder larvae" in the enclosures and allowing them to damage between 2-10% of the plant. Roslin *et al.* (2008) found that herbivorized plants in the field had an average of 5% of their leaves damaged (SD 4%). Each plant was damaged over the course of a single day. Early season herbivory treatments were implemented between 17 and 23 days prior to the beginning of the feeding trials when most larvae were in the third or fourth instar. Late season herbivory treatments were implemented 6-7 days prior to the beginning of the feeding trials when most larvae were in the late fourth or early fifth instar. The time taken to implement the herbivory treatments was longer for the early season treatment than for the late treatment due to poor weather conditions in May and June.

Forty-five fifth instar "test larvae" were divided into three treatment groups (n=15/treatment). Each larva was weighed prior to the trial and placed in an enclosure. Enclosures were topped with fine mesh while larvae were present to

prevent predation. Each larva was weighed daily and moved to a new, treated plant in a new enclosure when the plant was more than half eaten, generally every 1-2 days. Each larva had three treated plants available to it. The trials started on the same day and lasted between 3-10 days and their duration varied among individuals. Because of cool weather during the feeding trials, larvae did not feed some days.

Field feeding trials 2010

In June 2010, 200 *S. lanceolatum* plants were located on Powderface Ridge in the same manner as in 2009, and enclosed with fencing as in 2009. Enclosed plants were randomly divided into four treatment groups: ten days since herbivory, five days since herbivory, two days since herbivory or no herbivory. Treatment intervals were chosen to a) look at short-term (within-season) herbivory at a finer scale than the 2009 field trial and b) to incorporate the time scale that Roslin *et al.* (2008) used in their five day herbivory study. Plants were fed upon by "treatment larvae" until 5-10% of the plant was damaged. Each treatment plant was damaged two, five or ten days prior to its use in the feeding trial, so that all four treatments were available at the same time at the start of the experiment.

Forty fourth and fifth instar "test larvae" were divided into the four treatment groups (n=10/treatment). Larvae were starved for 6 hours and then weighed prior to the beginning of the feeding trials. Each larva was placed in a field enclosure, left for 24 hours, and was then weighed and placed on a new plant of the appropriate type in a new enclosure. Unlike the 2009 feeding trials, larvae were moved to a new plant daily whether they fed or not. Enclosures were topped with mesh as in 2009. Each larva had access to five treated plants, therefore the trials lasted five days (one plant per larva per day). All larvae began the trials on the same day. I recorded the number of leaves damaged by each larva for each plant. If a larva died or was missing during the first two days of the trial, it was replaced by another larva from the growth chamber the following day because it could still provide a "per day" growth estimate for the remaining three days.

Larvae lost after the second day, were not replaced. On the third day of the trials, after several larvae had been killed, all of the enclosures were encircled with baby powder (mom to mom™) to prevent ant predation. No larvae were killed after application of the powder.

I also tested the effect of herbivory on leaf carbon/nitrogen ratio in each treatment group at each time since herbivory interval (two, five and ten days since herbivory and undamaged plants, n=10 samples/treatment). Carbon/nitrogen ratio is often used as a metric of plant quality for an herbivore, and a lower C/N ratio generally means a higher quality plant for the herbivore (Ohnmeiss and Baldwin 1994). Plants used for chemical analyses were enclosed in the same manner and damaged by larvae at the same time as those used for the feeding trial. Plant samples (entire plant, minus the root system) were harvested, frozen and stored at the Biogeoscience Research Institute until they could be dried in a drying oven (40°C for 3 days) and finely ground. Between 2 to 3mg of sample was weighed and processed for carbon and nitrogen content using a CHN Analyzer (Control Equipment Corporation Model 440 Elemental Analyzer, Biogeochemical Analytical Lab, University of Alberta).

Between-year herbivory effects

Field feeding trials

In June 2009, 120 *S. lanceolatum* plants were enclosed on Powderface Ridge in the same fashion as in the previous studies. Enclosed plants were subjected to one of two treatments: a control treatment, whereby the plant was left undamaged, or an herbivory treatment. Treatment plants were damaged in the same way as in the previous studies. Each plant was damaged over the course of a single day. Plants remained enclosed until the following spring to prevent further feeding damage.

In July 2010, enclosed plants were used in feeding trials. Individual fifth instar larva were placed in enclosures on the same day (n=15 larvae/treatment) and left to feed for 24 hours. Each larva was provided with four plants (four days

of feeding trial). Larvae were starved for six hours prior to the pre-trial weighing, and were subsequently weighed daily and then moved to a new treated or control plant. Enclosures were topped with mesh and encircled with baby powder. The number of leaves eaten per larva per day was recorded. Damaged (partially eaten) leaves were counted as "eaten".

Larvae exhibiting pre-pupation behaviour (*i.e.* webbing, burying, cessation of feeding) during the course of the four days were removed from the trial and returned to the lab for pupation. No larvae died during this trial. Plants that did not survive the winter were excluded from the feeding trials. Control (undamaged) plants that had died over the winter were replaced by moving the enclosure to a nearby, undamaged plant.

Data analysis

All analyses were conducted with R (v. 2.9.2, R Development Core Team 2009). Linear mixed effects models ({nlme: lme}) were used to analyse data for all four studies because they account for the variation in the data that is due to repeated measures on individuals (subjects) by incorporating random effects (Pinheiro and Bates 2000). Each model had only one main fixed effect (treatment), therefore no terms were deleted from the final model. The models were fit using restricted maximum likelihood (REML). The significance of the model terms were analysed based on F-ratio tests (α=0.05). In all models, weight data were curtailed at the maximum weight for each individual because all larvae lost weight as they neared pupation regardless of the treatment or whether they pupated successfully or not.

Within-year herbivory effects

Laboratory feeding trials 2009

I used a linear mixed effects model (LME) to determine the relationship between larval time (days) and weight, with treatment (herbivory or no herbivory) as the fixed effect. The random effect in the model was date nested within caterpillar thereby accounting for repeated measures on individuals across time

and allowing for estimation of parameters for each individual. For the analyses, only newly moulted or very young (1-2 days) fifth instar larvae were used. Larvae that did not feed after moulting into fifth instar and that subsequently died (n=5) and larvae that went missing during the trial (n=3) were excluded from the analyses. The final analyses included 24 individuals in the control treatment and 22 in the herbivory treatment.

To estimate a treatment effect on the time scale (72 hours) used by Roslin *et al.* (2008), the LME analysis was repeated using only the first three weights of each trial larva.

Field feeding trials 2009

I used an LME to determine the relationship between time and larval weight, with treatment (early season herbivory, late season herbivory, or no herbivory) as the fixed effect. The random effect was date nested within caterpillar. Larvae that did not gain any weight during the trial, larvae that were predated, and those that went missing, were excluded. As a result, there were 9 individuals in the early season treatment, 9 in the late season treatment and 12 in the control treatment.

Field feeding trials 2010

As in the analyses described above I used LME to determine the relationship between time and larval weight. The random effect was date nested in caterpillar. The fixed effect was herbivory treatment (two-, five-, and ten-days since herbivory and no herbivory treatment). For the analysis, only fifth instar weights were used because the very few fourth instar larvae used in the trials were nearly ready to moult and therefore did not eat. For larvae that moulted during the trial, only the weights during the fifth instar were used. There were 9 larvae in the ten-day since herbivory treatment, 9 larvae in the five-day treatment, 11 in the two-day treatment, and 10 in the control treatment. Carbon/nitrogen ratios were compared across treatment groups with an ANOVA.

Between-year herbivory effects

I used LME to determine the relationship between time (day) and larval weight. The main fixed effect was treatment (herbivory or no herbivory). The random effect was date nested within caterpillar. Larvae exhibiting pre-pupation behaviour were excluded from the analysis (n=3).

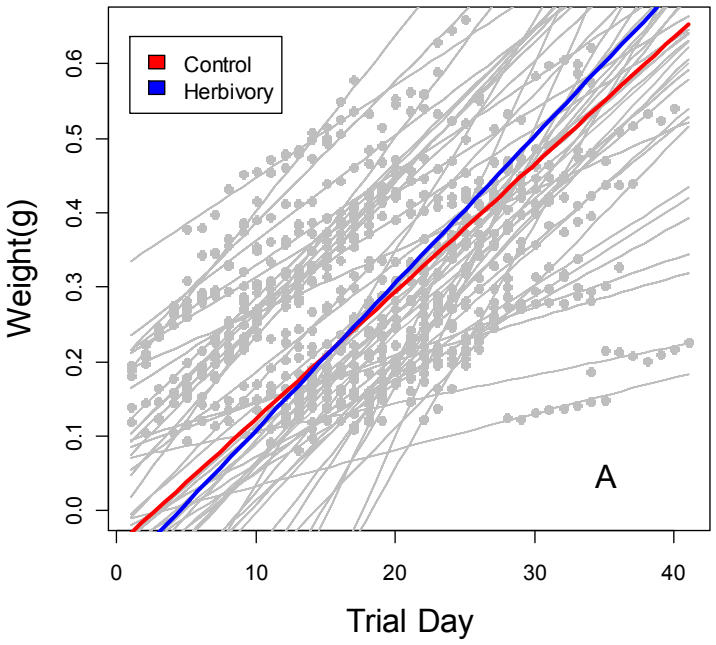
For both the within-year herbivory field study in 2010 and the between-year herbivory effects study, I repeated the analyses using "cumulative number of leaves eaten" instead of time (days) as the continuous dependent variable. This variable integrates the effect of both time and of amount eaten as the independent variable (Appendix A).

Results

Within-year herbivory effects

Laboratory feeding trials 2009

There was no significant effect of previous host-plant herbivory on weight gain measured to peak weight ($F_{1,44}$=0.33, P=0.57) nor over the first three days of fifth instar for each larva ($F_{1,44}$=1.71, P=0.20). Larvae gained weight over time in both treatments at both time scales (peak weight: $F_{1,664}$=201.26, P<0.001; three days: $F_{1,664}$=297.05, P<0.0001; Fig. 3.1). There was no significant interaction between treatment and date (time) at either time scale, suggesting that larvae in both treatment groups gain weight at the same rate (peak weight: $F_{1,664}$=1.05, P=0.31; three days: $F_{1,664}$=0.03, P=0.86). Larval growth rate when measured from the first day of fifth instar to peak weight was 0.020g/day ± 0.0037.



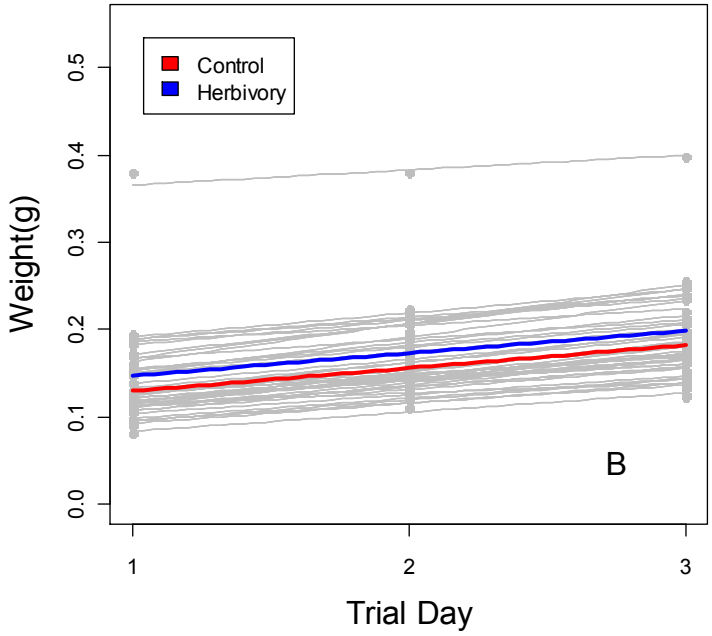


Figure 3.1: Larval weight gain in the laboratory for individual larvae (grey lines) and treatments (red line= control, blue line= herbivory treatment). Data were curtailed at a) the maximum weight or b) after three days.

Field feeding trials 2009

Neither early (plants fed upon 17-23 days prior to feeding trials) nor late season herbivory (plants fed upon 6-7 days prior to feeding trials) affected larval weight when compared to the control group ($F_{2,30}$ =0.11, P=0.90, Fig 3.2). Larvae gained weight in all treatment groups ($F_{1,133}$= 270.40, P<0.001, Fig. 3.2). There was no significant interaction between treatment and time, indicating that larvae in all treatments gained weight at the same rate (0.026g/day ± 0.0041; $F_{2,133}$= 0.40, P=0.674).

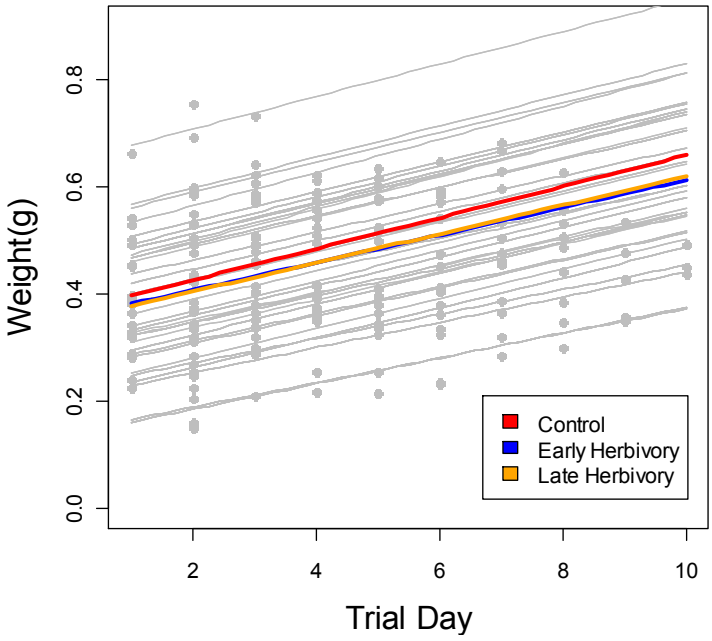


Figure 3.2: Larval weight gain in the field in 2009 for individual larvae (grey lines) and treatment effects (red line = control, blue line = early season herbivory, yellow line= late herbivory).

Field feeding trials 2010

Larval weight was not affected by previous herbivory at any time interval (2 day, 5 day, or 10 day) ($F_{3,39}$=0.15, P=0.93). Larvae gained weight in all treatments ($F_{1,116}$=334.97, P<0.001, Fig.3.3) and there was no interaction between the herbivory treatments and time; all larvae gained weight at the same rate regardless of treatment (0.038g/day ± 0.0095; $F_{3,116}$=0.81, P=0.49). Herbivory did not affect C/N ratio in leaf tissue at any time interval ($F_{3,37}$=2.26, P=0.10, Fig. 3.4).

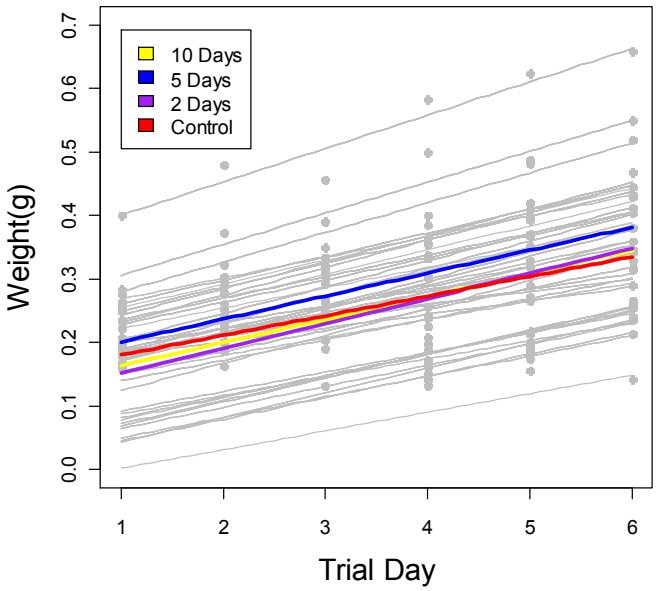


Figure 3.3. Larval weight gain in field feeding trials in 2010 for individual larvae (grey lines) and treatment effects (yellow line = 10 days since herbivory treatment, blue line = 5 days since herbivory, purple line = 2 days since herbivory, red line = control).

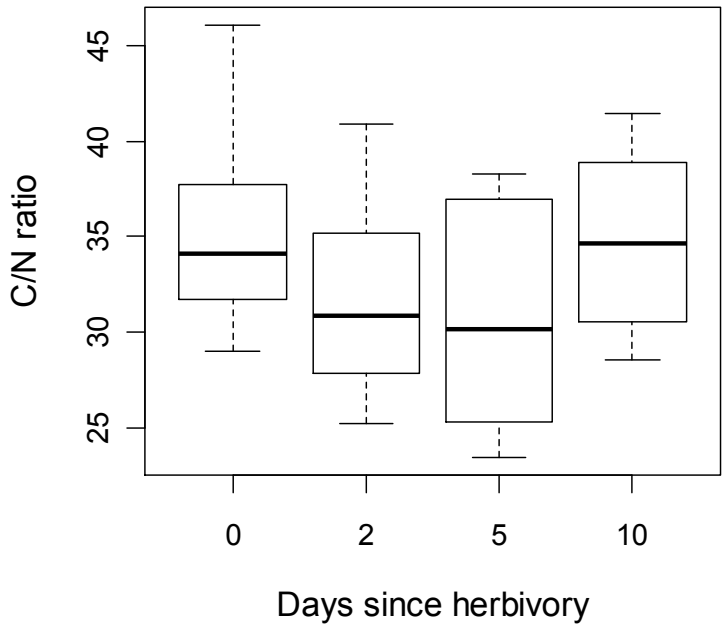


Figure 3.4*:* C/N ratios of leaf tissue from plants in four treatment groups: zero days since herbivory (control), two days since herbivory, five days since herbivory, and ten days since herbivory. The horizontal lines represent the median C/N ratio in each treatment (n=10 samples/treatment), and the lower and upper boundaries of the boxes represent the first and third quartiles, respectively. Whiskers include data within 1.5 times the interquartile range.

Between-year effects

Field feeding trials

Previous-season herbivory did not affect larval weight ($F_{1,25}$=0.53, P=0.47) and larvae gained weight during the trials ($F_{1,84}$=159.27, P<0.001., Fig. 3.5). Larvae gained weight at the same rate in both treatment groups (0.037g/day ± 0.014; $F_{1,84}$=1.16, P=0.28).

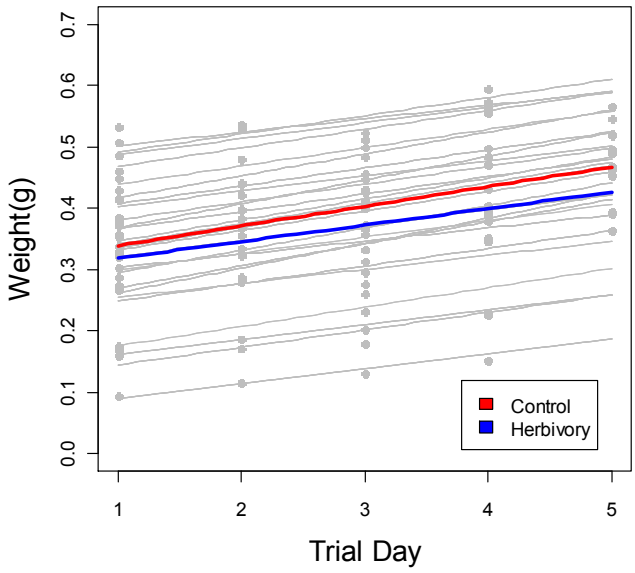


Figure 3.5*:* Larval growth over time for individual larvae (grey lines) and between-year treatment herbivory effects (red line = control, blue line = herbivory).

Cumulative number of leaves eaten

Similar to the above analyses, herbivory treatment had no effect on larval growth when "cumulative number of leaves eaten" was used as the continuous independent variable instead of time in both the 2010 within-year feeding trial and the between-year feeding trial (Appendix A). In addition to providing a better model fit, using time as the independent variable allowed me to estimate average larval growth rates for each study (weight as a function of time) (Table 3.2). Growth rates estimated for the 2009 field feeding trials were lower, although not significantly so, than those for 2010 (within-year and between-year herbivory effects field feeding trials (Table 3.2). The laboratory growth rate was significantly lower than the 2010 within-year field trials (Table 3.2).

Table 3.2. Estimated growth rates (± 95% confidence interval) of larvae in each feeding trial study. Growth rates were estimated as the slope of the regression line (effect of time) in each study because there was no significant treatment effect on the slope in any study, represented by the interaction between time and treatment.

Study	**Growth Rate (g/day) ± C.I.**
2009 Within-year herbivory effects laboratory	0.020 ± 0.0037
2009 Within-year herbivory effects field	0.026 ± 0.0041
2010 Within-year herbivory effects field	0.038 ± 0.0095
2009-2010 Between-year herbivory effects field	0.037 ± 0.014

Discussion

My goal was to evaluate whether previous herbivory affects the quality of *S. lanceolatum* as a host plant for *P. smintheus* larvae and, if so, to determine the time scale (within a season or between years) over which quality changes. The ultimate goal was to understand the relative importance of herbivory-induced changes in host-plant quality as a possible density-dependent mechanism that could influence *P. smintheus* dynamics. Herbivory as a mechanism contributing to observed patterns of population change (Fig 1.1) was investigated for several reasons: a) laboratory-based findings suggest a short-term induced defence (Roslin *et al.* 2008), b) larvae tend to feed in areas not previously damaged by herbivory (Illerbrun, *unpub*.), and c) there is little evidence of herbivory on *S. lanceolatum* by other organisms, suggesting either a constitutive or induced defence. Because *S. lanceolatum* is a long-lived perennial, either short-term or long-term effects of herbivory, or both, on host-plant quality are possible. However, I found that previous herbivory, be it in the same season or in the year prior, does not affect *P. smintheus* larval growth.

Implications for population dynamics

Decreases in host plant quality, such as an induced defence, that negatively affect the fitness of the current generation of herbivores can stabilize the herbivore population by driving it down (Underwood 2010, Karban and

Baldwin 1997, Haukioja 1980). In contrast, if a host plant is chemically unavailable for future generations of herbivores, the lagged negative effect can destabilize the population and contribute to population cycles (Underwood 2010, Karban and Baldwin 1997, Haukioja 1980). Although Roslin *et al.* (2008) found that *S. lanceolatum* quality decreased over a short period (2-5 days following herbivory), it was unclear whether or not this effect persisted on a longer time scale, or whether it was sufficiently strong in the field to influence *P. smintheus* dynamics. I found that herbivory from the previous year has no effect on the quality of *S. lanceolatum* as food for fifth instar larvae and is therefore not likely to contribute to the two-year pattern of *P. smintheus* population growth and decline.

Even if host plant quality affects herbivore fitness, the effect must be sufficiently strong to generate intra-specific competition, lagged density dependence, and hence the potential for cyclical dynamics (Cushing *et al.* 2002). Host plant/herbivore interactions are rarely considered the sole density-dependent mechanism generating insect population fluctuations and they often act in conjunction with host-parasitoid interactions (Haukioja 2005, Karban and Baldwin 1997) or with exogenous factors, such as climate (Hódar *et al.* 2004, Watt and Woiwod 1999). Parasitism is rarely observed in this system (Matter *et al.* 2011), although parasitoid eggs on *P .smintheus* larvae occur in conjunction with abnormally late summers (*pers. obs*.). Consequently, parasitism is unlikely to operate at a sufficiently large scale to dominate *P. smintheus* populations. Given that host-plant/herbivore interactions also appear to be weak in this system, endogenous processes alone are not likely driving the two-year population fluctuations. Although density dependence can strongly influence some butterfly populations (Nowicki *et al.* 2009, McLaughlin *et al.* 2002), my study leaves abiotic variables, particularly weather, which is severe and unpredictable in this system, or interactions between weather and endogenous processes, as the next logical avenues of research to explain *P. smintheus* dynamics.

Mechanisms explaining discrepancies between laboratory results

Contrary to expectations, previous herbivory had no effect on larval growth either within a season or between seasons. Especially surprising was the fact that laboratory feeding trials showed no effect of herbivory on larval growth, in contrast to findings by Roslin *et al.* (2008) who found that *P. smintheus* larvae fed previously-damaged plants grew more slowly than did those fed undamaged plants. There are several differences in the experimental protocol that may explain the different results. First, and most importantly, Roslin *et al.* (2008) used mechanical means to damage plants, whereas I used actual larval herbivory. Plants may respond differently to mechanical damage than to insect damage (Schultz 1988). For example, *Lolium perenne* L. (perennial rye grass) damaged by *Schistocerca gregaria* Forkal (locusts) showed a dramatic increase in silica concentration, whereas *L. perenne* damaged mechanically showed no such increase (Massey *et al.* 2007). Although the trend is that herbivory induces a stronger chemical defence than does mechanical damage (Massey *et al.* 2007, Karban and Baldwin 1997, Schultz 1988, Hartley and Lawton 1987), for both *Helicoverpa zea* (Noctuidae) feeding on *Nicotiana tabacum*, and *Manduca sexta* (Sphingidae) feeding on *Nicotiana attenuata,* larval saliva suppresses the induction of nicotine, which is normally produced as an herbivore deterrent (Musser *et al.* 2002, Kahl *et al.* 2000). Because *P. smintheus* larvae are specialists on *S. lanceolatum*, they may have evolved a similar mechanism to overcome the onset of an induced defence in *S. lanceolatum*, although data is currently lacking. The presence of such a mechanism could be determined by mechanically damaging the leaf tissue, then applying *P. smintheus* saliva to the plant wound.

S. lanceolatum plants damaged mechanically have lower carbon/nitrogen ratio than do those damaged by larval herbivory or undamaged plants (Kurt Illerbrun, *unpub.*, Appendix B). This pattern initially seems counterintuitive because tissues with lower C/N ratios are thought to be better food for herbivores (Ohnmeiss and Baldwin 1994). Kahl *et al.* (2000), however, found that *Nicotiana attenuata* does not produce nicotine, a nitrogenous compound normally used for

defence, in response to *M. sexta*, a specialist herbivore, damage. If a nitrogen-containing defence is suppressed in the presence of specialist herbivory but not with "generalist" (or mechanical) herbivory, one may expect a decrease in the C/N ratio in mechanically-damaged plants. We do not yet know whether *P. smintheus* larval saliva interferes with *S. lanceolatum* chemical defences, or if *S. lanceolatum* produces an induced nitrogenous defence in addition to its constitutive nitrogenous defence, sarmentosin, but future research could target this potential response. Given difference between my results and those of Roslin *et al.* (2008), along with evidence of damage type affecting the nitrogen content of leaf tissue (Illerbrun, *unpub.*, Appendix B), the exploration of larval saliva as a weapon in the evolutionary arms race between plant and herbivore could be an interesting area for future research.

Secondly, there is the chance of a climate-mediated "year effect", potentially altering host-plant quality in the years that the respective studies were done (Guppy and Sheppard 2001). The duration of snow cover is known to affect *Betula nana* (mountain birch) nitrogen content and, consequently, *Epirrita autumnata* larval herbivory and larval growth (Torp *et al.* 2010). In 2006, the year that Roslin *et al.* (2008) conducted their feeding trials, snow disappeared 36 days earlier than in 2009 when plants were collected for the current laboratory feeding trial (data from the Environment Canada meteorological station at Kananaskis, 51° 02'N, 115° 03'W, ~1391m). Given that *S. lanceolatum* was collected from the field and brought to the lab in both studies, there is a possibility that plant condition prior to the feeding trials affected larval growth. However, later snow melt reduces the quality of *S. lanceolatum* for *P. smintheus* larvae (Guppy and Shepard 2001, Illerbrun *unpub.*), so a "year-effect" of climate on the plants is a less likely explanation for the differences between my study and that of Roslin *et al.* (2008) than is mechanical damage compared to herbivory damage. However, if

Third, the two studies differed in the length of time that larvae fed on each treated plant; plants were not switched out for fresh, treated plants daily in my study, as they were by Roslin *et al.* (2008). They were instead replaced "as necessary" (*i.e.* when they were more than half-eaten), which was generally every

1-2 days. This difference could contribute to Roslin *et al.* (2008) finding an herbivory effect, while I did not, but only if there was a very quick-acting and short-term defence, on the time scale of one day. In this case, by the time larvae in my herbivory treatment ate plants damaged two or three days prior, the defence would be gone. Roslin *et al.* (2008), however, found the effect of herbivory to be strongest two and five days after induction, not one day.

Late instar larvae as study organisms

Fifth instar larvae were used in all of the feeding trials for two reasons. First, fifth instar larvae were used for consistency with the study by Roslin *et al.* (2008). Second, low density and small size of younger larvae makes finding first to third instars very difficult. Older instars are not only substantially larger than young instars, but they are also aposematically coloured, making them easier to find. *P. smintheus* larvae have yet to be reared from egg to pupation successfully in the lab and consequently using lab-hatched young larvae to study plant/herbivore interactions was not a possibility. The absence of information on plant effects on young instars presents a barrier to understanding the effects of herbivory on *P. smintheus.* Older larvae may not be affected by induced defences to the same extent as younger larvae (Van Dam *et al.* 2001). The late onset of aposematic colouration- yellow spots appear in the fourth instar - indicates that older larvae are likely more able to sequester and use *S. lanceolatum* chemicals as a defence compared to younger instars. Because my study uses only fifth instar larvae, I would not see an effect of induced defences if the defences affected younger larvae only. However, given that older larvae cause far more damage to a plant than younger larvae *S. lanceolatum* should defend against later instars if indeed it has an induced defence.

Relative importance of host plant quality in the field

There are only two possibilities: *S. lanceolatum* either does or does not change in response to herbivory. The more likely of the two possibilities is that *S. lanceolatum* quality changes in response to damage because mechanically

damaged plants negatively affected larval growth in lab (Roslin *et al.* 2008) and plant nutritional chemistry changes with mechanical damage (Illerbrun *unpub.*, Appendix B), which may represent generalist herbivores. If *S. lanceolatum* does produce a defence, then either the specialist *P. smintheus* larvae has overcome it, possibly by mitigating the response with their saliva (Musser *et al.* 2002, Kahl *et al.* 2002, *see above*) or the natural level of damage by *P. smintheus* is not sufficiently high to elicit a response (Underwood 2010). If host plant quality decreases in some other way, due to a reallocation of sugar (Awmack and Leather 2002), for example, the effect on larvae is not strong enough to affect their growth. Regardless of whether *S. lanceolatum* quality decreases with herbivory or not, herbivore-mediated changes in host plant quality play a minimal, if any, role in this system. Karban and Myers (1989) call for caution when comparing laboratory studies of herbivory-induced resistance in plants to field conditions, and to consider that even though plants may respond to herbivory, the herbivores may be faced with greater challenges. Alpine conditions are harsh, and abiotic interactions are unpredictable, with intense sun, snow, wind, rain and heat. In this specialized *P. smintheus*/*S. lanceolatum* system, it appears that larvae overcome, or tolerate, the defences of their host plant. Other, likely exogenous, factors probably contribute more to growth and mortality than does host-plant mediated density dependence. In this vein, future research can include testing explicitly for interactions between host plant quality and climate factors. Duration of snow cover (Torp *et al*. 2010, Guppy and Shepard 2001), drought-stress (Castillo 2003), UV exposure (Li *et al.* 2009) and temperature (Veteli *et al*. 2002) can all affect plant chemistry, and consequently the quality of plants for herbivores. Similarly, weather may alter the relationship between *P. smintheus* larvae and *S. lanceolatum* if host plant suitability varies with plant ontogeny, as it does in *Betula pubescens* ssp. *czrepanovii* (Virtanen and Neuvonen 1999). *Epirrita autumnata* (Geometridae) larval weight is negatively related to *B. pubescens* leaf age, accentuating any effect of asynchrony between leaf flush and insect development. If *P. smintheus* larvae develop more quickly or slowly in relation to *S. lanceolatum* in warmer or cooler years, weather could indirectly affect larvae

through host plant ontogeny, and consequently quality. The potential for weather-induced variation in *S. lanceolatum* quality should be addressed in future studies focusing on the effects of climate on *P. smintheus* populations.

Conclusions

Herbivory does not significantly affect the quality of *S. lanceolatum* as a host plant for *P. smintheus* larvae in the field, at least not for late instar larvae. These findings contradict previous studies and in doing so emphasize a) the differences between mechanically and naturally damaged foliage and b) the relative importance of host plant/herbivore interactions in field settings where abiotic conditions are highly stochastic compared to a controlled laboratory environment. Density-dependent mechanisms are unlikely to contribute to observed population dynamics in this system.

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Chapter 4

Effect of prior herbivory on female oviposition site preference

Introduction

Relatively immobile insect larvae depend on their mothers to have selected an oviposition site suitable for larval growth and survival (Awmack and Leather 2002, Bergman 1999, Renwick and Chew 1994). If host plant quality varies significantly across a landscape, females should maximize their own fitness by choosing the most suitable sites for their offspring to feed, develop and survive. It is generally accepted that adult female butterflies evaluate habitat quality prior to ovipositing (Bergman 1999, Honda 1995, Thompson and Pellmyr 1991), and that this choice can involve simple behaviours, such as identifying the preferred larval host plant species (Reudler *et al.* 2008), or more complex behaviours, such as assessing the concentrations of specific secondary chemicals within the host plant (Castells and Berenbaum 2008, Nieminen *et al.* 2003, Honda 1995). Although generalist herbivores may focus efforts on choosing the most suitable among several available species, specialist herbivores may be more sensitive to intraspecific differences in host plant suitability (Janz and Nylin 1997).

Parnassius smintheus Doubleday larvae are specialist herbivores of *Sedum lanceolatum* (Crassulaceae) at our site. Adult females oviposit near the larval host plant, but not directly on it, possibly as an anti-predation mechanism (Fownes and Roland 2002). Newly hatched larvae are therefore faced with the challenge of locating and accessing a suitable food source. Larvae of the closely-related *P. apollo* do not detect the presence of their host plant at a distance, and simply rely

on high host plant densities to locate plants after hatching (Fred and Brommer 2010). If *P. smintheus* larvae demonstrate a similar inability to find host plants, then the female's choice of oviposition site (with respect to larval resource abundance and distribution) will be of the utmost importance for the survival of her young.

Many plants release volatile chemicals when damaged by herbivores (Karban and Baldwin 1997). These volatiles can serve as oviposition deterrents, as for *Plutella xylostella* (Plutellidae) on *Brassica campestris* (Brassicaceae) (Lu *et al.* 2004) or *Pieris rapae* and *P. brassicae* on *Brassica oleracea* (Bruinsma *et al.* 2007) (but see also Goverde *et al.*2008). Among specialized Lepidopteran herbivores, there appears to be variation in how chemical defences affect oviposition decisions: some herbivores face either a constitutive or induced defence in their host plant, or both, which can either deter or stimulate oviposition (Table 4.1). This project aims to place *P. smintheus* in such a category by determining whether there is evidence for an induced defence, and whether prior host plant damage deters or stimulates oviposition. *S. lanceolatum* contains at least one chemical feeding deterrent, sarmentosin, that *P. smintheus* larvae sequester (Nishida and Rothschild 1995). Although sarmentosin is constitutive and not induced (Weber 2010), there is evidence of a short-term induced defence in *S. lanceolatum,* which negatively affects larval growth in laboratory (Roslin *et al.* 2008) (but see Chapter 3). If herbivory decreases the quality of *S. lanceolatum* as a larval host plant, larval fitness should decrease if females choose to oviposit near damaged plants.

Because larvae are specialized feeders and because there is evidence for an herbivory-induced defence, females should choose oviposition sites that have few herbivory-damaged plants (Roslin *et al*. 2008, Janz and Nylin 1997). Also, because larvae have generally short-range host-finding abilities, females should oviposit in sites of high host-plant density (Fred and Brommer 2010). In addition to assessing larval resources, females may also assess habitat quality based on their own food resource needs (Fred *et al.* 2006). Females may oviposit

preferentially in areas that meet both their own (nectar) and their offspring's (host plant) needs. To test whether female *P. smintheus* prefer undamaged *S. lanceolatum*, I followed females in the field, marked their oviposition sites, and assessed host plant damage levels and nectar resource abundance. In addition, I conducted laboratory-based oviposition preference trials with damaged and undamaged plants.

Table 4.1: Summary of 7 species of Lepidoptera whose host plant(s) has either a constitutive or induced chemical defence, whereby oviposition is deterred or stimulated by increasing concentrations of host plant chemical. Starred (*) species are known to sequester a toxin from their host plant. Note that some species' host plant has both a constitutive and induced defence, and that oviposition can be either deterred or stimulated, depending on the host plant (*e.g. P. xylostella*) or age of the host plant (*e.g. S. littoralis*).

	Constitutive	**Induced**
Oviposition Deterred	*Pieris brassicae*[1] *Pieris rapae*[1] *Plutella xylostella*[2]	*Pieris brassicae* *Pieris rapae* *Plutella xylostella* *Spordoptera littoralis*[3]
Oviposition Stimulated	*Battus archidamas**[4] *Euphydryas aurinia**[5] *Melitaea cinxia**[6]	*Euphydryas aurinia** *Melitaea cinxia** *Plutella xylostella* *Spordoptera littoralis*

1. Bruinsma *et al.* 2007; 2. Lu *et al.* 2004; 3. Anderson and Alborn 1999; 4. Pinto *et al.* 2009; 5. Peñeulas *et al.* 2006; 6. Niemenen *et al.* 2003

Methods

Study organism and location

Preference by *P. smintheus* females for either damaged or undamaged *S. lanceolatum* was evaluated both in the field and in a laboratory. Field studies were located on Jumpingpound Ridge, Kananaskis, AB (50°57'N, 114°54'W, ~2200m). The ridge is in the front range of the Rocky Mountains and consists of a series of open meadows surrounded by coniferous tree stands (for details of species, see Fownes and Roland 2002). *S. lanceolatum* is found in all of the meadows along this ridge. Adult *P. smintheus* females were collected in August 2010 from two of the ridge meadows and were used either for field oviposition

trials or taken to the laboratory at the nearby Biogeoscience Research Institute in Kananaskis, AB, for laboratory experiments on oviposition choice.

Field oviposition trials

Females were collected from late morning to mid-afternoon in early to mid-August and marked with an individual three letter code in conjunction with an ongoing mark-recapture program (Roland *et al.* 2000). Captured females were placed in a cool chamber for at least 10 minutes to reduce movement. They were then placed on a nectar flower plant and monitored for oviposition behaviour. Females that took flight from the nectar flowers were followed and marker flags were placed at each location that females oviposited. Most females oviposited more than once and therefore had several markers associated with them. Females were followed until they could no longer be seen or until they were no longer ovipositing, usually for about 1 hour. The number of undamaged *S. lanceolatum* plants, the number of plants damaged by herbivory, and the number of adult nectar flowers were recorded at three spatial scales around the egg: small (30cm dia.), intermediate (60 cm dia.), and large (100cm dia.). Three sizes of plot were included in an effort to evaluate the scale at which females assess the quality of their oviposition sites. Nineteen females were used for these trials with a total of 74 oviposition events. I also established thirty randomly selected sites in the same two meadows, wherein I recorded the same metrics as for the ovipoistion plots at the same spatial scales. Random plots were chosen by randomly selecting coordinates within the two meadows. This data was collected in collaboration with Kurt Illberbrun.

I used logistic regression analysis to test the effect of total and damaged *S. lanceolatum* plants and nectar flowers on the probability of a plot being an oviposition site compared to a random site, and did so at each of the three spatial scales. To check for correlations among independent variables, I used Pearson's correlation tests.

Laboratory oviposition trials

Females recaptured after the field oviposition trials were put in a cooling chamber and transported to the Biogeoscience Research Institute. Females were then placed in mesh-topped rectangular chambers 35cm long, 15cm wide and 13 cm deep. Mechanically-damaged *S. lanceolatum* was placed on one side of the chamber and undamaged *S. lanceolatum* was placed on the other. A paper towel with an artificial nectar source (4:1 water to white sugar) was placed in the centre of each chamber. Paper towels were wet daily. Chambers were placed in a growth chamber running at 21°C with 16h:8h light:dark. Eight females were used for the trials, which lasted from the day of capture until the females died (1-5 days). The number of eggs laid on each side of the chamber was recorded for each female. Eggs laid on the artificial nectar source were not included in the counts. Oviposition rates on damaged versus undamaged plants were compared using Wilcoxon ranked sums test, paired by butterfly. All analyses were conducted with R (v. 2.9.2, R Development Core Team 2009).

Results

Field oviposition trials

Total *S. lanceolatum* abundance and the number of damaged plants were correlated at all spatial scales (small: $r=0.57$, $P<0.0001$; medium: $r=0.48$, $P<0.0001$; large: $r=0.34$, $P=0.001$), so I assigned total host plant abundance as the first term in the model, thereby generating a conservative estimate of the added effect of herbivory on the probability of a plot being an oviposition site. The final model included the number of *S. lanceolatum* flowers and the number of other nectar flowers as two separate terms because the number of *S. lanceolatum* flowers was also highly correlated with total number of *S. lanceolatum* rosettes (small: $r=0.63$, $P<0.0001$; medium: $r=0.73$, $P<0.0001$; large: $r=0.38$, $P<0.0001$).

At the smallest spatial scale (30cm dia.), the total number of *S. lanceolatum* rosettes was the only significant predictor of oviposition site *vs.* random site ($\chi^2=3.90$, df=1, $P=0.048$; Fig. 4.1 (top)). None of the number of *S.*

lanceolatum flowers, the number other nectar flowers, or the number of herbivorized rosettes affected female oviposition choice (χ^2=1.24, df=1, P=0.26; χ^2=0.002, df=1, P=0.97; χ^2=0.43, df=1, P=0.51, respectively; Fig.4.1 (top)). At the intermediate spatial scale (60cm dia.), oviposition was again predicted by the total number of *S. lanceolatum* rosettes (χ^2=5.45, df=1, P=0.02; Fig. 4.1 (centre)) and also the number of nectar flowers (excluding *S. lanceolatum* flowers) (χ^2=6.80, df=1, P=0<0.01, Fig. 4.1 (centre)). Neither the number of *S. lanceolatum* flowers nor the number of herbivorized rosettes had an effect on oviposition at the intermediate scale (χ^2=0.0001, df=1, P=0.99; χ^2=0.002, df=1, P=0.96, respectively; Fig. 4.1 (centre)). At the largest scale (100cm dia.), only the number of *S. lanceolatum* rosettes was a significant predictor of oviposition (χ^2=12.13, df=1, P<0.001, Fig. 4.1 (bottom)). None of the other three variables: the number of *S. lanceolatum* flowers, the number other nectar flowers, or the number of herbivorized rosettes affected female oviposition choice at the largest spatial scale (χ^2=0.91, df=1, P=0.34; χ^2=1.08, df=1, P=0.30; χ^2=1.57, df=1, P=0.21, respectively; Fig.4.1 (bottom)). Because the first variable in the model was total number of *S. lanceolatum* rosettes, only a very strong effect of *S. lanceolatum* flowers or herbivorized rosettes on oviposition would be detected.

When I repeated the above analyses using only the first oviposition event for each female, the patterns were the same; multiple oviposition events from some of the females did not significantly affect the model estimates (Appendix C).

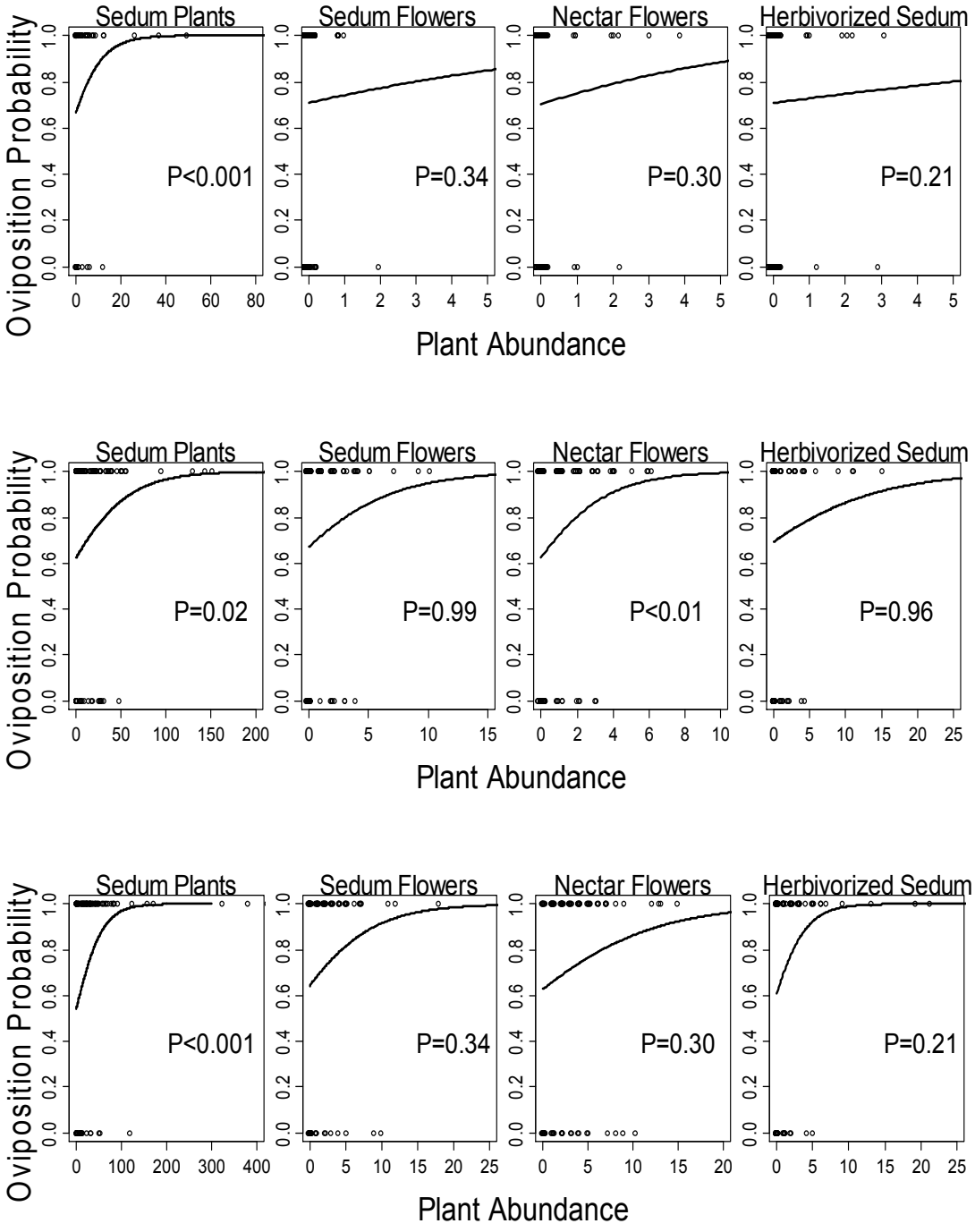

Figure. 4.1 Logistic regression estimates of the probability of a plot being an oviposition site *vs.* a random site, as a function of total number of *Sedum lanceolatum*, number of *S. lanceolatum* flowers, number of herbivorized *S. lanceolatum*, and number of adult nectar flowers (other than *S. lanceolatum* flowers) at small (top), intermediate (middle) and large (bottom) spatial scales. P-values for each predictor variable were calculated based on an analysis of deviance test (Chi-square). Data points are offset to show stacking.

Laboratory oviposition trials

Females showed no preference for ovipositing near damaged or undamaged *S. lanceolatum* plants (T=39, P=0.92, Fig. 4.2). The average (±SE) number of eggs laid on the "damaged" plant side of the chamber was 11.1±3.3 (median=6), compared to 11.3±3.6 (median=7) on the "undamaged" plant side.

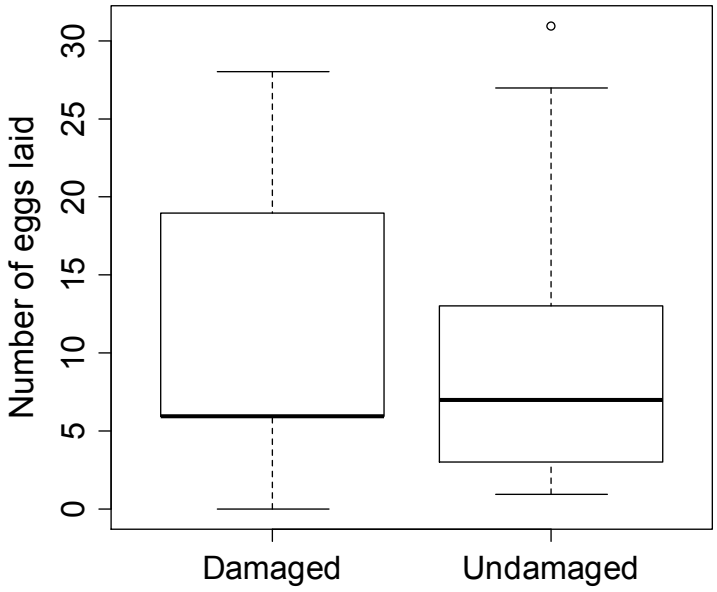

Figure 4.2: Number of eggs laid on each side of the growth chambers. Mechanically damaged *S. lanceolatum* was placed on one side of each chamber and undamaged *S. lanceolatum* was placed on the other. Total number of eggs laid was recorded for eight females. The horizontal line represents the median number of eggs laid on each side, and the lower and upper boundaries of the boxes represent the first and third quartiles, respectively. Whiskers represent 1.5 times the interquartile range and circles indicate outliers beyond that limit.

Discussion

Damage to the larval host plant did not affect female *P. smintheus* oviposition behaviour in the field or in the laboratory, suggesting that damaged plants are not considered lower quality food. Although this pattern is contrary to

my initial expectations, these results are not surprising in light of larval feeding trials (Chapter 3) in which herbivory did not affect larval growth over any time scale. Results from both the feeding trials and the oviposition trials suggest that females do not perceive herbivory as an indicator of habitat quality, presumably because it does not affect the fitness of their offspring (Chapter 3). These findings contrast with observations in *Pieris* sp. (Pieridae), for which chemicals associated with an induced defence in cruciferous host plants deter oviposition (Bruinsma *et al.* 2007). However, unlike *P. smintheus* larvae in my experiments, *Pieris* sp. larvae reared on induced host plants did have lower fitness because they developed more slowly and were exposed to natural enemies for longer (Bruinsma *et al.* 2007). *S. lanceolatum* damage did not stimulate oviposition either. In cases where larvae are not sensitive to secondary chemicals from the host plant, or where they sequester plant toxins, females may not respond to secondary chemicals when ovipositing. For example, *Agonopterix alstroemericana* (Oecophoridae) females, whose larvae sequester plant chemicals, do not show a preference for either high or low concentrations of piperidine alkaloids in *Conium maculatum* when ovipositing (Castells and Berenbaum 2008). Because *P. smintheus* oviposition is neither deterred nor stimulated by an induced defence, they do not fit into one of the categories presented in Table 4.1.

Although it appears there is no induced defence in *S. lanceolatum*, or any other decrease in plant quality due to herbivory, in the field (Chapter 3), my results are consistent with the plant maintaining a constitutive defence. No other animal eats *S. lanceolatum*, possibly due to the presence of sarmentosin, a chemical feeding deterrent that *P. smintheus* larvae have likely overcome by sequestration (Nishida and Rothschild 1995). *Plutella xylostella* (Lepidoptera) herbivory does not induce a defence in its host plants that have high levels of constitutive defences, which consequently does not deter oviposition (Lu *et al.* 2004). Herbivory can, however, deter oviposition on host plants that have low constitutive defences, a pattern attributed to a strong induced defence (Lu *et al.* 2004). It is therefore possible that *S. lanceolatum* has also evolved a trade-off strategy: a high constitutive defence for a low induced defence.

Although level of herbivory is not indicative of habitat quality in this system, females do none the less evaluate their habitat prior to ovipositing. We know that *P. smintheus* adults move to areas of high *P. smintheus* density (Roland *et al.* 2000) and to areas of high nectar flower abundance (Matter and Roland 2002). Fownes and Roland (2002) report that females only oviposit in the presence of *S. lanceolatum* and my study adds to these findings by showing that females also judge oviposition site quality based on the abundance of resources available to their offspring and themselves.

Host plant density is also a strong indicator of habitat quality for many Lepidopteran species (see Bergman 2001 for a review), affecting oviposition choices in *Melitaea cinxia* (Nymphalidae) (Kuussarri *et al.* 2000), *Polites mardon* (Hesperiidae) (Beyer and Schultz 2010), *Leuhdorfia japonica* (Papilonidae) (Hatada and Matsumoto 2008), *Lopinga achine* (Nymphalidae) (Bergman 1999), as it is for *P. smintheus*. If *P. smintheus* larvae, like *P. apollo* larvae, cannot detect their host plant, the positive response to *S. lanceolatum* abundance by the female butterfly will strongly benefit her larvae (Fred *et al.* 2006). It is clear that females respond to their larval host plant, but how *P. smintheus* are assessing host plant abundance remains to be determined. *P. smintheus* is among the few butterflies that oviposit off of their host plant (Fred and Brommer 2010, Fownes and Roland 2002, Scott 1986), a pattern that my findings support as well; no females were observed ovipositing on *S. lanceolatum* during the trials. Also, females rarely alight on *S. lanceolatum* prior to ovipositing (*pers. obs.*). Consequently, it is unlikely that females are using chemical cues picked up by their tarsi to judge host-plant quality, as do other species of butterfly (Nishida 2005, Thompson and Pellmyr 1991). They could, however, be using volatile olfactory cues emitted by *S. lanceolatum*. Wiklund (1984) posits that *P. apollo* females oviposit in response to the general "fragrance" of the very abundant *S. album* (the larval host plant) in their habitat. However, although *S. lanceolatum* abundance is relatively high at my site, the abundance of *S. lanceolatum* is clearly higher in oviposition sites, compared to random sites located in the same meadow, meaning that females are

responding at a finer scale than that of the entire meadow. Visual recognition is a possible mechanism for detecting host plants (Renwick and Chew 1994). *Eurema hecabe* (Pieridae), for example, can discern the pattern of its host plant leaves from 20cm away (Hirota and Kato 2001). This method of host plant recognition is a possibility for *P. smintheus,* although it remains untested. My data show that females do not respond to *S. lanceolatum* flower abundance, so any visual response would likely be to the leaf or rosette shape.

Nectar flower abundance is a predictor of oviposition at the intermediate scale (60cm) suggesting that females nectar and then oviposit soon after, a pattern commonly found for other butterfly species, like *Polyommatus icarus* (Lycaenidae) (Janz *et al.* 2005). Unlike *Polyommatus icarus*, however, *P. smintheus* butterflies move off of the nectar flower to oviposit (*pers. obs*.). After evaluating the abundance of host plants in the area, females could then be searching for a safe overwintering habitat for their eggs, which may be a substrate other than larval host plant either because of favoured microhabitat or risked predation (Wiklund 1984). Nectar resources also influence the distribution of eggs in *Euphydryas chalcedona* (Lepidoptera: Nymphalidae) and *Anthocharis cardamines* (Lepidoptera: Pieridae) systems (reviewed by Thompson and Pellmyr 1991). *P. apollo* butterflies in Finland are endangered and their habitat is actively managed. Female *P. apollo* distribute themselves and, consequently, their young based on nectar resource distribution, not larval host plants (Fred *et al.* 2006). Nectar and larval resources for *P. apollo* can be separated by over 900m, which could have consequences for population dynamics and conservation if that segregation is not taken into account when managing butterfly habitat (Fred *et al.* 2006). Although I did not determine whether *P. smintheus* females place priority for their resources over those of their larvae, adult nectar plants and *S. lanceolatum* are located in the same meadows and tend to be near each other (this study, Fownes and Roland 2002), and spatial separation of resources is not a problem. However, if larval host plants and adult nectar resources become decoupled in the future, it will be important to better understand female priorities when ovipositing.

Conclusions

Larval host plant density influences *P. smintheus* oviposition choices, a finding supported by other studies as well. However, *S. lanceolatum* herbivory does not have a significant effect on this decision-making process, likely because previous herbivory carries no negative consequences for the larvae. It remains unclear how females assess host plant density, but either visual or olfactory cues, or both, are possible. Oviposition is also stimulated by nectar flower resources and females are choosing sites that benefit themselves as well as their larvae. Future research should address whether or not females prioritize nectar or larval resources when ovipositing to better understand the consequences of resource separation for *P. smintheus* populations.

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Chapter 5

General discussion and conclusions

Alpine environments are often unpredictable. The threat of snow, frost, rain and wind is imminent and the growing season is short. For ectotherms, the role of endogenous (*i.e*. biotic) interactions in determining populations, especially in areas of capricious weather, such as the alpine, is often questioned. I explore the effects of temperature, an exogenous factor affecting populations, and host plant-herbivore interactions, an endogenous biotic process, on the growth and survival of *Parnassius smintheus* larvae. My aim was to help clarify potential importance of each in the dynamics of this alpine insect.

Summary

My research shows that ambient temperature affects the development of *P. smintheus* larvae more strongly than do host plant/herbivore interactions. The range of temperatures over which I studied larval growth was relatively narrow, but I did not detect a direct effect of temperature increase or decrease on larval survival, pre-pupal weight or on initiation of larval diapause. Because larval diapause appears to not be an option for *P. smintheus* in cool conditions, populations are not likely to experience double cohorts following cool years. Consequently, over the range of values at which I studied larvae, temperature does not directly influence patterns of *P. smintheus* growth and decline, or at least it does not do so at the larval stage. *P. smintheus* are, however, still sensitive temperature because larvae develop more quickly at warmer temperatures, but they may mitigate such effects through behavioural means.

Patterns of population growth and decline also cannot be explained by biotic interactions between larvae and their host plant. Previous host-plant

herbivory did not affect larval growth either within a season or between years, indicating that it cannot generate density-dependent population growth, and likely contributes little to *P. smintheus* population dynamics. Even though females do respond to the abundance of their host plant, herbivory of those plants does not affect their oviposition behaviour.

Directions of future research

The mechanisms explaining *P. smintheus* population growth and decline remain unknown. Although my research addressed the effects of moderate temperature shifts on *P. smintheus* larvae, exploring the effect of "climate" on *P. smintheus*, including precipitation and extreme weather events (Roland and Matter *in review*), was outside the scope of my project and warrants further study. Also, I studied the larval stage only, which is the stage most likely to be affected by density-dependent interactions (Nowicky *et al.*2009). Research should also include studying the effect of weather and climatic variables on eggs and pupae, life stages that have no behavioural means for thermoregulating.

Even the moderate increase in temperature used in my study increased larval growth rate and decreased time to pupation, indicating a potential for phenological shifts with future climate change. This indirect effect of temperature leaves several avenues open for further study. Firstly, the potential for adult emergence asynchrony in this protandrous system should be assessed in the field. Wheeler (2010) developed a theoretical model of the relationship between *P. smintheus* emergence and fecundity under different temperature regimes. Model results predict potential negative consequences for population if male and female emergence becomes asynchronous. Further, field-based research is necessary to quantify the effects of temperature on intraspecific synchrony.

Secondly, larval and adult interactions with their host and nectar plants could change with long-term changes in temperature (Ashton *et al.* 2009, Araújo *et al.* 2007). Yearly variation in weather, including precipitation and temperature, can alter host plant nutrient levels (Torp et al. 2010), defensive compounds

(Gianoli 2002) or the timing of herbivory relative to plant ontogeny (Virtanen and Neuvonen 1999), leading to between-year variability in host plant quality. For example, *S. lanceolatum* is reported to be toxic to *P. smintheus* during the winter months (anecdotal, Guppy and Shepard 2001). Although *S. lanceolatum* is perennial and technically available for larvae as soon as the snow melts in the spring, if it remains toxic into early spring, larvae developing and eating quickly could have difficulties finding enough non-toxic food. Kurt Illerbrun (University of Alberta) is currently addressing questions relating to snow and *S. lanceolatum* quality, which will hopefully lead to a better understanding of the risks for larvae in early spring. New interactions with mates and host plants are among some of the potential indirect consequences of a changing climate for *P. smintheus* populations.

Although *P. smintheus* larvae are unaffected by any potential induced defence produced by *S lanceolatum* and this interaction is unlikely to have population-level consequences, the co-evolution of plant and herbivore in this system may nonetheless pique the interest of researchers in the future. *P. smintheus* have overcome a constitutive defence by sequestering the plant toxin and using it to their advantage, and may also have developed a way to overcome an induced chemical response to herbivory. Further detailing the chemical interaction of *P. smintheus* larvae with their host plant across their range is interesting from both an evolutionary perspective and from a conservation perspective if this intimate interaction determines the range limit for this species.

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Appendix A

Cumulative number of leaves eaten as the continuous independent variable

Herbivory treatment had no effect on larval growth when "cumulative number of leaves eaten" was used as the continuous independent variable instead of time in both the 2010 within-year feeding trial and the between-year feeding trial. Larvae gained weight similarly in both treatments as they ate more leaves, as indicated by a lack of interaction between treatment and cumulative number of leaves eaten. As expected, larvae gained weight with the number of leaves eaten (Table A.1). In both trials, the models using time as the continuous fixed effect explained larval growth better than did those using cumulative number of leaves eaten, based on AIC values (Table A.2).

Table A.1: Parameters estimates (a) and significance in the model (b) from the linear mixed effect models for both the within-year herbivory field study in 2010 and the between-year herbivory study using "cumulative number of leaves eaten" as the independent variable instead of time.

a)

	Estimate (g) (S.E.)	**P-value**
Within-year trial 2010		
Intercept- 10 days since herbivory	0.19394 (0.0209)	<0.001
5 days since herbivory	0.03149 (0.0302)	0.30
2 days since herbivory	-0.00022 (0.0291)	0.99
control	0.02024 (0.0287)	0.48
Slope- 10 days since herbivory	0.00220 (0.0003)	<0.001
5 days since herbivory	-0.00019 (0.0004)	0.66
2 days since herbivory	-0.00049 (0.0004)	0.26
control	-0.00031 (0.0004)	0.49
Between-year trial		
Intercept- Control	0.35720 (0.0286)	<0.001
Herbivory	-0.02867 (0.0429	0.51
Slope- Control	0.00200 (0.0002)	<0.001
Herbivory	-0.00003 (0.0003)	0.93

b)

	df	F-stat	P-value
Within-year trial 2010			
Intercept	1,116	415.21	<0.001
Cumulative leaves eaten	1,116	170.85	<0.001
Treatment	3,39	0.23	0.87
Interaction	3,116	0.47	0.71
Between-year trial			
Intercept	1,84	379.39	<0.001
Cumulative leaves eaten	1,84	142.29	<0.001
Treatment	1,25	1.02	0.32
Interaction	1,84	0.0076	0.93

Table A.2: AIC values associated with the linear mixed effects models used to analyse the effect of within-year herbivory (2010 field feeding trial) and between-year herbivory (field feeding trial) on larval growth (weight) using either "cumulative number of leaves eaten" or "trial day" (time) as the independent variable.

Study	Cumulative leaves eaten	Trial day
2010 within-year herbivory effects	-509.71	-550.67
Between-year herbviory effects	-339.76	-341.98

Appendix B

Chemical analysis of plants damaged mechanically and with herbivory

Methods

In collaboration with Kurt Illerbrun (University of Alberta), I compared the response of *S. lanceolatum* to mechanical damage and larval herbivory using leaf carbon/nitrogen ratios as a rough indicator of plant quality for larvae. In July 2010, 210 greenhouse-grown *S. lanceolatum* plants were separated into three treatment groups: mechanical damage, herbivory damage, and no damage (control). The plants in the mechanical damage group were trimmed with scissors in a way that mimics larval herbivory patterns. The plants in the hebivory damage groups were fed upon by 5-10 fourth and fifth instar larvae. Five to ten percent of the plant was damaged in both the mechanical and herbivory groups. Plants were harvested at the base 1, 3, 5, 7, 9, 11 and 13 days after damage to see how C:N ratios changed with time since damage. After harvest, plants were frozen immediately.

Plants were dried in a drying oven, ground, and processed for carbon and nitrogen content using a CHN Analyzer (Control Equipment Corporation Model 440 Elemental Analyzer, Biogeochemical Analytical Lab, University of Alberta). The C:N ratios were compared between damage groups (mechanical, herbivory and control) and across time since herbivory intervals with a multi-factor ANOVA. Tukey's HSD post-hoc test was used to compare which groups differed from each other.

Results

The type of damage significantly affected C:N ratios of plant leaf tissue ($F_{2,189}$= 4.04, P=0.02). Mechanically-damaged plants had significantly lower C:N ratios than undamaged control plants (Tukey's HSD P=0.02) and marginally lower C:N ratios than herbivory-damaged plants (Tukey's HSD P=0.08). There is

no difference between herbivory-damaged plants and control plants (Tukey's HSD P=0.85). This pattern can be attributed to a change in percent nitrogen ($F_{2,189}$=5.38, P<0.01), not carbon ($F_{2,189}$=1.72, P=0.18). Time since damage affected C:N ratios ($F_{6,189}$=2.20, P=0.04, Fig B.1), and there was a significant interaction between damage type and time since damage ($F_{12,189}$=1.96, P=0.03, Fig B.1). Data show a general trend such that control plants have the highest C:N ratio, followed by herbivory-damaged plants, followed by mechanically-damaged plants until 9 days after herbivory, when the pattern breaks down (Fig B.1.). These results suggest that there is a nitrogenous defence produced by mechanical damage, and that it lasts for just over a week.

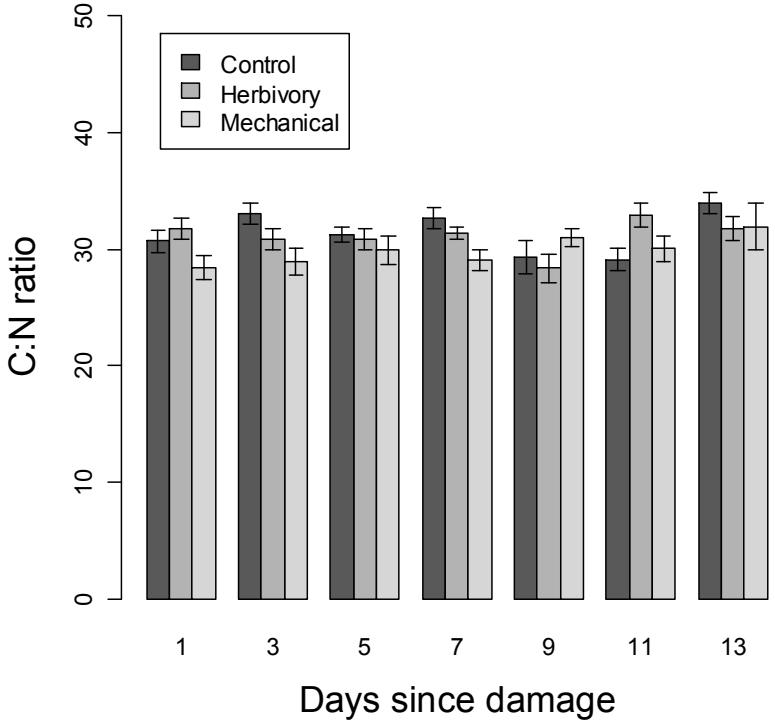

Figure B.1: Mean leaf C/N ratio (±S.E) of undamaged *S. lanceolatum* plants, plants damaged by larval herbivory and plants damaged mechanically (with scissors) as a function of days since damage.

Appendix C

Logistic regression with first oviposition events only

When repeating the logistic regression analyses using only the first oviposition event from each female, I used the same order of terms as with all oviposition events because the number of herbivorized plants was still correlated to the total number of *S. lanceolatum* rosettes at all spatial scales (small: r=0.67, P<0.0001; intermediate: r=0.48, P<0.01; large: r=0.74, P<0.0001). I once again included number of *S. lanceolatum* flowers as a separate term than number of other nectar flowers because of the high correlation between *S. lanceolatum* flowers and total *S. lanceolatum* rosettes (small: r=0.86, P<0.0001; intermediate: r=0.54, P<0.0001; large: r=0.56, P<0.0001).

At the smallest spatial scale (30cm), none of the model terms were significant predictors of oviposition (Table C.1). At the intermediate scale (60cm), nectar flower abundance was the only significant predictor of oviposition (Table C.2). At the largest scale (100cm), only the total number of rosettes was a significant predictor of oviposition (Table C.3).

Table C.1**:** Significance of logistic regression model terms as predictors of an oviposition site compared to a random site at the smallest spatial scale (30cm). Significance of the terms was determined with an analysis of deviance.

	χ^2	df	P
S. lanceolatum rosette abundance	1.36	1	0.24
S. lanceolatum flower abundance	2.44	1	0.12
Nectar flower abundance	0.58	1	0.45
Number of herbivorized rosettes	1.09	1	0.29

Table C.2: Significance of logistic regression model terms as predictors of an oviposition site compared to a random site at the intermediate spatial scale (60cm). Significance of the terms was determined with an analysis of deviance.

	χ^2	df	P
S. lanceolatum rosette abundance	2.08	1	0.15
S. lanceolatum flower abundance	<0.01	1	0.97
Nectar flower abundance	8.55	1	<0.01
Number of herbivorized rosettes	2.10	1	0.15

Table C.3: Significance of logistic regression model terms as predictors of an oviposition site compared to a random site at the largest spatial scale (100 cm). Significance of the terms was determined with an analysis of deviance.

	χ^2	df	P
S. lanceolatum rosette abundance	3.96	1	0.05
S. lanceolatum flower abundance	1.08	1	0.30
Nectar flower abundance	0.69	1	0.41
Number of herbivorized rosettes	1.80	1	0.18